

Effects of short-term irradiation on photoinhibition and accumulation of mycosporine-like amino acids in sun and shade species of the red algal genus *Porphyra*

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Abstract

The effect of irradiance (40 and 840 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) of short-term (48 h) irradiation on photosynthetic activity (estimated as oxygen evolution and as chlorophyll fluorescence), specific absorption and fluorescence excitation spectra, photosynthetic pigment accumulation (chlorophyll *a* and biliproteins) and UV-absorbing compounds (mycosporine-like amino acids, MAAs) was investigated in sun and shade species of the red algal genus *Porphyra* collected in Trondheimsfjord (Norway). In the sun type, high irradiance exposure (840 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) did not alter the Chl *a* concentration, however, exposure to a lower irradiance (40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 48 h significantly increased the chlorophyll concentration. The content of MAAs was significantly higher in the sun type than in the shade type algae. *Porphyra*-334 is the main MAA in this species followed by shinorine. The total content of MAAs significantly ($P < 0.05$) increased in the sun type after 48 h exposure to both high and low irradiances. However, in the shade type, *porphyra*-334 significantly decreased ($P < 0.05$) after both high and low irradiance exposure. Photosynthetic activity (as oxygen evolution) and the optimal quantum yield (F_v/F_m), as an indicator of photoinhibition, decreased under low and high irradiance in the shade type algae and no full recovery was observed when the algae were transferred to very low irradiation. The sun type algae presented a higher capacity of acclimation to increased irradiance than the shade type algae. This high acclimation of sun type algae to short term high irradiance exposure (48 h) is explained by the higher thermal dissipation. This was estimated as the ratio of nonphotochemical quenching related to the light dose (q_N :dose) and by the accumulation of MAAs.

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1. Introduction

Plants submitted to high exposure of solar radiation, both photosynthetic active radiation (PAR, 400–700 nm) and ultraviolet radiation (UVR, 280–400 nm) suffer photoinhibition. Two different kind of photoinhibition have been defined, dynamic and chronic photoinhibition [1]. In general, sun-adapted algae exhibit dynamic photoinhibition, i.e. a reversible photoprotective mechanism consisting in a downregulation of the photosystem II (PSII) in order

to handle excess energy, which increases thermal energy dissipation. In contrast, when shade-adapted algae are transferred to high irradiance e.g. shallow waters, chronic photoinhibition occurs. This phenomenon is characterized by photodamage of PSII reaction centres and subsequent proteolysis of the D1 protein [2]. Photodamage occurs when the rate of degradation of D1 proteins exceeds the rate of repair [3].

In this study, the extent of photoinhibition of sun and shade species of the red algal genus *Porphyra* submitted to short term high irradiance of PAR (48 h) is analysed. The photoinhibition is estimated as a decrease of both oxygen evolution and optimal quantum yield of Chl *a* fluorescence by using pulse amplitude modulated (PAM) technique. The

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algae subjected to very high irradiances have higher photoinhibition and recovery than those algae inhabiting shaded sites [4]. The recovery capacity, measured as increase in the quantum yield of fluorescence, is species-specific, i.e. it is more rapid in sun-adapted algae than in algae growing at deep or shaded locations and then transferred to the surface [5,6].

PAM chlorophyll fluorescence associated with PSII was primarily developed to assess stress-dependent changes in photosynthesis of higher plants [7]. The application of PAM fluorometry to macroalgae is also a useful tool for evaluation photosynthesis under different environmental conditions and in the laboratory under artificial lamps [4,8–10].

Chlorophyll fluorescence can function as an indicator of the different functional levels of photosynthesis such as photon capture by light harvesting pigments, primary light reactions, thylakoid electron transport reactions, dark-enzymatic stroma reactions and slow regulatory feedback processes [11]. The photochemical principles include two different types of competing de-excitation processes: (1) photochemical energy conversion at the photosystem II (PSII) centers and (2) nonphotochemical dissipation of excitation energy at the antenna and reaction center levels.

The macroalgae, as other plants, possesses different strategies for protecting their photosynthetic equipment against excess light [12]. The dynamic photoinhibition acts as a photoprotective mechanism [1], which decreases the quantum yield of PSII, so that excess absorbed energy is rendered harmless by thermal dissipation [13]. A relation between xanthophyll content in the antenna and energy dissipation is documented in green macroalgae [14–16] and in brown algae [17,18] but not in red algae. Other mechanisms for protection against UVR or excess PAR is the production of screen pigments such as the carotenoids or the UV-absorbing mycosporine-like amino acids (MAAs). MAAs have been reported in green, red and brown algae from tropical, temperate and polar regions [19–21].

In the last decade, a great number of studies of the environmental control (mainly light, salinity and temperature) of MAAs production and on the physiological role of MAAs have been reported [22,23]. Apical pieces of *Euchema striatum* have been shown to produce high concentrations of UV-absorbing pigments under UVR exposure. This is relative to the concentrations of unexposed pieces but growth was not depressed in the presence of UVR [24,25]. Macroalgae often contain several MAAs with different absorbance maxima in the ultraviolet-B (UV-B 280–315 nm) and ultraviolet-A (UV-A, 315–400 nm) radiation [20,26]. The concentration of MAAs has been correlated with depth zonation and UV exposure [27]. The fluorescence emissions of these compounds are at wavelengths that can be absorbed by chlorophyll with subsequent energy transfer into photosynthetic pigment but this is not relevant for photosynthesis because of the low UV irradiance under solar

radiation [28]. In addition, recently, Conde et al. [29] concluded that the very low quantum yields of fluorescence, intersystem crossing and photolysis are in agreement with a photoprotective role of porphyra-334 in living systems. There is little direct evidence that MAAs act only as UVR blockers and do not have other biological functions [22,30]. Microscale variations in the *in vivo* absorbance in turf algae has been directly related to the amount of extractable UVR absorbing compounds. The *in vivo* absorbance signature at 345 nm appears to provide a method to quickly and accurately gauge the potential UVR-shielding capacity of the primary producer [31]. The investigation of photoprotection mechanisms to high irradiances of PAR is of interest since these mechanisms could be operating under increased UV-B radiation. The increase of UV-B as a consequence of ozone depletion could have a great impact on the photosynthetic carbon fixation by plants and consequently, on the global climate change [32]. Thus, at the present time there is great interest in determining the effects of increased UV-B radiation on primary productivity in both terrestrial and aquatic ecosystems. Among plants, macroalgae can also be affected, mainly the intertidal algae submitted to drastic variations in environmental conditions. UV-B radiation can produce DNA damage, photoinhibition of photosynthesis or viability of spores [4,33,34].

In this study, the accumulation of MAAs, porphyra-334 and shinorine, in sun and shade species of the algal genus *Porphyra* and their role in photoprotection are evaluated in algae collected from Trondheimsfjord (Norway) exposed for a short period of time (48 h) to low and high irradiance produced by artificial lamps.

2. Material and methods

2.1. Algal species

Two *Porphyra* species were harvested in mid-August 1999 on the rocky shores just in front of the Trondheim Biological station in Trondheimsfjord, Norway (63°N), *Porphyra purpurea* growing in the upper part of the intertidal system and another species of *Porphyra* still under taxonomical identification [35] growing in the lower eulittoral.

Porphyra purpurea emerges during low tide, however, the other *Porphyra* species remain below the water surface during low tide. Both species grow epilithically on stones. According to the exposure in its natural growth site and its pigment composition (Table 1), *Porphyra purpurea* is denominated as sun type algae and the other *Porphyra* species is a shade type algae.

2.2. Light treatments

After harvesting, the algae were incubated at 14 °C in darkness for 12 h in seawater collected from the fiord. The

Table 1

Concentration of photosynthetic pigments, chlorophyll *a* (Chl *a*) and biliproteins as phycoerythrin (PE) and phycocyanin (PC) expressed in mg m^{-2} before and after exposure to artificial lamps under high irradiance (HI, $840 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and low irradiance (LI, $40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) of two *Porphyra* species, a sunlight type (*Porphyra purpurea*) and a shade type (*Porphyra* sp.)

	Sun type <i>Porphyra</i>				Shade type <i>Porphyra</i>			
	Chl <i>a</i>	PE	PC	PE/PC	Chl <i>a</i>	PE	PC	PE/PC
0 h	20.6±2.1 ^a	2.4±0.15	0.7±0.05 ^a	3.4±0.36 ^a	72.7±6.5 ^a	6.1±0.52 ^a	5.5±0.59 ^a	1.1±0.12 ^a
HI-24 h	25.6±1.8 ^b	1.0±0.08 ^b	0.2±0.01 ^b	4.7±0.45 ^b	77.3±6.9 ^a	3.9±0.36 ^b	1.7±0.21 ^b	2.3±0.25 ^b
HI-48 h	20.7±1.9 ^a	2.0±0.15 ^c	0.4±0.03 ^c	4.5±0.46 ^b	51.8±4.8 ^b	2.4±0.26 ^c	1.0±0.09 ^c	1.0±0.09 ^c
LI-24 h	30.0±2.6 ^c	1.1±0.09 ^b	0.4±0.04 ^c	3.0±0.34 ^a	80.3±7.8 ^a	2.4±0.28 ^c	1.2±0.15 ^c	2.0±0.23 ^b
LI-48 h	32.2±2.8 ^c	1.8±0.12 ^c	0.6±0.05 ^a	3.1±0.34 ^a	62.0±6.5 ^b	2.6±0.27 ^c	1.2±0.16 ^c	2.1±0.24 ^b

Different letters indicate significant differences ($P < 0.05$) for each variable (comparing initial with high and low irradiance treatments).

temperature of the seawater was maintained at 14 °C by continuous circulation of seawater taken directly from the fiord using a pump system. After this pretreatment, algae were submitted for 48 h to high irradiance ($840 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and low irradiance ($40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) using high pressure halide lamps (Optimarc, Duro test, NJ, USA). After 12 h in darkness and after 24 and 48 h exposure three samples of algae were taken to determine light absorption of the thallus, pigment concentration: chlorophyll and biliprotein (phycoerythrin and phycocyanin), concentration and composition of MAAs, effective and optimal quantum yield of fluorescence and photosynthetic activity by oxygen evolution.

2.3. Light absorption of thallus and fluorescence excitation spectra

In vivo thallus absorption was measured in 2-cm diameter discs of algal tissue mounted in plastic Petri dishes containing filtered seawater. This method is analogous to the filter-pad technique used in phytoplankton studies [36] and previously reported by Grymski et al. [37]. The Petri dish was placed close to a photomultiplier and scanned from 350 to 800 nm in a Hitachi 150-20 double beam spectrophotometer (Hitachi, Tokyo, Japan) with a clear Petri dish as a reference. Fluorescence excitation spectra from a Hitachi F-3000 spectrofluorometer, at emission of 730 nm according to Neori et al. [38], were measured by placing a rectangular piece of alga into a 1-cm quartz cuvette filled with filtered seawater at a 45° angle to the emission detector and scanned from 400 to 700 nm with and without the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (final concentration 30 μM). The algae were incubated under actinic light for 3–5 min to allow the DCMU to close PSII reaction centres. To scale the relative fluorescence excitation spectrum, it is necessary to first collect a quantum-corrected spectrum by using Basic Blue dye 3 [39].

Verification of the quantum correction was done by comparing the shape of light-absorption spectra from authentic standards of Chl *a* with the corresponding quantum corrected fluorescence excitation spectra [40].

2.4. Pigment analysis

Chl *a* was extracted with 90% pH-neutralised (sodium carbonate) acetone during 24 h at 4 °C in the dark and after grinding of the thalli in a mortar. Its concentration was determined spectrophotometrically by using the equation of Jeffrey and Humphrey [41].

Phycoerythrin (PE) and phycocyanin (PC) were extracted at 4 °C in phosphate buffer 0.1 M (pH 6.5) containing 10 mM EDTA- Na_2 and 4 mM phenylmethylsulphonyl fluoride (PMSF). Biliprotein concentration was determined spectrophotometrically using the equations of Beer and Eshel [42].

2.5. Analysis of mycosporine-like amino acids

Air-dried algal samples (10–20 mg dry weight) were extracted for 2 h in screw-capped centrifuge vials filled with 1 ml 20% aqueous methanol (v/v) and incubated in a waterbath at 45 °C. After centrifugation at 5000 *g* for 5 min, 700 μl of the supernatants were evaporated to dryness under a vacuum (rotovapor). Dried extracts were redissolved in 700 μl 100% methanol and vortexed for 30 s. After passing through a 0.2- μm membrane filter, samples were analysed with a Waters HPLC system, W600 Powerline (Waters Cromatografía, Barcelona, Spain). The mobile phase was 5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water, run isocratically at a flow-rate of 0.5 ml min^{-1} .

Sample volumes of 10 μl were injected into the Spherclone C_8 column with precolumn (5 m packing; 250×4 mm I.D.). MAAs were detected online with a Waters photodiode array detection system at 330 nm and absorption spectra (290–400 nm) were recorded each second directly on the HPLC-separated peaks. Identification of the MAAs was by comparison of the absorption spectra and retention times with various standards (*Mastocarpus stellatus*, *Bostrychia scorpioides*, *Porphyra yezoensis* and fish lenses of the coral trout *Plectropomus leopardus*) kindly provided by Dr. Ulf Karsten, University of Rostock, Germany.

2.6. Fluorescence measurements

In vivo chlorophyll fluorescence of PSII was measured with a portable pulse modulation fluorometer (PAM 2000, Waltz, Effeltrich, Germany). For measurements of quantum efficiency, optimal quantum yield (F_v/F_m), plants were first incubated in darkness for 15 min F_v being the difference between the maximal fluorescence (F_m), that is the emission when all PSII reaction centres are reduced and the initial fluorescence (F_0) when all PSII reaction centres are oxidised. For each measuring series (initial, sunlight exposure and after three subsequent recovery periods), 8–10 individual plants were taken, which were then removed from the system. As outlined above, fluorescence emission in red algae is complicated by the presence of phycobilisomes: the onset of maximal fluorescence (after a saturating light pulse) occurs very quickly, which is associated with excitation of photosystem I (PSI) and delay of the F_m decline. Thus, we applied a modified protocol described by Hanelt et al. [43]: first, samples were submitted to 5 s of low irradiance ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$) far-red pulse followed by 5 min of darkness. To ensure the stabilisation of the fluorescence signal, a short red actinic pulse (5 s, $8 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 655 nm) was given. An additional 5 s far-red pulse was given again to fully oxidise the electron transport chain. Finally, F_0 was recorded after a pulsed, red measuring light ($0.3 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 650 nm), while F_m was induced with a saturating white light pulse (0.4 ms, approx. $9000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). This procedure was repeated in triplicate for each sample with intervals of 5 min of darkness to avoid incomplete recovery of fluorescence quenching parameters such as energy quenching or those derived from state transitions [43].

Nonphotochemical quenching (q_N) was calculated according to the expression [7]: $q_N = (F_m - F'_m)/(F_m - F'_0)$ where F'_0 is the minimum fluorescence yield of a light-acclimated plant to a given irradiance (preilluminated sample) and F'_m is the maximal fluorescence of light adapted algae. These coefficients vary between 0 and 1 and under increasing irradiances. An accurate measurement of F'_0 requires the application of far-red pulses to re-oxidise the reaction centres that still remain reduced.

2.7. Photosynthetic rate as oxygen evolution

Thalli (0.15–0.2 g FW) were taken and put into a measurement chamber (10 ml) fitted with a Clark-type electrode and a magnetic stirrer by using OXY M-5 equipment (Real Time Computer, Erlangen, Germany) and with the optic fibre of the PAM-2000 fluorometer. The chamber contained filtered seawater and was maintained at a temperature of $\sim 15^\circ\text{C}$. Two Durotest lighting white light lamps (Duro-Test, New Jersey, USA) were used as light sources. The irradiances reaching the thallus surface were monitored using a spherical PAR quantum sensor

(Zemoko, The Netherlands) specially designed for small measuring chambers. Thalli were exposed for 5–10 min to an initial dark respiration period and then photosynthetic rate was determined for 15–30 min in the growth irradiance, $840 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the high irradiance treatment and at $40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the low irradiance treatment.

2.8. Statistics

Mean values and standard deviation were calculated from at least eight replicates per treatment for PAM measurements and from at least three replicates for photosynthetic determination in independent samples for each treatment. Pigments were extracted in triplicate samples. Statistical significances of means were tested with a model 1 one-way ANOVA followed by a multirange test by Fisher's protected least significance difference (LSD) [44].

3. Results

The specific absorption coefficient in the 400–700 nm interval is higher in the sun type than that in the shade type *Porphyra* (Fig. 1). This is due to the about 3.5 times higher chlorophyll concentration and higher package effect in the shade type than in the sun type (Table 1). The PE concentration in the shade type was about 2.5 times higher than that in the sun type, whereas PC was 8 times higher (Table 1). Consequently the PE/PC ratio was about three times higher in the sun type than in the shade type.

The fluorescence excitation spectrum (+DCMU) was also different in shade compared to sun type algae (Fig. 1). In the shade type the contribution of the fluorescence emission at 730 nm after excitation at 565 nm (absorbed by PE) was similar to that due to PC (650 nm), however, in the sun type the contribution of PC was much lower than that of PE. On the other hand, the contribution of excited chlorophyll *a* (680 nm) was higher in the sun type than in the shade type algae.

The difference spectra (absorbance minus fluorescence excitation spectra) was higher in the sun type than in the shade type algae (Fig. 2) indicating that a great part of the energy absorbed is not used in photosynthesis but dissipated. The difference between shade and sun type algae were higher in algae submitted to high irradiance compared to those incubated under low irradiance (Fig. 2).

Optimal quantum yield before exposure was higher in the shade type than in the sun type algae (Fig. 3). Under low irradiance exposure of shade type algae, F_v/F_m significantly decreased over time. However, in the sun type no significant variations were observed (Fig. 3a). Under high irradiance exposure, F_v/F_m decreased in both sun and shade type algae (Fig. 3b).

The decrease of F_v/F_m was significantly higher ($P < 0.01$) under high than under low irradiance (Fig. 4). In the

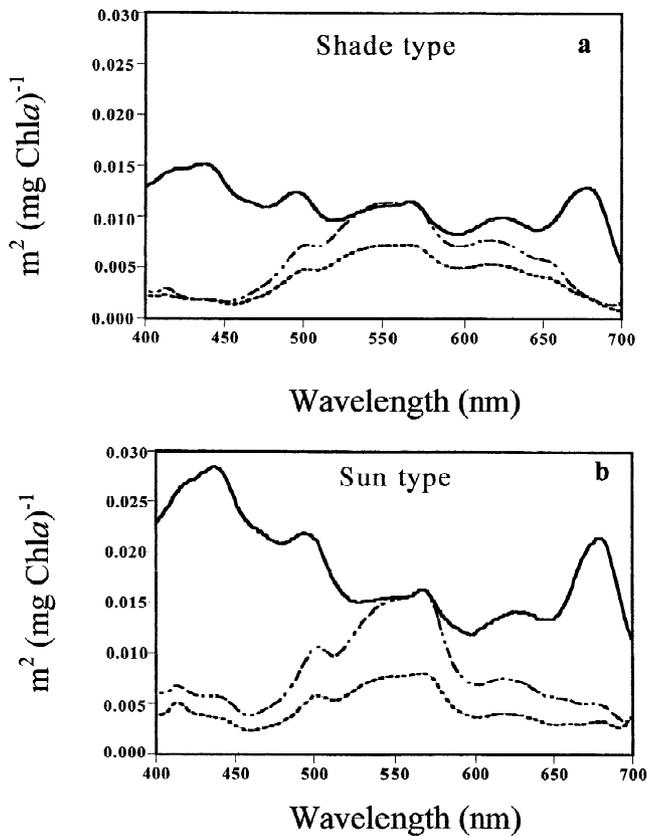


Fig. 1. Chlorophyll specific absorption spectra (thick line), fluorescence excitation spectra (emission at 730 nm) (pointed line) and fluorescence excitation spectra with the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (final concentration 30 μ M) in shade type (*Porphyra* sp.) and sun type algae (*Porphyra purpurea*) harvested in mid-August 1999 on the rocky shores just in front of the Trondheim Biological station in Trondheimsfjord, Norway (63°N). The data are expressed as $m^2 (mg Chl a)^{-1}$.

shade type algae after 48 h exposure, the inhibition was about 60% under low irradiance exposure and 97% under high irradiance exposure (Fig. 4a). In the sun type, after 48 h exposure, the decrease was only 6% in low irradiance and about 80% in high irradiance treatment (Fig. 4a). The kinetics of photoinhibition were different under high irradiance compared to low irradiance. The decrease of F_v/F_m was very drastic in the first 24 h exposure reaching a steady state after 48 h exposure. However under low irradiance the slope of F_v/F_m versus time was higher between 24 and 48 h than that in the first 24 h (Fig. 4a).

After 48 h exposure the algae were transferred to very low irradiance (20 μ mol photons $m^{-2} s^{-1}$) in order to analyse the extent of recovery of photosynthesis, estimated as the recovery of F_v/F_m values to those determined before exposure. Thus, sun type algae fully recovered after both low irradiance and high irradiance exposure. Under low irradiance F_v/F_m was even higher than determined before exposure. Under high irradiance the recovery was about 100% (Fig. 4b). In shade algae no full recovery was produced, in low irradiance treatment the recovery was

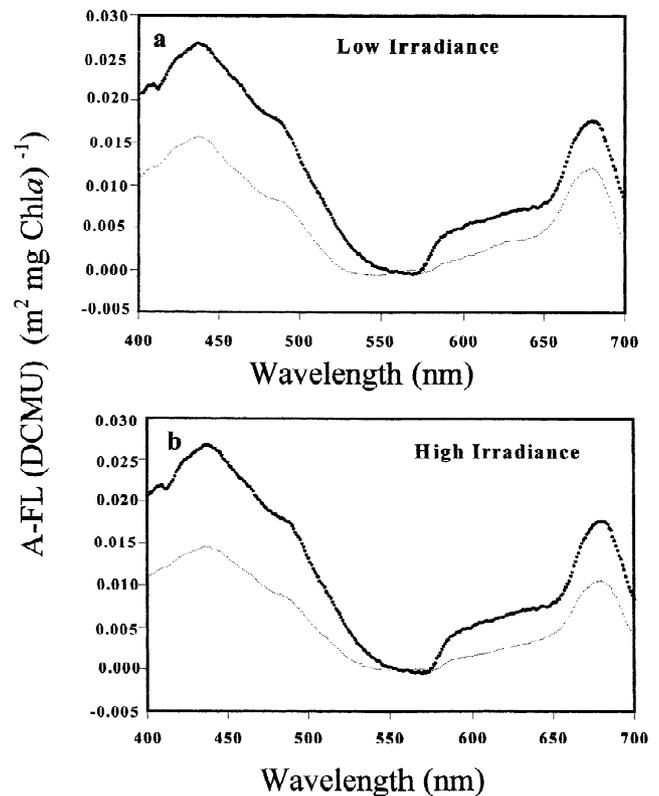


Fig. 2. Difference spectra between chlorophyll specific absorption and fluorescence excitation spectra (emission at 730 nm) with the addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (final concentration 30 μ M) in shade type (*Porphyra* sp.) (thin line) and sun type algae (*Porphyra purpurea*) (thick line) after 48 exposure to white light at (a) low irradiance (40 μ mol photons $m^{-2} s^{-1}$) and (b) high irradiance (840 μ mol photons $m^{-2} s^{-1}$).

about 75% meanwhile in the high irradiance treatment no recovery was observed and the algae was drastically damaged by the radiation (Fig. 4b).

Photosynthetic rate expressed as oxygen evolution, before exposure, was significantly higher in the shade than in the sun type algae (Fig. 5). Photosynthetic rate significantly decreased in both types of algae through the exposure time. No significant differences in photosynthetic rate after low irradiance exposure in either type of algae were observed (Fig. 5a). However, after 48 h exposure to high irradiance the decrease of photosynthetic rate was significantly ($P < 0.01$) higher in the shade than that in the sun type algae (Fig. 4b). The decrease of oxygen evolution was significantly ($P < 0.01$) higher under high irradiance exposure than that under low irradiance (Fig. 5). The decrease of photosynthetic rate after 48 h exposure was linearly and positively correlated ($r^2 = 0.95$) to the percentage decrease of F_v/F_m after 48 h exposure (data not shown).

In the sun type, high irradiance exposure did not alter the Chl *a* concentration, however, exposure to lower irradiances (40 μ mol photons $m^{-2} s^{-1}$) for 48 h increased significantly ($P < 0.01$) the chlorophyll concentration

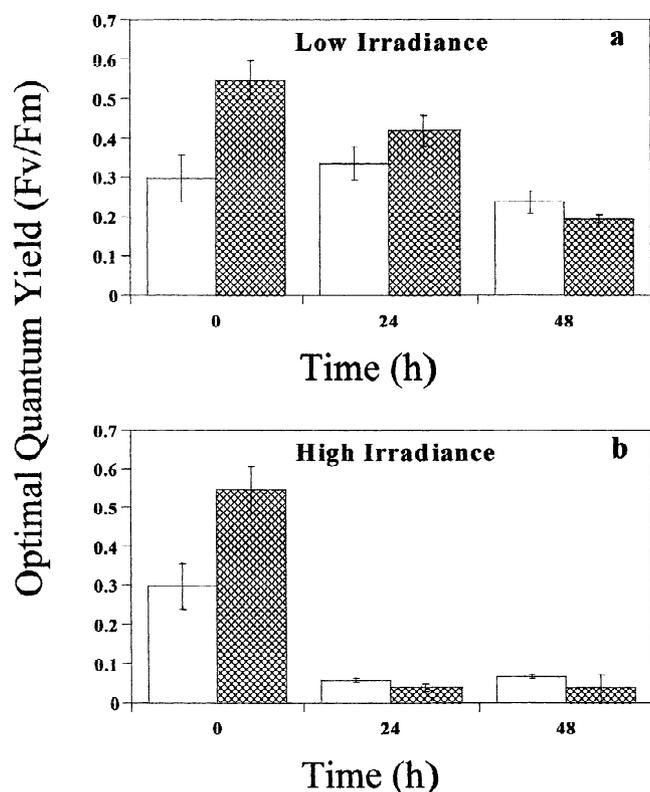


Fig. 3. Optimal quantum yield (F_v/F_m) in shade type (*Porphyra* sp.) (black histograms) and sun type algae (*Porphyra purpurea*) (open histograms) harvested in mid August 1999 on the rocky shores just in front of the Trondheim Biological station in Trondheimsfjord and F_v/F_m after 24 and 48 h exposure to white light (halide lamps) at (a) low irradiance ($40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and (b) high irradiance ($840 \mu\text{mol photons m}^{-2} \text{s}^{-1}$).

(Table 1). Both PE and PC concentrations slightly decreased after exposure both to high and low irradiances. However, in the shade type after 48 h exposure Chl a, PE and PC concentrations decreased significantly (Tukey's test, $P < 0.01$) after both high and low irradiances (Table 1).

The content of MAAs was significantly higher ($P < 0.05$) in the sun type than that in the shade type *Porphyra* (Table 2). *Porphyra*-334 is the main MAAs in this species followed by shinorine. The concentration of porphyra-334 was about 10 times higher than that of shinorine in the sun type and about 17 times higher in the shade type. In the sun type, porphyra-334 increased significantly ($P < 0.05$) but shinorine did not significantly change after both high and low irradiance exposure. Thus the total content of MAAs significantly ($P < 0.05$) increased in the sun type after 48 h exposure to both high and low irradiances (Table 2). The increase was significantly higher ($P < 0.05$) after high than after low irradiance exposure (Table 2). On the other hand, in the shade type porphyra-334 concentration significantly decreased ($P < 0.05$) after both high

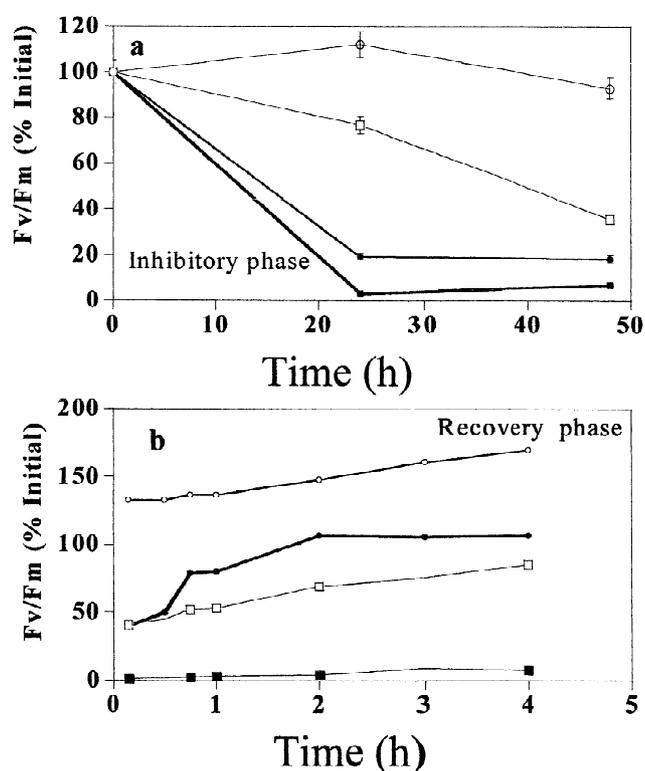


Fig. 4. (a) Percentage of F_v/F_m respect to the initial values after 24 and 48 h exposure at high irradiance ($840 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (close symbols) and low irradiance ($40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (open symbols) of shade type (*Porphyra* sp.) (squares) and sun type algae (*Porphyra purpurea*) (circles). (b) Percentage of F_v/F_m with respect to the initial values through the time exposure to low irradiance ($20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (recovery phase) after 48 h exposure to low and high irradiance of shade and sun type symbols (symbols as explained above).

and low irradiance exposure. The decrease was significantly higher under low than under high irradiance exposure. Shinorine did not vary significantly with the exposure to both high and low irradiances (Table 2). Thus, the content of total MAAs significantly ($P < 0.05$) decreased after 48 h exposure to high and low irradiances.

The percentage of decrease of F_v/F_m in terms of integrated irradiance (dose) after 48 h exposure decreased exponentially as function of the increment of MAAs after 48 h exposure (Fig. 6). Thus, in the case of the highest decrease in MAAs (shade type algae under high irradiance exposure), the highest photoinhibition was observed, whereas in the case of the highest accumulation of MAAs (sun type algae under high irradiance), the lowest photoinhibition was observed (Fig. 6a). This exponential function can be also shown with the nonphotochemical quenching per dose after 48 h exposure. The highest nonphotochemical quenching per dose is related to the highest decrease of MAAs (Fig. 6b) and the highest photoinhibition.

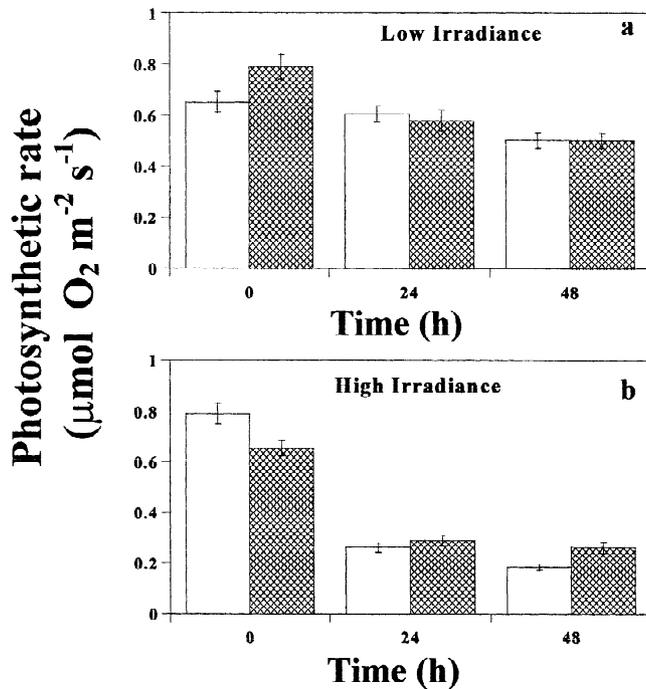


Fig. 5. Photosynthetic rate, expressed as $\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$, in shade type (*Porphyra* sp.) (black histograms) and sun type algae (*Porphyra purpurea*) (open histograms) harvested in mid-August 1999 on the rocky shores just in front of the Trondheim Biological station in Trondheimsfjord and photosynthetic rate after 24 and 48 h exposure to white light (halide lamps) at (a) low irradiance (40 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and (b) high irradiance (840 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$).

4. Discussion

The sun type *Porphyra purpurea* was more resistant to photoinhibition than the shade type *Porphyra* sp. after short-term exposure to high irradiance (48 h). The acclimation in the sun type to high irradiance is evidenced because of the lower decrease in both optimal quantum yield and oxygen evolution as well as the higher recovery than that observed in the shade type. Since photoinhibition rates have been shown to represent a combination of rates of

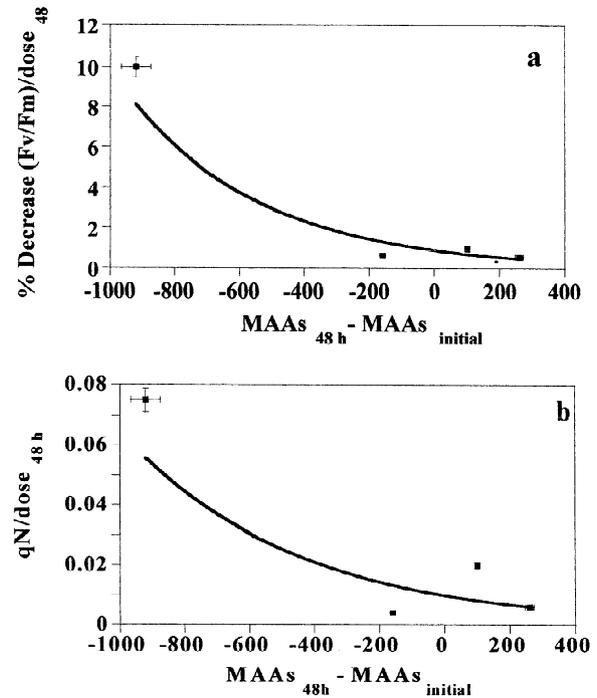


Fig. 6. (a) Percentage of the decrease of F_v/F_m per integrated irradiance after 48 h (dose) versus accumulation of mycosporine-like amino acids in the same time period of *Porphyra* species submitted to 48 h exposure under low irradiance (40 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and high irradiance (840 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). (b) Nonphotochemical quenching per dose (48 h) as function of the accumulation of mycosporine-like amino acids.

damage and simultaneous repair [45], this greater capacity could represent a mechanism by which the sun type *P. purpurea* is more resistant to photoinhibition than the shade type *Porphyra* sp. Macroalgae as well as higher plants possess different strategies for protection of the photosynthetic apparatus against excess light [12]. Dynamic photoinhibition acts as a photoprotective mechanism [1], which decreases the quantum yield of PSII, so that excess absorbed energy is rendered harmless by thermal dissipation. At subinhibitory fluence rates thermal energy

Table 2

Concentration of the mycosporine-like amino acids porphyra-334 and shinorine expressed in mg g^{-1} algal dry weight (DW) before and after exposure to artificial lamps under high irradiance (HI, 840 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and low irradiance (LI, 40 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) of two *Porphyra* species, a sun type (*Porphyra purpurea*) and a shade type (*Porphyra* sp.)

	Sun type <i>Porphyra</i> (gDW)			Shade type <i>Porphyra</i> (gDW)		
	Porphyra-334	Shinorine	Total MAAs	Porphyra-334	Shinorine	Total MAAs
0 h	1.83±0.11 ^a	0.17±0.01 ^a	2.00±0.12 ^a	1.75±0.12 ^a	0.10±0.007 ^a	1.85±0.12 ^a
HI-24 h	1.60±0.12 ^{a,b}	0.12±0.009 ^b	1.72±0.13 ^b	1.68±0.10 ^a	0.17±0.010 ^b	1.85±0.11 ^a
HI-48 h	2.10±0.10 ^c	0.16±0.008 ^a	2.26±0.11 ^c	156±0.11 ^a	0.13±0.009 ^c	1.69±0.10 ^a
LI-24 h	1.64±0.09 ^{a,b}	0.14±0.007 ^c	1.78±0.09 ^b	1.01±0.09 ^b	0.12±0.011 ^c	1.13±0.10 ^c
LI-48 h	1.96±0.12	0.14±0.008 ^c	2.10±0.13 ^{a,c}	0.85±0.06 ^b	0.08±0.005 ^d	0.93±0.06 ^d

Different letters indicate significant differences ($P < 0.05$) for each variable (comparing initial with high and low irradiance treatments).

dissipation is mainly controlled by the light-induced formation of a thylakoid pH gradient (energy-dependent quenching, q_E), and it accelerates the rate of energy supplied by photosynthesis to the Calvin cycle [46]. At higher photoinhibitory fluence rates, photoinactivation due to photoinhibition opens an additional path of thermal energy dissipation, thus optimising the rate of photochemistry and diminishing the rate of photodamage. Photoinhibition has been proposed as a strategy of photoprotection against high irradiance [1,10]. The susceptibility to photoinhibition has consequences for the ecology of macroalgae and depends on the irradiance dose, season, water transparency and the presence of algal canopies. Under exposures to unfiltered direct solar radiation at the surface almost all macroalgae show a pronounced photoinhibition after a certain time of exposure, at least at high zenith angles [47,48]. Even algae harvested from rock pools, normally exposed to extreme solar irradiances, show signs of photoinhibition after prolonged periods [4,49]. Deep-water algae and those adapted to shaded environments are inhibited even faster when exposed to direct solar radiation [4].

Although the red macroalgae show a greater diversity of responses to high irradiances, the most severe photoinhibition in intertidal red algae occurs when low tide coincides with the intense solar radiation at noon [50]. This is especially stressful in species from Southern Spain, where irradiances higher than $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ have been measured. *Porphyra leucosticta* showed a decrease of 30% in the effective quantum yield at noon due to UVR [49]. In this paper the different acclimation to high irradiance was also related to the growth light environments, the sun type *P. purpurea* acclimated to high irradiance exposure better than the shade type grown *Porphyra* sp. Recovery of photosynthesis was also higher in the sun type grown *P. purpurea* than that of shade type *Porphyra* sp. Jiménez et al. [51] showed that the degree of photoinhibition and recovery in different red macroalgae from Southern Spain was also related to the light exposure history. The algae subjected to very high irradiances had higher photoinhibition and recovery than that of algae inhabiting shaded sites. As mentioned above, the recovery capacity, measured as increase in the quantum yield of fluorescence, is species-specific and is more rapid in sun-adapted algae than in algae growing at deep or shaded locations and then transferred to the surface. In the eulittoral red algae *Porphyra leucosticta* [49], *Asparagopsis armata* and *Feldmannophycus rayssiae* [51] from Southern Spain, the recovery of photosynthesis occurs immediately after a decrease of only 10–20% of solar radiation in the afternoon.

In addition to photoinhibition, several other mechanisms have been proposed in macroalgae related to the accumulation of carotenoids. The photoprotective role of the xanthophyll cycle has been investigated mostly in microalgae [52,53] and to a lesser extent in macroalgae, e.g. the green

algae *Ulva rotundata* [8,14,15] and *Ulva lactuca* [16] and the brown algae *Dictyota dichotoma* [17] and *Lobophora variegata* [18]. The red algae do not show the xanthophyll cycle.

Other physiological mechanisms which reduce the absorption cross section and are stimulated by excess light are chloroplast movements [54]. At high irradiances the phaeoplasts in the brown alga *Dictyota dichotoma* move to a parallel position with respect to the light beam avoiding photodamage of the photosynthetic apparatus.

Finally, another mechanism of protection against UVR or excess PAR is the production of screening pigments such as UV-absorbing MAAs [22,23]. In this study, the accumulation of MAAs after 48 h exposure was observed only in the sun type algae. In any case, the initial MAA content in algae collected from the fiord is very high. In *Porphyra umbilicalis*, no induction of the accumulation of MAAs by UVR was observed, this result was justified due to the high initial MAA content in this species [55].

A negative exponential relationship was observed between decreased F_v/F_m per dose and the accumulation of MAAs. Thus, it can be suggested that, in addition to the photoinhibition, the accumulation of MAAs can have a photoprotective role in the sun type *P. purpurea*. Although most of these compounds absorb mainly within the UV-A region (310–360 nm) and hence, they would protect more efficiently against UV-A radiation, a stimulation in the concentration of these UV-compounds by increasing PAR has been reported for the dinoflagellate *Alexandrium excavatum* [56] and in the red alga *Chondrus crispus* [26].

MAAs have been reported in green, red and brown algae from tropical, temperate and polar regions [19–21]. Wood [24] reported that apical pieces of *Eucheuma striatum* produced high concentrations of UV absorbing pigments under UV exposure, but growth was not depressed in the presence of UV [25]. The absorption maxima of MAA occur in both the UV-A and UV-B regions [23,21]. Macroalgae often contain several MAAs with different absorbance maxima [21]. The concentration of MAAs has been correlated with depth zonation and UV exposure [19].

The main role suggested for MAAs is the photoprotection against UV radiation. However, there is little direct evidence that they act only as UV blockers and do not have other biological functions [23]. In tropical algae, enhanced levels of carotenoids and UV-absorbing compounds were found in tissues from the canopy compared to tissues from understory sites indicating a pattern of remarkably sensitive photoacclimation over distances of <10 cm in turf-forming rhodophytes such as *Ahnfeltiopsis concinna* and *Laurencia modernidiae* [31]. Microscale variations in the in vivo absorbance in the turf algae was directly related to the amount of extractable UV absorbing compounds. The in vivo absorbance signature at 345 nm appears to provide a method to quickly and accurately gauge the potential UV-shielding capacity of primary producers [31].

The accumulation of MAAs is a dose dependent process evidenced because they present a higher accumulation under high than under low daily irradiances i.e. different latitudes and the accumulation is greater in intertidal than subtidal algae [20,57,58]. The accumulation of MAAs is a wavelength-dependent process [26,57,59]. A UV-B-mediated increment has been shown in a variety of algae [25,60,61]. Thus, for example in *Chondrus crispus*, both UV-A and UV-B stimulated a strong accumulation of shinorine whereas the content of palythol and palythine was mainly stimulated by PAR dose, indicating an MAA-specific induction triggered by UVR or PAR [26]. Thus, although MAAs are mainly UV-A absorbing compounds, they can also be stimulated by high irradiance of PAR.

The shade type *Porphyra* however did show a drastic reduction of MAAs and it suffered chronic photoinhibition. Photodamage occurs when the rate of damage of the reaction centre protein D1 exceeds the rate of repair processes [3]. Recently, the term ‘chronic photoinhibition’ has been used for this phenomenon, which also results in a waste of excess photons as heat and hence, may be a type of photosynthetic protection mechanism in shade-grown plants [1].

In summary, the higher resistance to high irradiance of sun type algae could be due to a high thermal dissipation i.e. high q_N :dose ratio and as well as the accumulation of photoprotective substances i.e. porphyra-334. Bose et al. [62] found that the recovery of the sun type *Porphyra perforata* was more complete than that of subtidal *Porphyra nereocystis* after very short exposure (1 h) to high irradiance (2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). This greater capacity for recovery was suggested as a mechanism by which *P. perforata* is more resistant to photoinhibition than *P. nereocystis*. The resistance to photoinhibition of sun type *Porphyra purpurea* compared to that of the shade type *Porphyra* sp. can be explained by the distribution of both species in the intertidal zone.

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