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Effects of solar UV radiation on photosynthesis and enzyme activities (carbonic anhydrase and nitrate reductase) in marine macroalgae from southern Spain

Efectos de la radiación solar UV sobre la fotosíntesis y actividades enzimáticas (anhidrasa carbónica y nitrato reductasa) en macralgas marinas del sur de España

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ABSTRACT

The effects of solar ultraviolet (UV) radiation during daily cycles on photosynthesis and two key enzymes involved in carbon incorporation, the carbonic anhydrase, and in inorganic nitrogen reduction, the nitrate reductase, of macroalgae from southern Spain are presented. During daily cycles, photoinhibition in several intertidal macroalgae, expressed as decrease in the effective quantum yield from the morning to noon time, was linearly dependent on the daily integrated irradiance. However, recovery, expressed as the increase in the effective quantum yield from noon to the afternoon, presented a different pattern; full recovery was found below daily integrated irradiance of 1.0×10^4 kJ m^{-2}. However, recovery reached only 50 % at higher irradiances. The existence of daily photoinhibition and full recovery in intertidal algae suggests that photoinhibition is a photoprotective mechanism against high solar radiation as in higher plants, and that patterns of photoinhibition and recovery are affected by accumulative doses. Activities of carbonic anhidrase and nitrate reductase were determined in three marine macroalgae (*Plocamium cartilagineum*, *Ulva rigida* and *Fucus spiralis*) under full (PAR + UV-A + UV-B) and excluded UV solar radiation (PAR). Under PAR + UV-A + UV-B, peaks of enzyme activity were found in *P. cartilagineum* during the evening, and accordingly to data previously published for other red macroalgae. This situation was modified by the absence of UV radiation since the increase in the activities was delayed several hours. In the three macroalgae and under full solar radiation, a significant and negative correlation was found only when data from nitrate reductase activity was shifted in time during at least four hours. This correlation is lost in *Ulva rigida* when UV radiation is excluded. The existence of these daily variations with a negative correlation of both enzyme activities could reflect a complex regulatory link between carbon and nitrogen metabolism under solar radiation. Considering the absence of a significant correlation in *U. rigida* and the delay observed in maximal activities of *P. cartilagineum* in the absence of UV, it is suggested that UV radiation acts as an environmental signal involved in the control of cycles. The presence of feedback processes that control nitrogen assimilation as a function of carbon content is proposed.

Key words: carbonic anhydrase, chlorophyll fluorescence, effective quantum yield, nitrate reductase, ultraviolet radiation.

RESUMEN

En este trabajo se presenta el efecto de la radiación solar ultravioleta (UV) durante el ciclo diario de luz sobre la actividad fotosintética y la de dos enzimas claves en la incorporación de carbono, la anhidrasa carbónica, y en la reducción de nitrógeno inorgánico, la nitrato reductasa, en macroalgas del sur de España. Durante el ciclo diario de luz, la fotoinhibición, expresada como porcentaje de disminución del rendimiento cuántico efectivo desde la mañana al mediodía, estuvo linear y negativamente correlacionada con la irradiancia integrada diaria. Sin embargo, la recuperación, expresada como porcentaje de incremento del rendimiento cuántico efectivo desde el mediodía hasta el atardecer siguió un patrón diferente. Se observó recuperación completa a irradiancias integradas diarias menores a 1.0×10^4 kJ m^{-2}. Sin embargo, a irradiancias diarias mayores, la recuperación fue sólo del 50 %. La existencia de fotoinhibición y de una recuperación diaria completa en macroalgas hace pensar que la fotoinhibición está funcionado como un mecanismo de fotoprotección contra radiaciones solares altas como ocurre en plantas terrestres. Además, los patrones de fotoinhición y recuperación tienen relación con la dosis acumulada diaria. Las actividades enzimáticas (anhidrasa carbónica y nitrato reductasa) fueron determinadas en tres macroalgas marinas (*Plocamium cartilagineum*, *Ulva rigida* y *Fucus spiralis*) bajo radiación solar completa (PAR + UV-A + UV-B) y excluyendo la radiación UV (PAR). Bajo PAR + UV-A + UV-B se observó un máximo en la actividad de ambas enzimas en *P. cartilagineum* por la tarde, lo que concuerda con datos publicados en otras algas rojas. La situación fue modificada en ausencia de UV ya que el incremento de ambas actividades fue retrasado en el tiempo (varias horas). En las tres macroalgas bajo radiación solar completa se encontró

una correlación significativa y negativa sólo cuando los datos de la actividad nitrato reductasa fueron desplazados al menos 4 horas respecto a los de la anhidrasa carbónica. Esta correlación se pierde en *Ulva rigida* cunado se excluye la radiación UV. La existencia de variaciones diarias y la correlación negativa entre la actividad de ambas enzimas podría reflejar una compleja regulación conjunta entre el metabolismo del carbono y del nitrógeno bajo radiación solar. Debido a que esta correlación se pierde en ausencia de UV en *Ulva* o los máximos se retrasan en *Plocamium*, se sugiere que la radiación UV podría ser una señal medioambiental implicada en el control de los ciclos enzimáticos. Se sugiere la existencia de procesos de retroalimentación que controlan el metabolismo del nitrógeno en función del contenido de carbono.

Palabras clave: anhidrasa carbónica, fluorescencia de la clorofila, nitrato reductasa, radiación ultravioleta, rendimiento cuántico efectivo.

INTRODUCTION

Stratospheric ozone depletion and the consequent increase in the penetration of ultraviolet-B radiation (reviewed in Madronich et al. 1995) has prompted considerable interest in evaluating the effects of UV-B on different aspects of plant biology. In aquatic systems, impairments of growth and primary productivity of different phytoplankton assemblages by short wavelengths (280-315 nm), especially in southern oceans, have broadly been documented, since carbon fixation in oceanic waters is crucial in the regulation of global climate change (Helbling et al. 1992, Neale et al. 1994, Holm-Hansen et al. 1994). However, the contribution of coastal marine macrophytes to the global carbon fixation and the effects of UV radiation on primary production of these macroalgae have not been comprehensively determined (Larkum & Wood 1993, Dring et al. 1996, Franklin & Forster 1997). Recent studies reveal that macroalgae may suffer DNA damage and various alterations in the photosynthetic apparatus (reviewed in Franklin & Forster 1997). Such detrimental effects are species-specific and related to a series of morphological characteristics as well as to patterns of vertical distribution (Dring et al. 1996, Franklin et al. 1996, Hanelt et al. 1997, Bischof et al. 1998, Hanelt 1998).

In temperate regions, characterised by high solar radiation (e.g., the southern Mediterranean coast of Spain), macroalgae are exposed to high doses of UV radiation (Häder & Figueroa 1997). The high irradiance and the transparency of the shallow waters in this region suggest that macroalgae have developed more efficient photoprotective mechanisms to tolerate light stress than species from other biogeographical regions. In the last years a number of investigations have focused on changes in photosynthesis related to natural UV radiation of selected species in the coast of southern Spain (Häder et al. 1996, 1997, Figueroa et al. 1997, Flores-Moya et al. 1998, Gómez et al. 1998, Pérez-Rodríguez et al.1998). These studies revealed the occurrence of photoinhibition of photosynthesis under high solar radiation, which depends on daily changes in irradiance, vertical light attenuation, or on a combination of both factors. However, data on comparative photosynthesis of macroalgae under different scenarios of UV climate are scarce and there is only a diffuse picture of their photoadaptive strategies. Taking into account the distinct origin and the morpho-functional diversity of these species, a common adaptive strategy is unlikely.

Intertidal algae under moderate desiccation present high photosynthetic rates (Mercado et al. 1998), but, on the other hand, they are the first candidates to suffer the effects of excess UV solar radiation and increased CO_2 levels. The uptake and accumulation of inorganic carbon in algae are energy dependent processes that are affected by both exposure to the air and by excess solar radiation (PAR and UV, Figueroa 1998).

Although growth rate is dependent not only on carbon assimilation, but also on the incorporation and assimilation of nitrogen, sulfur or micronutrients, only a few studies on the effect of UV radiation on nutrient incorporation have been conducted. Nitrogen is needed for the synthesis of nucleic acids, amino acids, proteins, and pigments (Wheeler 1983). It has been reported that UV radiation affects nutrient uptake and metabolism of nitrogen in different species of microalgae (Döhler 1984, Behrenfeld 1995, Döhler 1997) and macroalgae (Döhler at al. 1995). Recently, Fauchot et al. (2000) showed that NO_3^-, NH_4^+, and urea uptakes increase in natural assemblages of estuarine phytoplankton (just below the water surface) when a short-time exposure (4 h) under UV-B radiation exclusion is applied. However, and under longer exposure, the phytoplankton community (dominated by diatoms and experiencing vertical mixing in a mesocosm) is able to withstand UV radiation enhancements without any perceptible effect on nitrogen uptake (Fauchot et al. 2000). In addition, treatments of varying UV-B radiation do not affect the internal organic nitrogen composition as internal urea, free amino acids, and proteins (Fauchot et al. 2000).The discrepancy between short-time uptake measure-

ments at the surface and long-term effects in mesoscosms emphasises the importance of vertical mixing of UV-B radiation on nitrogen uptake (Fauchot et al. 2000), as it occurs during carbon fixation (Helbling et al. 1994, Neale et al. 1998). The effect of vertical mixing on carbon and nitrogen metabolism in macroalgae has been studied as related to nutrient availability (Wheeler 1988), but not in relation to the penetration of UV radiation in the macroalgal growth site.

On the other hand, the influence of UV on enzymes involved in carbon metabolism, such as the carbonic anhydrase, or those involved in nitrogen metabolism, such as the nitrate reductase, have been analysed only in a few number of algae (Flores-Moya et al. 1998, Gómez et al. 1998). In this study we report the effects of solar UV radiation during daily cycles on photosynthesis, and on two key enzymes involved in carbon uptake (carbonic anhydrase) and in inorganic nitrogen reduction (nitrate reductase) of macroalgae from southern Spain.

MATERIAL AND METHODS

Algal material and site of collection

The effective quantum yield, conducted during daily cycles and under solar radiation, was conducted in the followed species:

Red algae: *Porphyra leucosticta* Thuret in le Jolis collected in Lagos (Málaga; Southern Spain), *Rissoella verruculosa* (Bertholoni) J. Agardh collected in Marina del Este (Granada, southern Spain), *Corallina elongata* Ellis et Solander, *Asparagopsis armata* Harvey, *Gelidium sesquipedale* Clemente Bornet et Thuret, *Felmanophycus rayssae*, *Laurencia pinnatifida* (Hudson) Stackhouse, and *Falkenbergia* (Tetrasporophytic phase of *Asparagopsis armata*) collected in Punta Carnero (Cádiz, southern Spain).

Green algae: *Enteromorpha muscoides* (Clemente) Cremades, *Ulva rigida* C. Agardh, *Ulva rotundata* Bliding, and *Codium adhaerens* C. Agardh collected in Punta Carnero (Cádiz, southern Spain) and *Dasycladus vermicularis* (Scopoli) Krasser collected in El Playazo de Rodalquilar (Almería, southern Spain).

Brown algae: *Cystoseira usneoides* (L.) M. Roberts, *Cystoseira tamariscifolia* (Hudson) Papenfuss, *Cladostephus spongiosus* (Hudson) C. Agardh, *Padina pavonica* (L.) Thivy, and *Dictyota dichotoma* (Hudson) J. V. Lamouroux collected in El Playazo de Rodalquilar (Almería, southern Spain).

The effects of UV radiation on the activity of enzymes was analysed on thalli of the green alga *Ulva rigida*, the brown alga *Fucus spiralis*, and the red alga *Plocamium cartilagineum* collected in July 1997 in Punta Carnero (Cádiz, southern Spain). This area corresponds to the northern margin of the Alboran sea, which extents from Gibraltar to the Cabo de Gata in Almeria. Hydrographically, the upper water mass (up to 150 m depth) constitutes the Atlantic component characterized by temperatures higher than 20 °C in summer and salinities lower than 36.5 ‰. Down to an interface of 100-150 m it is possible to distinguish the deepest Mediterranean water layer (12.9 °C and 38.4 ‰), which is denser than the upper ones. Therefore, the photic zone and especially the littoral assemblages in the Alboran sea have basically an Atlantic influence. Although this region is considered an oligotrophic system, upwelling events caused by remobilization of deeper water masses may occur in some zones such as the east side of Gibraltar and off coast of Málaga.

After sampling, algae were transported to the laboratory in an ice-chest, cleaned out from epibiota and acclimated for several days in 5 l plexi-glass beakers containing filtered seawater at 16 °C. Illumination (80 µmol photon m^{-2} s^{-1}) was provided by daylight lamps (Osram L20W/10S) in a 12:12 h light:dark regime. The algae were maintained in these conditions for three days before subjected to experimental conditions.

Experimental design

Daily-cycle experiments were carried out using a tank-like system placed on a roof at the campus of Málaga University (36° 47' N, 0.4° 19' W), which was exposed to full solar radiation (PAR + UV-A + UV-B), or deprived of UV radiation (PAR) during a whole day. After three days under laboratory conditions, samples were submerged into 0.15 m high white PVC tanks, which were placed in an illuminated place inside the tank-like system previously reported (Gómez et al. 1998). In the PAR treatment, the tank was covered with an Ultraphan filter (Digefra, GmbH, Munich, Germany) with a transmittance of $\lambda > 395$ nm. Because the filter reduced incident irradiance by about 10 % in the PAR waveband, the irradiance of controls (PAR + UV-A + UV-B) was reduced in 10% with an Ultraphan filter with transmittance of $\lambda > 295$ nm. To prevent the formation of water droplets on the lens, filters were positioned under water...

The spectral transmittances of Ultraphan-395 and Ultraphan-295 filters have been previously described (Figueroa et al. 1997). The irradiance and the doses received in the different spectral during the exposure period are shown in Table 1.

Photoinhibition as determined by in vivo Chlorophyll-a fluorescence

Chlorophyll fluorescence was measured using a portable pulse amplitude modulated PAM-2000 fluorometer (Walz, Effeltrich, Germany) according to Schreiber et al. (1986, 1995).

Photoinhibition of photosynthesis was expressed as the percentage of decay of effective quantum yield from the morning to noon values. The effective quantum yield was calculated as:

$$(F_m' - F_t)/F_m' = \Delta F/F_m',$$

where F_m' is the maximal steady-state fluorescence, and F_t the measured fluorescence yield at any given irradiance. This expression was developed by Genty et al. (1989) to assess the overall quantum yield of photochemical energy conversion, and it can be correlated with the quantum yield of carbon assimilation.

The experimental procedure to determine the effective quantum yield has previously been reported (Figueroa et al. 1997, Jiménez et al. 1998). Because the onset of maximal fluorescence after a saturating light pulse in red algae occurs very quickly (associated with excitation of PSI and delay of the F_m decline), a modified PAM protocol described by Hanelt et al. (1997) was used in the case of red macroalgae. Firstly, a 5 s low irradiance (30 μmol m^{-2} s^{-1}) far-red pulse was applied, and followed by a 5 min dark period. To ensure the stabilisation of the fluorescence signal, a short red actinic pulse (5 s, 8 μmol m^{-2} s^{-1} at 655 nm) was given. An additional 5 s far-red pulse was given to re-oxidise the electron transport chain. Finally, F_o was recorded after a pulsed, red measuring light (0.3 μmol m^{-2} s^{-1} at 650 nm), while F_m was induced with a saturating white light pulse (0.4 ms, approximately 9,000 μmol m^{-2} s^{-1}). This procedure was repeated three times for each sample at intervals of 5 min darkness to avoid incomplete recovery of fluorescence quenching parameters such as energy quenching or those derived from state transitions (Hanelt et al. 1997).

Photoinhibition was expressed as the percentage of decrease in $\Delta F/F_m'$ from the morning to noon in each macroalgae, whereas recovery was expressed as the percentage of increase in $\Delta F/F_m'$, from noon to the afternoon during a daily cycle.

TABLE 1

Daily integrated irradiance at three spectral bands to which the different algae were exposed during experiments conducted during June 1997: photosynthetic active radiation (PAR, λ = 400-700 nm), ultraviolet A (UV-A, λ = 315-400 nm), and ultraviolet B (UV-B, λ = 280-315)

Irradiancia diaria integrada (kJ m^{-2}) en tres bandas espectrales a las cuales se expuso a las algas durante junio de 1997: radiación fotosintéticamente activa (PAR, λ = 400-700 nm), ultravioleta A (UV-A, λ = 315-400 nm) y ultravioleta B (UV-B, λ = 280-315)

Species	PAR	UV-A	UV-B
Plocamium cartilagineum	9,228	1,110	13.0
Ulva rigida	7,508	921	10.5
Fucus spiralis	7,508	921	10.5

Determination of nitrate reductase activity

Samples for nitrate reductase assays (E. C. 1.6.6.1.) were placed in liquid nitrogen during the field work. Nitrate reductase activity was determined as described by Corzo & Niell (1991). Samples of approximately 0.15 g fresh weight were incubated for 30 min at 30 °C in a solution containing 0.3 M KNO_3 and 0.5 mM glucose. The alga was removed from the assay medium, and then frozen in liquid nitrogen. After thawing at room temperature, the concentration of NO_2^- was determined calorimetrically by adding 1 ml of extract to 1 ml of N-N'-1, naphthylethylenediamine and 1 ml of sulphanilamide (Snell & Snell 1949).

Determination of carbonic anhydrase activity

Total carbonic anhydrase (E. C. 4.2.1.1.) activity was measured in the laboratory using algal material previously stored in liquid nitrogen in the field. The determination followed the potentiometric method described by Haglund et al. (1992), which consists of measuring the time taken for a linear drop of pH from 8.5 to 7.5 at 0-2 °C. From the stored algal stock, samples of 10-20 mg fresh weight were thawed at room temperature, ground in a mortar, and then transferred to a cuvette containing 3 ml of buffer (50 mM Tris, 25 mM isoascorbic acid, 5 mM EDTA, pH 9.0). The reaction was started by

introducing 1 ml of ice-cold CO_2-saturated distilled water, obtained previously by bubbling water with pure CO_2. Relative enzyme activity (REA) was expressed as $(t_o/t_c) - 1$, where t_o and t_c were the time for pH change of the non-catalysed (buffer only) and the catalysed (in the presence of the extract) reactions, respectively.

Statistical analysis

Data were compared by means of two-way (time of day and solar radiation) model I ANOVA. Homogeneity of variances was assessed by the F_{max}-test (in experiments with five replications) or the Barlett's test (in experiments with six replications). Pairwise comparisons between treatments were performed only after finding significant differences, using the LSD test calculated at a 95 % confidence level. Pearson correlation coefficients were also calculated to compare patterns of variation of both enzyme activities during the daily cycles. Statistics followed Sokal & Rohlf (1995).

RESULTS AND DISCUSSION

Photoinhibition of photosynthesis

During daily cycles, photoinhibition in the different intertidal macroalgae, expressed as the decrease in the effective quantum yield from the morning to noon time, was linearly dependent on the daily integrated irradiance (Fig. 1). However, the recovery, expressed as the increase in the effective quantum yield from noon to the afternoon, presented a markedly different pattern; full recovery was found below a daily integrated irradiance of 1.0 x 10^4 kJ m^{-2}, but at higher irradiances recovery reached only 50 % (Fig. 2).

The bio-optical properties of the water column where the algae were collected have already been characterised (Häder & Figueroa 1997, Figueroa 1998). The irradiance of both PAR and UV throughout the year in this geographical area is relatively high (Häder et al. 2000). The attenuation coefficient of downward radiation at 305 nm ($K_{d,305}$ as determined by using a PUV-500 radiometer, Biospherical Instruments) was 0.7-0.8 m^{-1}. The coastal waters where the algae were collected are very clear throughout the year (Type I in the Jerlov classification, Jerlov 1976), with light of 305 nm reaching 0.1 % of surface levels at 19 m in summer, and 10 m in winter; for PAR, the corresponding depths were 72 m in summer, and 64 m in winter.

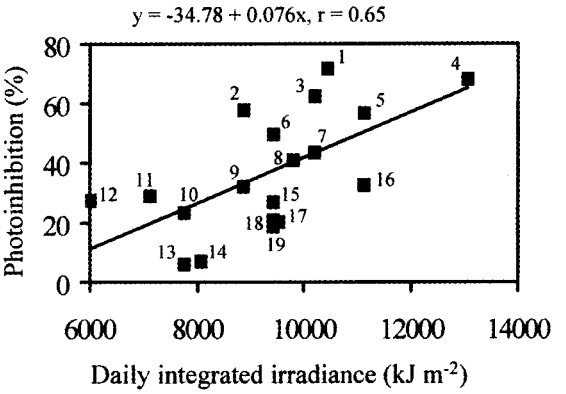

Fig. 1: Photoinhibition versus daily integrated irradiance in different macroalgae from southern Spain during daily cycles. Photoinhibition is expressed as the percentage of decrease in the effective quantum (ΔF/Fm') from the morning to noon time during daily cycles. The different species analysed are listed as follows: (1) *Felmanophycus rayssae*, (2) *Corallina elongata*, (3) *Dasycladus vermicularis*, (4) *Rissoella verruculosa*; (5) *Laurencia pinnatifida*, (6) *Enteromorpha muscoides*, (7) *Cystoseira tamariscifolia*; (8) *Porphyra umbilicalis*, (9) *Asparagopsis armata*, (10) *Cystoseira usneoides*, (11) *Gelidium sesquipedale*, (12) *Dasycladus vermicularis*, (13) *Cladostephus spongiosus*, (14) *Dictyota dichotoma*, (15) *Ulva rotundata*, (16) *Falkenbergia rufolanosa*, (17) *Ulva rigida*, (18) *Padina pavonica*, and (19) *Codium adhaerens*.

Fotoinhibición frente a dosis diaria de luz medida en diferentes macroalgas del sur de España durante diferentes ciclos diarios de medida. La fotoinhibición se expresó como el porcentaje de caída del rendimiento cuántico efectivo de emisión de fluorescencia (ΔF/Fm') al mediodía comparado con los valores de las primeras horas del día en cada ciclo diario. Las diferentes especies analizadas fueron las siguientes: (1) *Felmanophycus rayssae*, (2) *Corallina elongata*, (3) *Dasycladus vermicularis*, (4) *Rissoella verruculosa*; (5) *Laurencia pinnatifida*, (6) *Enteromorpha muscoides*, (7) *Cystoseira tamariscifolia*; (8) *Porphyra umbilicalis*, (9) *Asparagopsis armata*, (10) *Cystoseira usneoides*, (11) *Gelidium sesquipedale*, (12) *Dasycladus vermicularis*, (13) *Cladostephus spongiosus*, (14) *Dictyota dichotoma*, (15) *Ulva rotundata*, (16) *Falkenbergia rufolanosa*, (17) *Ulva rigida*, (18) *Padina pavonica* y (19) *Codium adhaerens*.

When exposed to unfiltered direct solar radiation at the surface, most macroalgae show a pronounced photoinhibition after various times of exposure at least at high zenith angles (Häder et al. 1996, Häder et al. 1997). Even though harvested from rock pools (where they are exposed to extreme solar irradiances), algae show signs of photoinhibition after extended periods of exposure (Figueroa et al. 1997, Häder & Figueroa 1997). As expected, deep-water algae and those

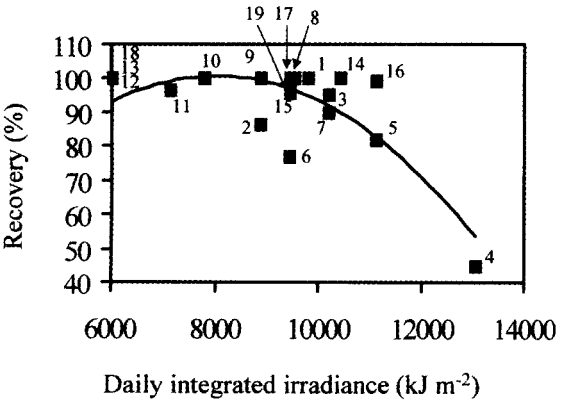

Fig. 2: Recovery of photosynthesis versus daily integrated irradiance in different macroalgae from southern Spain during daily cycles. Recovery is expressed as the percentage of increase in the effective quantum (ΔF/Fm') from noon to the afternoon during daily cycles. The species analysed are listed as follows: (1) *Felmanophycus rayssae*, (2) *Corallina elongata*, (3) *Dasycladus vermicularis*, (4) *Rissoella verruculosa*; (5) *Laurencia pinnatifida*, (6) *Enteromorpha muscoides*, (7) *Cystoseira tamariscifolia*; (8) *Porphyra umbilicalis*, (9) *Asparagopsis armata*, (10) *Cystoseira usneoides*, (11) *Gelidium sesquipedale*, (12) *Dasycladus vermicularis*, (13) *Cladostephus spongiosus*, (14) *Dictyota dichotoma*, (15) *Ulva rotundata*, (16) *Falkenbergia rufolanosa*, (17) *Ulva rigida*, (18) *Padina pavonica*, and (19) *Codium adhaerens*.

Recuperación de la fotosíntesis frente a dosis diaria de luz medida en diferentes macroalgas del sur de España durante diferentes ciclos diarios de medida. La recuperación se expresó como el porcentaje de incremento del rendimiento cuántico efectivo de emisión de fluorescencia (ΔF/Fm') desde el mediodía hasta el atardecer durante ciclos diarios de medida. Las especies analizadas fueron las siguientes: (1) *Felmanophycus rayssae*, (2) *Corallina elongata*, (3) *Dasycladus vermicularis*, (4) *Rissoella verruculosa*; (5) *Laurencia pinnatifida*, (6) *Enteromorpha muscoides*, (7) *Cystoseira tamariscifolia*; (8) *Porphyra umbilicalis*, (9) *Asparagopsis armata*, (10) *Cystoseira usneoides*, (11) *Gelidium sesquipedale*, (12) *Dasycladus vermicularis*, (13) *Cladostephus spongiosus*, (14) *Dictyota dichotoma*, (15) *Ulva rotundata*, (16) *Falkenbergia rufolanosa*, (17) *Ulva rigida*, (18) *Padina pavonica* y (19) *Codium adhaerens*.

adapted to shaded conditions are inhibited even faster when exposed to direct solar radiation (Häder & Figueroa 1997).

Under a daily course of solar radiation, macroalgae usually exhibit photoinhibition at midday. High irradiances of PAR are regarded as the most important wavelength components in this decline of photosynthetic rates (Hanelt 1996, Häder & Figueroa 1997, Franklin & Forster 1997). However, UV-B (280-315 nm) and UV-A (315-400 nm) radiation have an important role in photoinhibition and further inhibition of recovery processes in intertidal and subtidal red macroalgae, even though their energetic contribution relative to PAR, is much lower (Wood 1989, Larkum & Wood 1993, Dring et al. 1996, Hanelt et al. 1997, Figueroa et al. 1997, Gómez & Figueroa 1998). Recent studies indicate that UV-B, under environmental conditions of high PAR, may have beneficial effects on photobiological processes in macroalgae. For example, in the brown alga *Dictyota dichotoma* from southern Spain, UV-B promotes recovery after a stressful photoinhibition of photosynthesis (Flores-Moya et al. 1999).

Two different kinds of photoinhibition have been defined, dynamic and chronic photoinhibition (Osmond 1994). In general, sun-adapted algae exhibit dynamic photoinhibition, a reversible photoprotective mechanism consisting in a down-regulation of the PSII in order to handle excess energy increasing thermal energy dissipation. In contrast, when shade-adapted algae are transferred to high irradiance environments (e.g., to shallow waters), chronic photoinhibition occurs. This phenomenon involves photodamage to PSII reaction centres and subsequent proteolysis of the D1 protein (Critchley & Rusell 1994). Thus, photodamage occurs when the rate of degradation of D1 proteins exceeds the rate of repair (Aro et al. 1993). Photoinhibition has been proposed as a strategy of photoprotection against high irradiance (Osmond 1994, Hanelt 1996). The susceptibility to photoinhibition has consequences for the ecology of macroalgae and depends on the irradiance doses, season, water transparency and the presence of algal canopies.

Although macroalgae show a great diversity of responses to high irradiances, the relatively large rate of photoinhibition in intertidal algae occurs when low tide coincides with the onset of solar radiation at noon (Franklin & Forster 1997, Häder & Figueroa 1997). This is especially stressful in species from southern Spain, where irradiances higher than 2,000 μmol photon m^{-2} s^{-1} have been measured. For example, the differential acclimation potential to UV radiation of two intertidal species of *Gelidium* was associated to their growth light environments; *Gelidum latifolium* growing on rocky shores exposed to full solar radiation showed more efficient metabolic adjustments (dynamic photoinhibition) to cope with solar radiation than *Gelidum sesquipedale* that inhabits shaded crevices and suffers chronic photoinhibition (Gómez & Figueroa 1998).

Jiménez et al. (1998) showed that the extent of photoinhibition and recovery in different red macroalgae from southern Spain is related to the history of light exposure. Algae subjected to very high irradiances had higher photoinhibition and recovery than algae inhabiting shaded sites. As mentioned above, the recovery capacity, measured as an increase in the quantum yield of fluorescence, is species-specific (i.e., more rapid in sun adapted algae than in algae growing in deeper waters or in those transferred to the surface from shaded locations). While the brown alga *Padina boryana* shows recovery after a 30 % irradiance decline, *Sargassum polycystum* do so only after a reduction of 70 % in the incident radiation (Hanelt et al. 1994). In the red eulittoral red algae *Porphyra leucosticta* (Figueroa et al. 1997), *Asparagopsis armata*, and *Felmanophycus rayssae* (Jiménez et al. 1998) from southern Spain, the recovery of photosynthesis occurs immediately after a decrease of only 10-20 % of solar radiation. In eulittoral red algae *Gelidium sesquipedale* transferred from shaded crevices to full solar radiation, the recovery is much slower than in *Gelidim pusillum*, an alga commonly found at exposed sites (Gómez & Figueroa 1998). Thus, the kinetic of recovery gives insights into the photo-adaptive strategies of macroalgae and their light-stress tolerance capacity: algae with dynamic photoinhibition during enhanced solar radiation and a rapid recovery during the afternoon have competitive advantages in relation to algae without any efficient photoprotection mechanism.

In terms of tolerance to UV radiation and PAR, the enhanced capacity for dynamic photoinhibition and subsequent recovery observed in algae from southern Spain underlies clear sun-adaptation mechanisms. Inhibition of photosynthesis, expressed as a decline in effective quantum yield in algae grown under full solar radiation, ranged between 71.6 % in *Felmanophycus rayssae* and 2.5 % in *Asparagopsis armata* (this study), and 100 % recovery was observed in various other species (Jiménez et al. 1998). Only *Rissoella verruculosa,* an endemic Mediterranean species, shows relatively low recovery values (Flores-Moya et al. 1998). A comparison between *Porphyra leucosticta* and *Rissoella verruculosa*, species with similar zonation patterns at intertidal sites, suggests different photo-protective strategies. Differences could be due to different light absorption properties related to morphology and pigment composition. *Porphyra* has a thin, monolayered-cell thallus with a rapid and homogenous light transmission towards the light harvesting complexes. In contrast, *Rissoella* has a thicker thallus structure, in which scattering of photons through the multi-cell layers (self-shading) could take place. This seems to be the case under UV-B radiation: short wavelengths accounted for about 30 % of the whole photoinhibition in *Porphyra* (Figueroa et al. 1997), whereas the full solar spectrum including UV-B did not increase photoinhibition in *Rissoella.*

The usefulness of PAM fluorescence for estimating photosynthetic activity has been widely demonstrated (Figueroa 1998), but in several cases, no correlation with oxygen evolution has been found. A relationship between effective quantum yield of fluorescence and carbon assimilation or oxygen production has been demonstrated in higher plants (Genty et al. 1989), micro (Büchel & Wilhelm 1993, Schreiber et al. 1995, Flameling & Kromkamp 1998), and macroalgae (Hanelt et al. 1994). Such relationship validates chlorophyll fluorescence as an indirect determination of photosynthesis, but it is necessary to conduct more experiments to elucidate the cause of this loss of correlation between PAM fluorescence and oxygen evolution (Holmes et al. 1989, Flameling & Kromkamp 1998).

Carbonic anhydrase and nitrate reductase activities

There was a clear daily course for both enzymatic activities in *P. cartilagineum* that was modified by the nature of radiation received. In situ activity of nitrate reductase followed a pattern of initial decrease and recovery during the rest of the day. This pattern is clear in the PAR + UV-A + UV-B treatment (Fig. 3a), but it is delayed when UV radiation (PAR treatment) is lacking (Fig. 3b).

A peak of total carbonic anhydrase activity was found at 18:00 h, regardless of experimental conditions. During the next day total activity of carbonic anhydrase was again lower when the plants had been exposed to UV radiation.

Activity of both enzymes also revealed daily variations in the green alga *Ulva rigida* (Fig. 4) and in the brown alga *Fucus spiralis* (Fig. 5). When both enzyme activities were compared no correlation was found. However, a significant and negative correlation was found under full solar radiation when data on nitrate reductase activity were shifted 4 h in relation to data on carbonic anhydrase activity (Table 2). Nevertheless, no significant correlation was detected in the case of *U. rigida* subjected to the PAR treatment, which suggests that UV radiation is a signal for nitrate reductase.

If activity of carbonic anhydrase influences activity of nitrate reductase, a temporal delay is

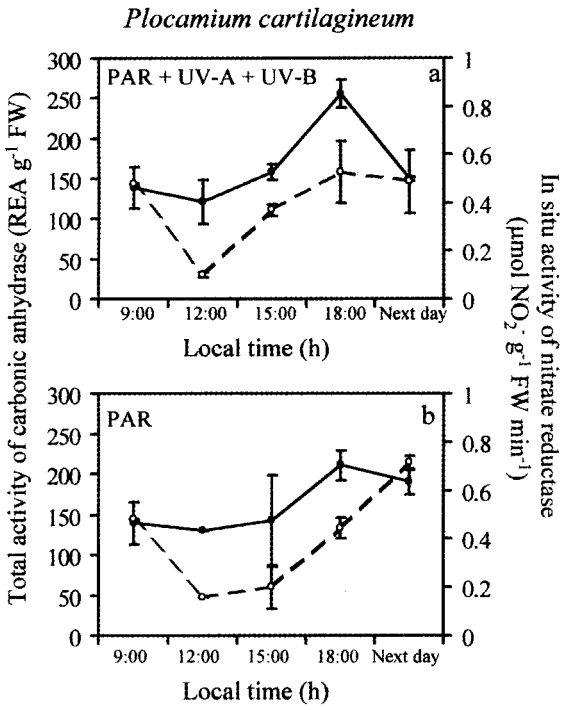

Fig 3: Total carbonic anhydrase (continuous line, black circles) and in situ nitrate reductase (dotted lines, open circles) activities under (a) full (PAR + UV-A + UV-B) and (b) PAR solar radiation during daily cycles in the red alga *Plocamium cartilagineum*. Vertical bars correspond to standard deviations.

Actividad de anhidrasa carbónica total (línea continua, círculos oscuros) y de nitrato reductasa in situ (línea discontinua, círculos claros) bajo (a) radiación solar completa (PAR+UV-A+UV-B) y (b) sin UV (PAR) durante ciclos diarios medidos en el alga *Plocamium cartilagineum*. Las barras verticales corresponden a desviaciones estándar.

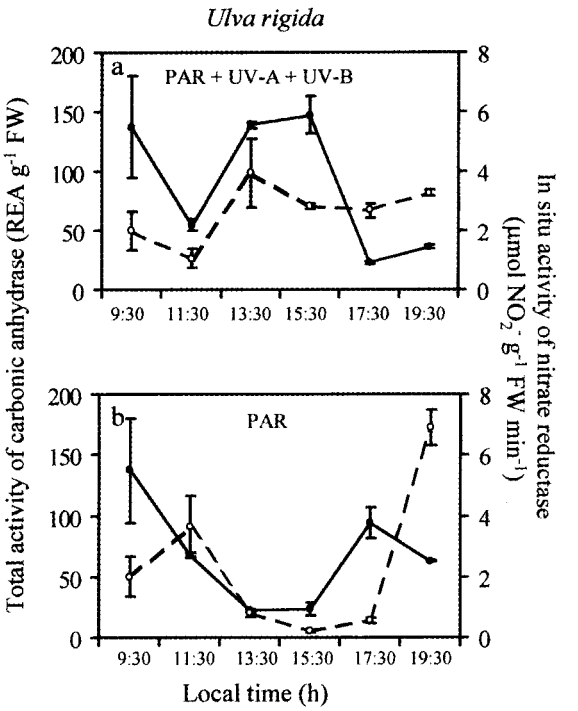

Fig. 4: Total carbonic anhydrase (continuous line, black circles) and in situ nitrate reductase (dotted lines, open circles) activities under (a) full (PAR+UV-A+UV-B) and (b) PAR solar radiation during daily cycles in the green alga *Ulva rigida*. Vertical bars correspond to standard deviations.

Actividad de anhidrasa carbónica total (línea continua, círculos oscuros) y nitrato reductasa in situ (línea discontinua, círculos claros) bajo (a) radiación solar completa (PAR + UV-A + UV-B) y (b) sin UV (PAR) durante ciclos diarios de medida en el alga *Ulva rigida*. Las barras verticales corresponden a desviaciones estándar.

expected for the whole process to occur. Thus, the temporal shifting applied to the data is not an artificial manipulation, but the expression of its accommodation to temporal processes occurring inside the cells. Our results revealed that UV radiation influences daily variations in enzymatic activities related to nutrient uptake and to assimilation. Thus, the occurrence of maximum activity of nitrate reductase during late in the day (something reported previously for red macroalgae, Lopes et al. 1997) was noted also for total activity of carbonic anhydrase. Exclusion of UV radiation appears to be a key factor in the case in *P. cartilagineum* since the recovery of nitrate reductase activity from midday is delayed in the PAR treatment. Similar variations in total carbonic anhydrase and in situ-measured nitrate reductase activities were found in the Mediterranean endemic red alga, *Rissoella verruculosa* (Flores-Moya et al. 1998). Total activity of carbonic anhydrase in this alga decreased gradually over the day in thalli exposed to PAR, but tended to increase in the afternoon under full solar radiation paralleling the increase of nitrate reductase activity. The increase in total carbonic anhydrase activity during the first half of the day could function to increase the supply of carbon skeletons. Subsequently, as internal sources of carbon and nitrogen become depleted, activities of carbonic anhydrse and nitrate reductase increase again. In general, activity of nitrate reductase in photosynthetic organisms is strongly light dependent, peaking 6 to 8 h after the beginning of illumination (Gao et al. 1992). For example, in the red alga *Gracilaria tenuistipitata* var. *liui* Zhang et Xia, activity of nitrate reductase follows

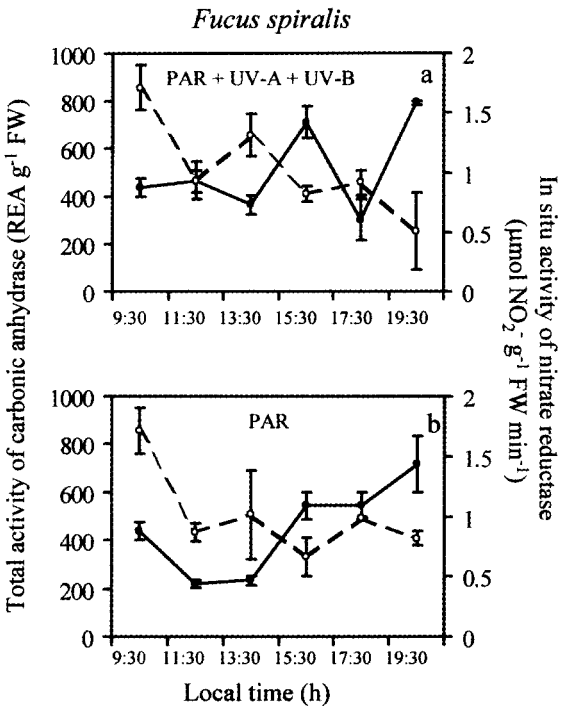

Fig. 5: Total carbonic anhydrase (continuous line, black circles) and in situ nitrate reductase (dotted lines, open circles) activities under (a) full (PAR + UV-A + UV-B) and (b) PAR solar radiation during daily cycles in the brown alga *Fucus spiralis*. Vertical bars correspond to standard deviations.

Actividad de anhidrasa carbónica total (línea continua, círculos oscuros) y nitrato reductasa in situ (línea discontinua, círculos claros) bajo (a) radiación solar completa (PAR + UV-A + UV-B) y (b) sin UV (PAR) durante ciclos diarios medidos en el alga *Fucus spiralis*. Las barras verticales corresponden a desviaciones estándar.

a diurnal rhythm with highest values during day (Lopes et al. 1997). During the present study effort was made to avoid additional nutrient depletion throughout the day. This included the setting of a high medium volume:biomass ratio in the tanks, and the full renovation of seawater every 30 min. Thus, stimulation of nitrate reductase activity due to NO_3^- depletion seems unlikely. Nevertheless, whether short wavelengths act as signals in the activation or deactivation of carbon and nutrient uptake remains unclear. According to Döhler et al. (1995), UV radiation affects nutrient uptake and nitrogen metabolism in different species of macroalgae. Undoubtedly, more effort is needed to fully understand the role of UV radiation on enzyme activity, and on the daily acclimation of this activity in algae growing under extreme light conditions.

In the green alga *Dasycladus vermicularis*, UV-A seems to stimulate carbonic anhydrase activity (Figueroa 1998). Exclusion of UV-B, and of both UV-A and UV-B decrease in carbonic anhydrase activity. In the marine angiosperms *Posidonia oceanica* and *Cymodocea nodosa*, activity of carbonic anhydrase is higher in summer than in winter (an energy-dependent response, Viñegla 2000). The effect of UV radiation vary with season (Figueroa 1998). Thus, during summer, the highest carbonic anhydrase activity in both marine angiosperms was found under PAR, whereas the lowest activity occurred under PAR + UV-A. During winter, however, minimal activity was measured under PAR in both plants, and, in the case of maximal activity, it took place under full solar radiation (PAR + UV-A + UV-B) in *Posidonia*, but indifferently under PAR + UV-A + UV-B and PAR + UV-A in *Cymodocea*.

TABLE 2

Pearson coefficient of correlation obtained from the correlation between the activity of carbonic anhydrase and that of nitrate reductase for three algal species under full (PAR + UV-A + UV-B) and PAR solar radiation. Statistical significance ($P < 0.05$) is indicated by asterisks

Coeficiente de correlación de Pearson obtenido después de correlacionar las actividades enzimáticas de la anhidrasa carbónica y la nitrato reductasa en tres especies de algas mantenidas bajo radiación solar completa (PAR + UV-A + UV-B) y PAR. Los asteriscos indican significancia estadística ($P < 0,05$)

Species	Treatment	No shifting	4-h shifting
Plocamium	(PAR + UV-A + UV-B)	0.383 (n = 25)	-0.939* (n = 15)
cartilagineum	PAR	0.451 (n = 25)	-0.985* (n = 15)
Ulva rigida	(PAR + UV-A + UV-B)	-0.901* (n = 30)	-0.934* (n = 20)
	PAR	-0.402 (n = 30)	-0.764* (n = 20)
Fucus spiralis	(PAR + UV-A + UV-B)	-0.375 (n = 30)	-0.891* (n = 20)
	PAR	-0.140 (n = 30)	-0.976* (n = 20)

Enzyme activities measured in *D. vermicularis* revealed that during increasing solar irradiance between 9:30 and 13:30 h, activities of nitrate reductase and carbonic anhydrase varied antagonistically, with nitrate reductase decreasing in relation to carbonic anhydrase (Gómez et al. 1998). Activity of nitrate reductase increased strongly towards the afternoon, specially in plants exposed to full solar radiation. In contrast, activity of carbonic anhydrase decreased gradually under PAR + UV-A + UV-B, but strongly under PAR + UV-A towards the afternoon. PAR irradiance did not significantly influence carbonic anhydrase activities throughout the day. On the other hand, activity of nitrate reductase peaked 6 h after exposure to sunlight, and it occurred in the three light treatments. This agrees with the diurnal rhythms reported for other macroalgae such as *Ulva fenestrata* (Gao et al. 1992) and *Gracilaria tenuistipitata* (Lopes et al. 1997). Although similar data on macroalgae from other geographical regions are not available for comparison, our findings revealed that current levels of UV radiation in southern Spain affect the activity of these enzymes. Also, one can argue that enhanced solar UV act simultaneously on the activity of nitrate reductase and carbonic anhydrase, as both processes of nitrogen and carbon assimilation are metabolically related (Turpin 1991). We speculate that the increase in carbonic anhydrase activity around noon time, which was significantly induced by UV radiation, resulted from an increased requirement for carbon-skeletons, probably required for the synthesis of UV-absorbing compounds or involved in the turnover of nitrogen-rich organic substances (e.g., PS II proteins) during photoinhibition. Undoubtedly, these enzymes are also sensitive to other environmental factors, including nutrient availability (Wheeler & Weinder 1983, Davison & Stewart 1984), and we do not reject any possible influence of these factors on the variations observed in the present study.

The most important feature expressed here is the clear interrelation between the enzymatic activities involved in carbon and nitrogen uptake in the three algae investigated. In this sense, activity of carbonic anhydrase and assimilation of carbon appear to influence the active incorporation of nitrogen. Thus, activity of nitrate reductase during the day is influenced by prior the levels of carbonic anhydrase (at least for four hours), suggesting a feedback process where nitrogen is incorporate to molecular skeletons that have been formed with carbon previously incorporated due to the activity of the carbonic anhydrase. This relation can also be expressed for protein content and nitrate reductase, which supplies inorganic nitrogen for the synthesis of these molecules. The level of nitrate reductase in cells is determined by the difference between the rate of synthesis and degradation. However, a second step of postranslational regulation could be linked to carbon levels inside cells as it has been demonstrated in microalgae (Vergara et al. 1998). Thus, the time period between peaks of enzymes is reflecting the complex nature of processes that are taking place in relation to carbon and nitrogen metabolism under solar natural conditions. These processes are down-regulated by the external and internal signals of nutrients content, and such signals might not be processed if the triggering environmental factor (UV-B radiation) is excluded.

Circadian rhythms are known to be present in macroalgae for different physiological processes. Moreover, daily rhythms of variation in photosynthetic activity have been reported frequently (Flores & Herrero 1994, Mori et al. 1996). Thus, daily variations of enzymatic activities related to carbon and nitrogen metabolism reflect a process of competition for energy supplied by photosynthesis, which also changes daily.

The daily cycles in enzyme activities determined in this study suggest that UV radiation can modulate changes in carbon and nitrogen assimilation as it occurs in *Rissoella verruculosa* (Flores-Moya et al. 1998), and in *D. vermicularis* (Gómez et al. 1999). Therefore, the question whether short wavelengths act as triggers on the activation or deactivation of carbon and nitrogen uptake mechanisms, as it occurs during energy dissipation in PSII, remains open. Further studies manipulating UV radiation and ambient nutrient concentration simultaneously will provide new insights into the mechanisms of daily acclimation of enzyme activity under enhanced UV radiation in these macroalgae.

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