

Photoacclimation of *Ulva rigida* and *U. rotundata* (Chlorophyta) arranged in canopies

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ABSTRACT: Photosynthetic acclimation of *Ulva rigida* and *U. rotundata* arranged in canopies was assessed by combining laboratory and field experiments. Changes in the light field caused by the self-shading of *Ulva* thalli resulted in an instantaneous effect on the photosynthetic parameters. Photosynthetic efficiency decreased from the top of the canopy to the bottom as the photosynthetic active radiation/photosynthetic usable radiation ratio (PAR/PUR) increased. In contrast, the light compensation point showed an opposite pattern. Other photosynthetic parameters, light-saturated rate of photosynthesis and dark respiration, were unaffected. Both species acclimated to light gradients within the canopies, both in the laboratory and in the field, by changes in capacity of light capture and in photosynthetic performance: total chlorophyll concentration and absorbance decreased in the upper *Ulva* thalli, while the C:N ratio showed an opposite pattern. With regard to photosynthetic performance, area-normalized photosynthesis rate under saturating and subsaturating light increased towards the bottom of the canopies, while dark respiration was higher in the upper surface of the mats. The photoacclimation of canopies in which the thalli were randomly mixed 2 times per day showed a highly variable response: *U. rigida* thalli, submitted to similar mean irradiances and with comparable fluctuations in the light regime, showed high variability in chlorophyll content and growth rate, which, in turn, were not correlated. Canopies of *U. rigida* were also set up in the field. Total chlorophyll displayed a vertical profile similar to those obtained under laboratory conditions. Growth rate decreased exponentially through the canopy, but remained positive despite the low photon fluence rate reaching the bottom of the mat. Chlorophyll profiles in natural *U. rotundata* canopies suggested a structured arrangement of thalli in the field. Light attenuation through the *Ulva* canopies and photoacclimation due to this arrangement should be included in any model of primary production in these systems.

KEY WORDS: Canopy · PAR/PUR ratio · Photoacclimation · Photosynthesis · *Ulva rigida* · *Ulva rotundata*

INTRODUCTION

Soft sediments and mudflats of shallow coastal zones undergoing eutrophication, such as some estuaries and bays, are often dominated by green algae of the order Ulvales, mainly *Ulva* and *Enteromorpha* (Schramm & Nienhuis 1996, Valiela et al. 1997). *Ulva* thalli are frequently arranged in mats, canopy-like structures where a high thallus area index (TAI; a concept analogous to the leaf area index for vascular plant sporophytes) is observed in the field (Hernández et al. 1997). In these circumstances, the canopies undergo marked

changes in light climate caused by a self-shading effect (Vergara et al. 1997). The persistence of *Ulva* canopies can be controlled by tidal movements. In mudflats and flumes, the water motion and turbulence during ebb and flow may cause mixing of the layers within the canopy or even loss of the whole structure. Conversely, in some areas the canopies may be stable, keeping their structure during high tide, even as floating thick mats.

Light is also strongly attenuated over the canopy by the water column, especially at high tides. Recently, Vergara et al. (1997) showed that *Ulva* blades arranged in canopies are not optically neutral, since they preferentially absorb the blue and red regions of the light spectrum. Thus, the light spectrum becomes progressively more green-enriched as it passes through the

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mats. Consequently, the change in the spectral composition of the light reduces the photosynthetic active radiation/photosynthetic usable radiation ratio (PAR/PUR) (Morel et al. 1987) through the canopies.

In spite of numerous reports related to self-shading effects in phytoplankton (see Kirk 1994), and photoacclimation in vascular plant canopies (Terashima & Hikosaka 1995), studies on photosynthetic metabolism within canopies in marine macroalgae are scarce. Measurement of light attenuation in *Codium bursa*, a balloon-like green alga, indicated that it absorbs most incident light (Geertz-Hansen et al. 1994). Microsensor studies for light and oxygen distribution were carried out by Lassen et al. (1994) in *C. fragile*, due to its siphonal and soft thallus. These authors reported steep light and oxygen gradients within a thallus on a scale of millimetres. Salles et al. (1996) showed changes in the spectral composition of light under canopies of intertidal macroalgae. In their study, *Porphyra leucosticta* growth rates were lowered by a canopy of 1 blade, despite the same light intensity, which is indicative of a lower PAR/PUR ratio under the canopy. Recently, Krause-Jensen et al. (1996) showed that productivity declined with depth in mats of *Chaetomorpha linum*, reflecting a pronounced self-shading within these mats.

Ulva species are ubiquitous on the sediments of the shallow coastal zones of southern Spain. Ecophysiological studies in different coastal zones of this region have focused on the photoacclimation of *Ulva* species under laboratory conditions (Pérez-Lloréns et al. 1996), the biomass, the dynamics of growth and net primary production of this species (Hernández et al. 1997) and the seasonal photosynthetic performance and light attenuation in *Ulva* canopies (Vergara et al. 1997). In these studies, a main conclusion was that light attenuation within *Ulva* canopies may be an important factor in the regulation of primary productivity, while photoacclimation of *Ulva* thalli to a canopy arrangement could enhance productivity compared to a hypothetical constant response within the mat.

The aim of this work was to assess the photoacclimation effect on photosynthetic performance, thallus elemental composition and growth within *Ulva* canopies. Canopies were maintained both under laboratory conditions and in the field. To simulate the possible mixing caused by tidal movements, the study was conducted in 2 ways: (1) by keeping a structured canopy during the whole period of study and (2) by randomly mixing the thalli within the mat twice a day. This work was performed in 2 *Ulva* species that bloom in shallow coastal zones of southern Spain: *U. rigida* and *U. rotundata*. The results obtained in this study indicate that the photoacclimation of *Ulva* arranged in canopies must be considered when planning a global model to estimate the primary productivity of these systems.

MATERIAL AND METHODS

Field sampling. Two *Ulva* species that bloom on the soft sediments of the coastal zones of southern Spain were selected. *U. rigida* C. Agardh was collected from a creek at Los Toruños, a salt marsh belonging to the Cádiz Bay Natural Park, in the vicinity of the Faculty of Marine Sciences (Puerto Real, Cádiz). *U. rotundata* Bliding was collected from the Palmones river estuary (Algeciras Bay, Cádiz), a eutrophic coastal ecosystem where a green tide episode has been thoroughly studied (Hernández et al. 1997).

Instantaneous effect of a canopy arrangement on photosynthesis. The first laboratory experiment was conducted to study the immediate effect of a change in the light field on the photosynthetic parameters. This change can be followed from variations in the PAR/PUR ratio, which increases through *Ulva* canopies (Vergara et al. 1997). The PUR, according to Morel (1978), is the product of PAR (λ) with a weighting function $[A(\lambda)]$ describing the probability that a photon of a given wavelength will be absorbed by the thallus. The dimensionless variable is derived from the absorption spectrum of *Ulva* by dividing the spectrum by the maximal absorption (A_{\max}), which occurs *in vivo* at 440 nm (Vergara et al. 1997). Prior to the experiment, *U. rigida* and *U. rotundata* thalli were maintained for 2 d in filtered natural sea water (Whatman GF/C) in a Koxka EC-540F incubation chamber. Culture conditions were 15°C, white light of low intensity (20 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, Philips TLD36W/54), and 12 h light: 12 h dark L/D photoperiod cycle. Photosynthesis-photon fluence rate (P-PFR) curves were calculated using *U. rigida* and *U. rotundata* discs. A variable number of *Ulva* layers (from 0 to 3) were placed between the light source and the oxygen-electrode chamber. These layers were sandwiched between 2 transparent glass plates and wetted with sea water to prevent desiccation (Fig. 1A). This device changed the PAR/PUR ratio of the incident light, as it reduced the amount of photons of blue and red wavelengths (Vergara et al. 1997). Readings of the actual PFR reaching the *Ulva* discs in the electrode chamber were carried out by placing the detector (2π collector LiCor LI 192 SA connected to a LiCor LI-1000 radiometer) behind the '*Ulva* sandwich'. Photosynthetic measurements were immediately performed in a Hansatech polarographic O_2 electrode at 15°C, as previously described (Pérez-Lloréns et al. 1996), and P-PFR curves were fitted according to Jassby & Platt (1976).

Photoacclimation in the laboratory. In this second design, *Ulva* blades were arranged in canopies either in a permanent position or by random changes of the thalli (2 mixings per day), simulating the 2 possible conditions to which the algae can be subjected in the

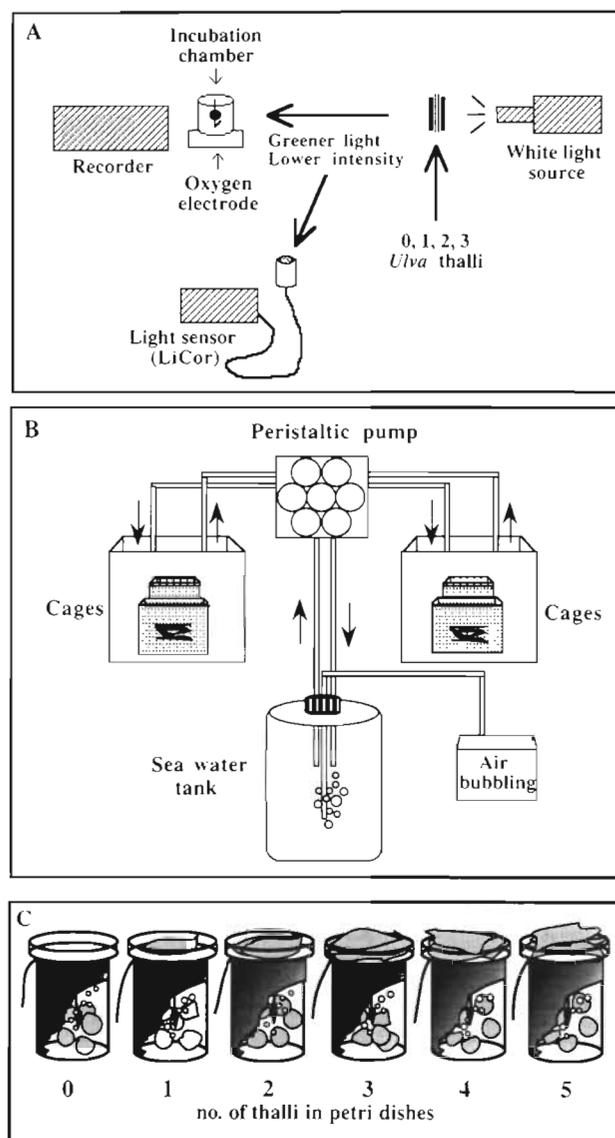


Fig. 1. Experimental designs to study (A) the immediate effect of the change in the light field on the photosynthetic parameters, (B) the photoacclimation in *Ulva* canopies under laboratory conditions, and (C) the culture of *Ulva* covered by a variable number of thalli, simulating a dynamic light supply

field (Fig. 1B). Six *Ulva* thalli of 10×10 cm area were superimposed within white plastic cages ($16 \times 16 \times 5$ cm) of 1 cm mesh size. Cages ($n = 4$) were immersed in 2 methacrylate aquaria filled with filtered (Whatman GF/C) natural air-bubbled sea water. Independent replicates of each treatment were conducted. Each aquarium ($n = 2$) contained 2 cages, one with *Ulva* thalli in a permanent position and another one with a random arrangement. A continuous closed-flow system was kept by means of a peristaltic pump (Watson-Marlow) at a flow rate of 5 l d^{-1} , with a reservoir of 15 l. Incubations were conducted at 15°C . Incident PFR on

the canopies was $160 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Sylvania GRO-LUX, F-36W/GRO-T8) in a 12 h light:12 h dark photoperiod. The incubation period was 13 d for *U. rigida* and 7 d for *U. rotundata*. This period was shorter in *U. rotundata*, as some signs of sporulation were observed in the margin of some thalli. However, this period of time is sufficient to complete the photoacclimation process in *Ulva* species (Pérez-Lloréns et al. 1996). After the incubation, growth rate, photosynthetic performance, chlorophyll concentration, wide band absorbance, and C:N atomic ratio were determined as previously described (Pérez-Lloréns et al. 1996), and compared with the initial values. Since the acclimation responses for chlorophyll *a* and *b* were similar (data not shown), total chlorophyll data (*a* plus *b*) were reported. Because the time to complete P-PFR curves (2 replicates \times 2 treatments \times 6 thalli) was a limiting step, photosynthesis was measured at light saturation ($715 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, P_{max}), at a sub-saturating light level ($48 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, P_{subsat}), indicative of photosynthetic efficiency at low light levels (as indicated in previous P-PFR curves) and in darkness (R_d).

The design shown above did not allow us to record the sequence of changes of the PFR reaching the different *Ulva* layers in the random arrangement (i.e. the 'light history'; the mean PFR and its variance). Therefore, a further experiment was necessary to follow these PFR changes. The design of this experiment is shown in Fig. 1C. *U. rigida* discs of 10 mm diameter ($n = 6$) were placed in 250 ml aerated beakers ($n = 24$) with filtered natural sea water, which was replaced every 2 d. The incubations were carried out for 8 d at 15°C with an incident PFR of $160 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in a 12 h light:12 h dark photoperiod, as described above. Petri dishes were fitted on the top of the beakers. The petri dishes contained a variable number of superimposed *Ulva* thalli (0 to 5), simulating *Ulva* canopies of different number of layers (1 to 6). Beakers were wrapped in black plastic bags to prevent light entering other than from the top. *Ulva* thalli placed in petri dishes were immersed in sea water to prevent desiccation. The position of the petri dishes on the beakers remained fixed in the treatment simulating a stable canopy arrangement, while they were randomly changed (2 times per day in the light period; about 3 and 9 h after the onset of the light period) in the random treatment. Two replicates per treatment were carried out. After the incubations, growth rate and chlorophyll concentration were measured. The control of the PFR reaching the beakers and the measurement of growth rate and total chlorophyll were performed as described above. The specific attenuation coefficient for PFR was calculated for each petri dish, assuming an exponential model, and was applied to the incident

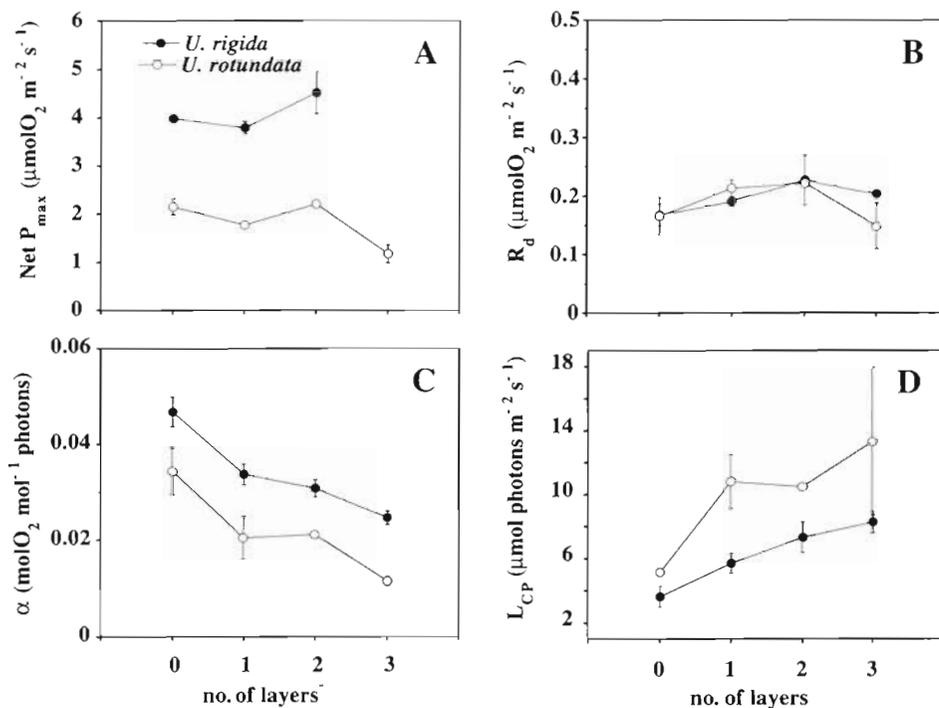


Fig. 2. *Ulva rigida* and *U. rotundata*. (A) Light-saturated rate of photosynthesis (net P_{max}); (B) dark respiration (R_d); (C) photosynthetic efficiency (α); and (D) light compensation point (L_{CP}) as a function of the number of *Ulva* layers placed between the light source and the incubation chamber. Data are means \pm SE ($n = 2$). P_{max} under 3 thalli was not computed in *U. rigida* as light was strongly attenuated and no saturating PFRs were obtained

PFR on each treatment in the simulated mixed canopies. This experimental design was also useful for interpreting possible effects of a slow water diffusion within the canopy. This was achieved by comparing the responses of thalli within the canopy (first design) to those of free-floating discs under a simulated-canopy arrangement (second design).

Photoacclimation in the field. Canopy devices ($n = 3$) for *Ulva rigida*, like those described in the laboratory experiments (Fig. 1B), were placed at the main flume of a creek in Los Toruños. This location was selected because its proximity to the laboratory allowed daily inspection. To avoid *Ulva* thalli being lost, cages were covered with a grid of fishing line. The enclosures were attached to plastic rods, which were sunk into the sediment near the low water mark. The incubation period was 12 d. The chlorophyll concentration, absorbance and specific growth rate on an area basis were determined as described above. The field inspection in the Palmones river estuary suggested the stability of canopy arrangements. Thus, 3 random spots of natural canopies of *U. rotundata* were sampled. To analyze the chlorophyll profiles, discs were carefully punched from different layers in the canopies. Immediately after collection, the discs were placed in acetone (24 h, 4°C in darkness), and chlorophyll concentration was estimated in the laboratory according to Jeffrey & Humprey (1975).

Statistical analysis. Significant effects of canopy structure on photoacclimation were assessed by

a 1-way analysis of variance (ANOVA). Student's *t*-tests were applied to compare the means of structured versus randomly mixed canopies. The significance of the instantaneous effect of canopy arrangement on photosynthetic parameters was assessed by a simple linear regression test. In all cases the statistical significance was set at 5% probability (Zar 1984).

RESULTS

Instantaneous effect of the canopy arrangement on photosynthesis

The aim of this experiment was to investigate the instantaneous effect of the self-shading on the photosynthetic parameters of *Ulva rigida* and *U. rotundata*. In both species, the light-saturated rate of photosynthesis (P_{max}) and the dark respiration (R_d) were unaffected by changes in the PAR/PUR ratio (Fig. 2A, B). In contrast, the photosynthetic efficiency (α) and the light compensation point (L_{CP}) were affected by the spectral change in the light field. In the 2 species, increased self-shading resulted in a decrease of α (Fig. 2C), whereas the L_{CP} increased significantly in *U. rigida* and in *U. rotundata* (Fig. 2D). Interspecific differences were observed for P_{max} , α and L_{CP} : *U. rigida* showed significantly higher values of P_{max} and α than *U. rotundata*.

Photoacclimation of *Ulva* canopies in the laboratory

The *Ulva* thalli acclimated to a canopy arrangement in the laboratory. Total chlorophyll concentration decreased towards the top of the canopies in both species (Fig. 3A, C). As a result, the upper thalli of the mat exhibited lower absorbance values than deeper ones (Fig. 3B, D). In *U. rigida*, total chlorophyll concentration suggested a bell-shaped curve (with a maximum in the fifth layer). This species exhibited higher absorbance values than *U. rotundata*. This resulted in a drastic reduction of the light availability in the bottom layer of the *U. rigida* canopies, which was about 3 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, close to the L_{CP} for this species. In *U. rotundata*, the PFR reaching the bottom layer was much higher (ca 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$), as absorbance was lower than in *U. rigida*. Mean chlorophyll concentration and absorbance of thalli from randomly mixed canopies were lower than those in the permanently arranged canopies in *U. rigida*, but not in *U. rotundata*.

The effect of photoacclimation on the photosynthetic characteristics of the canopy arrangement is shown in Fig. 4. P_{max} and P_{subsat} (indicative of photosynthetic

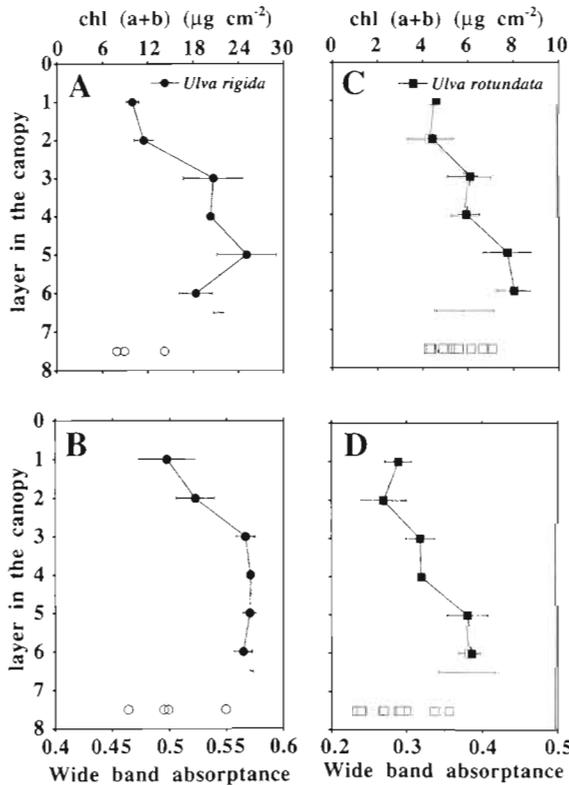


Fig. 3. (A, B) *Ulva rigida* and (C, D) *U. rotundata*. (A, C) Total chlorophyll concentration and (B, D) wide band absorbance as a function of the arrangement within *Ulva* canopies or as a result of a random disposition (open symbols at the bottom). Vertical dotted lines represent initial values. Data are means \pm SE (n = 2)

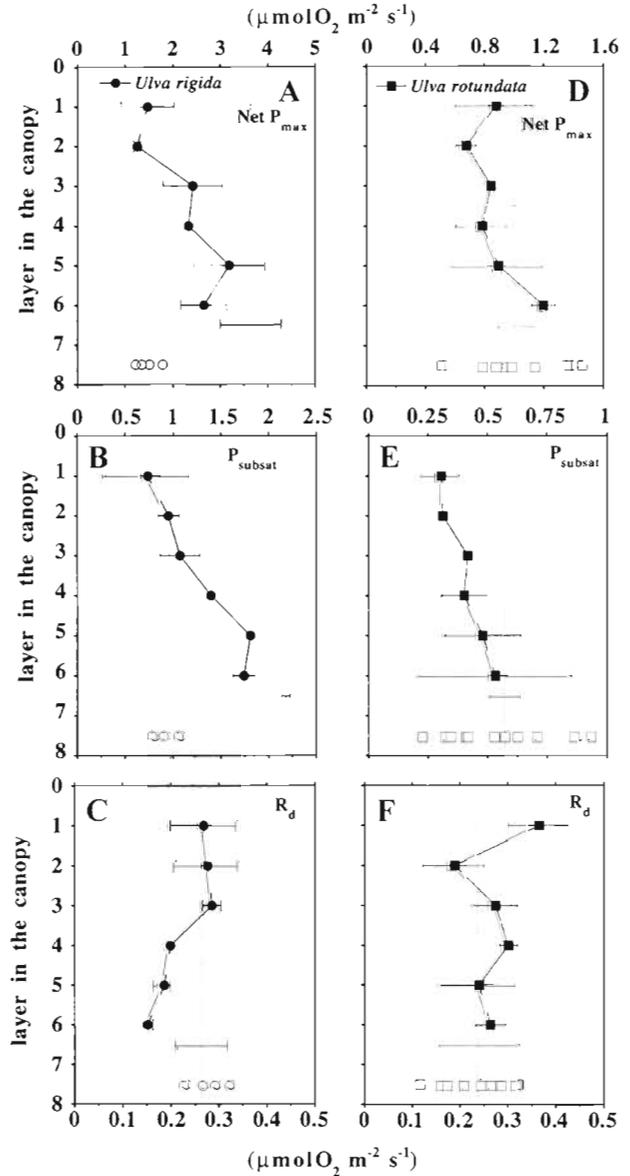


Fig. 4. Effect of canopy disposition on the rate of photosynthesis of (A–C) *Ulva rigida* and (D–F) *U. rotundata* at (A, D) light saturation (net P_{max}), (B, E) subsaturating light level (P_{subsat}), and (C, F) dark respiration (R_d). Open symbols at the bottom represent values corresponding to randomly mixed canopies. Vertical dotted lines represent initial rates. Data are means \pm SE (n = 2)

efficiency at low light levels) of *Ulva rigida* decreased towards the top of the canopy. The same trend was observed in *U. rotundata* for P_{subsat} , while P_{max} was not significantly affected. Conversely, R_d in *U. rigida* decreased from the fourth layer to the bottom as compared to the upper layers. In *U. rotundata* mats, R_d did not change, except for the higher value in the uppermost layer. P_{max} and P_{subsat} were significantly affected by treatment (permanent vs mixed canopies) in *U.*

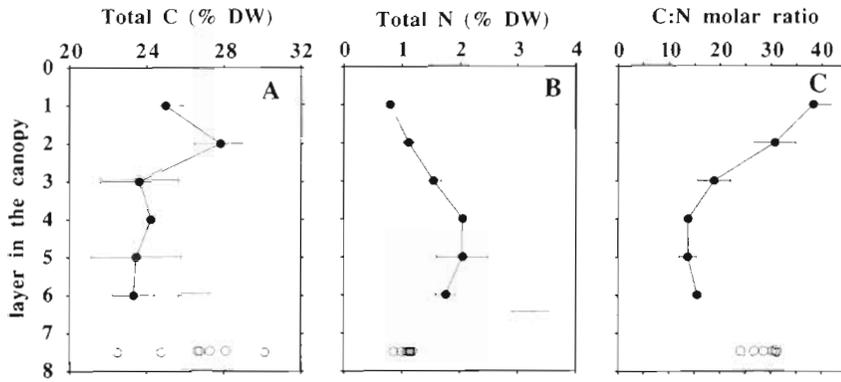


Fig. 5. *Ulva rigida*. (A) Total internal C, (B) total internal N, and (C) C:N molar ratio as a function of the arrangement within canopies or as a result of a random disposition (open symbols at the bottom). Vertical dotted lines represent initial values. Data are means \pm SE (n = 2). DW: dry weight

rigida, but not in *U. rotundata*. In contrast, R_d showed similar values in mixed and permanent canopies in both species.

The C:N atomic ratio exhibited a vertical profile in *Ulva rigida* that decreased from the top to the fourth layer and from there remained fairly constant down to the bottom layers of the mat (Fig. 5). This pattern was mainly due to the trend of N content (Fig. 5B). Nitrogen content and the C:N ratio were significantly affected depending on the degree of stability of the canopies. In randomly mixed canopies, values were close to those achieved in the first upper layers of the permanent canopies.

Specific growth rate was a function of the incident PFR within the canopy, decreasing from top to bottom layers. However, growth rates showed a minimum in the middle of the mat in both species (fourth or fifth thallus), increasing again towards the bottom layers (Fig. 6). Randomly mixed thalli displayed higher growth rates than the mean value attained in structured canopies.

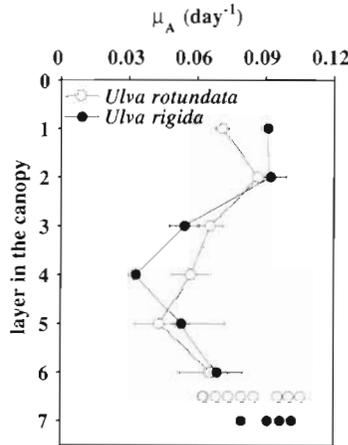


Fig. 6. *Ulva rigida* and *U. rotundata*. Specific growth rate (μ_A) as a function of the position within the canopies or in randomly mixed canopies (symbols at the bottom). Data are means \pm SE (n = 2)

Fig. 7 shows the growth rate and the chlorophyll content of *Ulva rigida* discs incubated in beakers, simulating a permanent canopy disposition (i.e. petri dishes with 'Ulva screens' always on the top of the same beaker) and a mixed canopy (the position of petri dishes were randomly changed twice per day). As stated in 'Material and methods', this experimental design allowed us to quantify the mean PFR (and its variability) reaching each one of the beakers. Growth rate and the chlorophyll content were affected by PFR under a stable canopy arrangement. Growth rate saturated with increasing PFRs. Chlorophyll concentration peaked at a PFR = ca $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, decayed

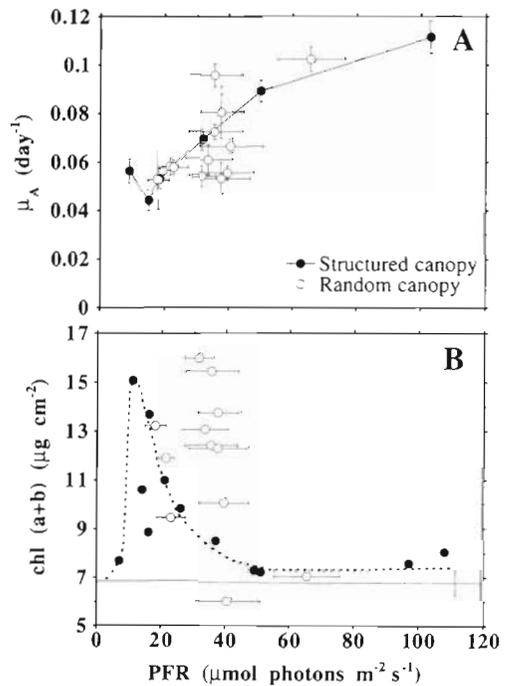


Fig. 7. *Ulva rigida*. (A) Specific growth rate (μ_A) and (B) total chlorophyll as a function of incident (PFR) within structured or randomly mixed canopies. Data for chlorophyll content represent mean values of 5 to 6 *U. rigida* discs per treatment. Horizontal line in (B) represents the initial value

exponentially at higher PFRs, and decreased sharply at very low irradiances.

In those treatments simulating a mixed canopy, the growth rate and the chlorophyll content were highly variable, despite the fact that almost all the beakers received a similar mean PFR (between 30 and 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) and a similar degree of PFR variability (Fig. 7). Growth rates were higher, similar or lower than those attained by thalli receiving a similar but uniform PFR. Chlorophyll content was also highly variable, generally displaying higher values than those attained by thalli receiving a similar and constant light dose. Besides this variability, growth rate and chlorophyll content were not correlated ($p > 0.05$); that is, the higher chlorophyll contents were not associated with higher or lower growth rates. In addition, chlorophyll content showed no relationship with the last PFR experienced by the thalli. Furthermore, no clear trends were observed in growth rate and chlorophyll content with respect to the order of light fluctuation (data not shown).

Photoacclimation under field conditions

To evaluate the photoacclimation in response to a canopy arrangement in the field, *Ulva rigida* canopies were settled in the vicinity of the laboratory (see 'Material and methods'). The vertical profiles of total chlorophyll (Fig. 8), and, similarly, the wide band absorbance (data not shown), indicated that photoacclimation to a stable canopy arrangement was accomplished in the field. The *in situ* growth rate was a function of incident PFR. These rates were close to those achieved in the laboratory. From the third layer to the bottom, growth was constant at about 0.04 d^{-1} , despite a much lower PFR.

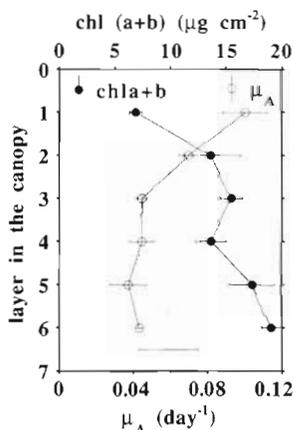


Fig. 8. *Ulva rigida*. Total chlorophyll and specific growth rate (μ_A) of canopies maintained in rectangular cages in the field at Los Toruños creek. Data are means \pm SE ($n = 3$). Vertical dotted line represents the initial total chlorophyll value

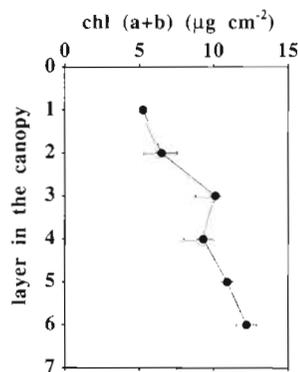


Fig. 9. *Ulva rotundata*. Vertical profile of total chlorophyll in canopies sampled in Palmones river estuary. Data are means \pm SE ($n = 3$)

For *Ulva rotundata*, 3 independent canopy arrangements were randomly sampled in Palmones river estuary. The profile of chlorophyll concentration through the canopy showed evidence of a structured arrangement of the canopies in natural assemblages (Fig. 9), similar to those found in laboratory studies.

DISCUSSION

The primary production of macroalgal populations depends largely on the photosynthetic performance and on the light availability within macroalgal canopies. Both factors have been assessed in previous studies in *Ulva* (Vergara et al. 1997) and the rhodophytes *Ahnfeltiopsis concinna* (J. Ag.) and *Laurencia mcdermidiae* (J. Ag.) (Beach & Smith 1996a, b). However, studies of the seasonal variations of light attenuation, pigment content and photosynthesis in *Ulva* do not consider the photoacclimation to a canopy structure (Vergara et al. 1997). Besides a variable light climate caused by tides in a highly turbid environment, light is further attenuated as a consequence of the canopy arrangement of *Ulva* thalli (Vergara et al. 1997). In these mats, light is not only attenuated, but also spectrally filtered. If the PAR/PUR ratio increased through the canopy (Vergara et al. 1997), it would be expected that α would decrease and the L_{CP} would show the opposite pattern, while P_{max} and R_d would be unaffected. The results presented in this study (Fig. 2) corroborate these expectations: there is less photosynthetically usable radiation (PUR; sensu Morel 1978) with depth through the canopies.

The culture of *Ulva* thalli arranged in permanent canopies showed evidence of photoacclimation. In this study, chlorophyll content of both species acclimated in much the same way as observed in response to irradiance (Pérez-Lloréns et al. 1996). There was an

inverse relationship between chlorophyll content and incident PFR, as shown by Henley & Ramus (1989a) and Markager & Sand-Jensen (1994) in *Ulva* species, with an eventual minimum at very low light levels, as in Pérez-Lloréns et al. (1996). The profile of the C:N ratio showed a pattern that was similar to that reported in mats of *Chaetomorpha linum* arranged in the laboratory (Krause-Jensen et al. 1996). Thus, bottom layers will be more light limited, while upper layers will be predominantly N limited (Lapointe & Duke 1984, Krause-Jensen et al. 1996). This profile agrees with the chlorophyll data, as chlorophyll concentrations reflect not only the light regime but also the macroalgal N status (Bird et al. 1982, Lapointe & Duke 1984).

With regard to the photosynthetic parameters, thalli placed deeper in the canopy, and hence submitted to a lower PFR, showed higher photosynthesis rates in comparison to blades located in the upper layers of the mats. This acclimation to low light levels can be considered as adaptive (sensu Givnish 1988, Pérez-Lloréns et al. 1996). Dark respiration, in contrast, was higher at the upper *Ulva* layers, which is directly related to a higher metabolic requirement for growth (Geider & Osborne 1992, Pérez-Lloréns et al. 1996). The increase in respiration at the upper layer may be also attributed to the cost of photooxidative damage (Long et al. 1994).

The minimum growth rate was found in the middle of the canopies and may seem, at first, rather unexpected (Fig. 6). To explain this result, the effect of a persistent laminar flow between 2 *Ulva* layers must be considered (Wheeler 1988). With a mean coefficient of molecular diffusion of $10^{-9} \text{ m}^2 \text{ s}^{-1}$ for nutrients in sea water (Mann & Lazier 1996), the time it would take a nutrient to cause an effect over 5 cm (from the edge to the middle of a thalli of 10 cm length, in arranged canopies) is about 5000 s (between 1 and 2 h). Thus, this stagnant sea water between the *Ulva* blades will have a low renewal rate and will become impoverished in inorganic carbon and nutrients, while the pH will increase and the oxygen will be oversaturated. This explanation has been suggested in *Chaetomorpha* mats (Krause-Jensen et al. 1996). The relative changes in the proportions of CO_2 and O_2 may influence the activity of Rubisco (Woodrow & Berry 1988), negatively affecting photosynthesis and hence growth. In fact, this minimum was not achieved under natural conditions, where the hydrodynamic conditions were stronger than in the laboratory experiments. Thus, besides a light gradient within the mat, the lack of an adequate renewal of sea water may decrease growth in *Ulva* thalli located inside the mat.

The experiment conducted to assess the effect of a random organization of the thalli within a canopy showed that, in structured canopies, PFR affected

growth rate and chlorophyll concentration, whereas the random arrangement displayed high variability despite a similar mean light dose. There is little knowledge of the effect of low frequency (hours) changes in light exposure in marine macroalgae. Henley & Ramus (1989b) studied the response of light fluctuation on the order of hours on growth and chlorophyll content in *Ulva rotundata*. They found a relationship between chlorophyll content and total daily radiation, while growth rate was dependent on the temporal distribution of light fluctuation. The effects of sunflecks of high frequency (in the time scale of seconds) on photosynthesis have been investigated by Greene & Gerard (1990) and Kübler & Raven (1996) in the rhodophytes *Chondrus crispus*, *Palmaria palmata* and *Lomentaria articulata*. However, the mechanisms involved at this time scale will differ from those occurring at longer ones, as physiological responses may depend on the time scale of the fluctuation (Cullen & Lewis 1988). In microalgae, several studies have been devoted to analyze the primary productivity under a light fluctuation of lower frequency (hours), as vertical mixing may subject phytoplankton populations to a dynamic light supply. These frequencies are usually shorter than the generation time of the cells (Prézelin 1992). Dynamic light with a period of fluctuation of 1 h has been compared to static light regimes in *Chlorella* (Kroom et al. 1992a, b). In these studies, dynamic light supply caused a lower chlorophyll concentration as well as reduced P_{max} and α . In phytoplankton, the effects of mixing and variable light have been equivocal (Mallin & Paerl 1992). These authors used a 'light field simulator' to mimic vertical mixing of phytoplankton community. There was a stimulation of growth rate under mixing conditions and a lower chlorophyll concentration in comparison to static light fields. In constant light regimes, at high light intensities, photoinhibition may reduce growth, and, additionally, growth may be restricted under low light (Marra 1978, Gallegos & Platt 1982). Thus, mixing will alleviate these 2 constraints, stimulating productivity (Harding et al. 1987). In contrast to these arguments, the steep and abrupt changes of PFR applied in our study, simulating a random mix of the thalli, will cause an intermittent and unpredictable stimulation-repression of acclimation processes. Thus, the photosynthetic structures (reaction centres, photosynthetic antenna complexes, electron transport components and enzymatic catalysts) would experience a much higher and variable turnover than under constant light, which may raise the maintenance cost for growth (Raven 1984, Pérez-Lloréns et al. 1996).

The results obtained in the field corroborate those achieved in the laboratory. The chlorophyll profile suggested clear signs of photoacclimation in *Ulva rigida*

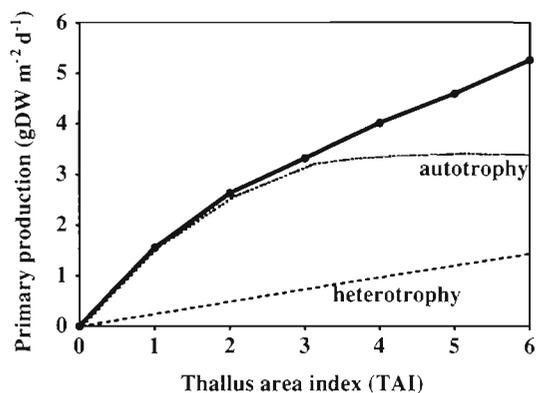


Fig. 10. *Ulva rigida*. Primary production of canopies settled in the field as a function of the thallus area index (TAI). The experimental data can be interpreted as the sum of a light-saturated function (autotrophy) plus a light-independent linear one (heterotrophy). DW: dry weight

canopies maintained in cages. With regard to growth, the typical saturation curve of primary production of the population versus TAI was not achieved in *U. rigida* (Fig. 10). Rather, the slope was smoothed as thalli were placed deeper in the canopy, despite a quite low PFR at the bottom layers of the mat, especially at high tide in a highly turbid environment. This could be the result of a double kinetic, a light-dependent saturation curve plus a light-independent linear one, indicative of heterotrophic growth at low light levels (Fig. 10; see also Markager & Sand-Jensen 1990). This possibility has already been suggested for natural *Ulva* populations (Vergara et al. 1997), as a TAI as high as 17 was occasionally recorded in Palmones river estuary (Hernández et al. 1997). Under such circumstances, light will be completely attenuated through the mat, and the bottom layers completely darkened. Thus, organic substrates, which are abundant in highly productive environments, such as shallow bays and estuaries (Valiela 1995), may be an additional source for primary production of *Ulva* species.

Besides field acclimation in *Ulva rigida*, the vertical chlorophyll gradient found in *U. rotundata* canopies from a natural assemblage is evidence of a stable arrangement of *Ulva* blades within the stands, although random mixing may occur sporadically in some parts of the Palmones river estuary. Acclimation may have a certain time scale to occur (from hours to days) (Falkowski & La Roche 1991), and it is clear from the laboratory experiments that chlorophyll concentrations of randomly mixed canopies were not related to the mean light dose received (Fig. 7) nor to the last light regime in the experiments (data not shown).

In conclusion, while the light attenuation caused by *Ulva* canopies may be included in any model of primary production in these systems (Vergara et al. 1997),

the incorporation of the photoacclimation characteristics of *Ulva* to a canopy arrangement may refine the predictions of the model.

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