The effect of photoacclimation on the photosynthetic physiology of *Ulva curvata* and *Ulva rotundata* (Ulvales, Chlorophyta)

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The effects of photoacclimation on growth, photosynthesis, pigment content and elemental composition of *Ulva curvata* and *Ulva rotundata*, which grow together in eutrophic areas of southern Spain, were investigated. Cultures were grown for 6 days at different photon fluence rates (PFR) ranging from darkness up to 200 μmol m^{-2} s^{-1} under nutrient-sufficient conditions (artificial seawater supplemented with ammonium and phosphate). Growth rates were not light-saturated (up to 200 μmol m^{-2} s^{-1}), reaching a value close to 0·2 d^{-1}. Growth rates based on mass, area or C content were equivalent, except in darkness and very low light levels (2 μmol m^{-2} s^{-1}), where thallus expansion occurred by diluting internal biomass and C. Chlorophyll and absorptance showed a bell-shaped PFR–response curve, with maxima at 30–60 μmol m^{-2} s^{-1} and lower values at light saturation and under light-limiting conditions. Although net NH_4^+ incorporation was not affected by growth-PFR, there was inefficient assimilation of N at low light levels, which may restrict chlorophyll, protein and membrane synthesis. The light-saturated photosynthesis rate (P_{max}) displayed a bell-shaped PFR–response curve, when expressed on an area basis, whereas it was saturated from 11 μmol m^{-2} s^{-1} when scaled to internal N. This suggests that ribulose-1,5-bisphosphate carboxylase/oxygenase activity could be the rate-limiting step underlying P_{max} for high-light-acclimated algae, whereas electron transport chain elements may limit P_{max} under light-limiting conditions. P_{max} and tissue C were always higher for *U. curvata*. Dark respiration rates were positively correlated with growth rates, and photon yield of net growth declined with increasing growth-PFR. The results are also discussed in relation to cell size, since *U. curvata* cells are smaller than those of *U. rotundata*.

Key words: growth, light, photoacclimation, photosynthesis, *Ulva curvata*, *Ulva rotundata*

Introduction

Eutrophication of estuarine and coastal waters, accompanied by intense mass growth of algae, has been recorded in the past few decades in many areas around the world. Developing eutrophication modifies biotic and abiotic factors in such a way that the growth of some key species is promoted. Many of these species belong to the genus *Ulva*, although conflicting data concerning the success of *Ulva* species in eutrophicated areas have also been reported (Rivers & Peckol, 1995).

Dense populations of *Ulva rotundata* Bliding and *Ulva curvata* (Kützing) De Toni occur in the Palmones river estuary (Southern Spain), with spatial and, possibly, seasonal segregation. These species 'bloomed' a few years ago, in parallel with observed physico-chemical changes in the estuary, as a consequence of the construction of a reservoir upstream, together with a severe drought period (1992–5). These populations overgrew and replaced other macrophyte populations such as the rhodophyte *Gracilaria bursa-pastoris* (S. G. Gmelin) Silva and, particularly, the aquatic angiosperm *Zostera noltii* Horneman, which formed dense meadows (Pérez-Lloréns & Niell, 1993).

Self-shading, as a consequence of the dense growth of these sheet-like species, modifies both the intensity and the spectral composition of light reaching these macrophytes. Superimposed on this are daily and seasonal variations, and modifications due to tidal mixing: during high tide, turbid waters considerably reduce the light available for photosynthesis, since most algae remain attached to the surface of the sediment. Although at low tide an excess of light can reach individuals in the upper layers, the bottom layers may receive low light as a consequence of the high attenuation coefficient resulting from thick muddy *Ulva* mats. Consideration of shifts in the species composition and primary productivity of seaweed assemblages in response to anthropogenic disturbance is often required to predict accurately the effects on other ecosystem compartments (McCormick & Cairns, 1994). A first step in the understanding of their ecology is the study of physiological responses to different environmental conditions.

This paper is dedicated to Prof. Dr F. Tomás y Valiente, killed the day we finished our manuscript.

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Photoacclimation experiments have been carried out mainly on terrestrial plants and aquatic unicellular algae (Björkman, 1981; Falkowski & LaRoche, 1991). The photosynthetic physiology of *Ulva* species has been studied previously, as well as growth, pigment content and chemical composition (Duke *et al.*, 1986, 1989; Ramus & Venable, 1987; Sand-Jensen, 1988*a*, *b*; Henley & Ramus, 1989*a*, *b*; Henley *et al.*, 1991*a*, *b*; Levavasseur *et al.*, 1991; Henley, 1992; Geertz-Hansen & Sand-Jensen, 1992; Markager & Sand-Jensen, 1992, 1994; Markager, 1993; Osmond *et al.*, 1993; Franklin, 1994). However, current knowledge of the physiology of key species associated with eutrophication (mostly Ulvales) is limited (EUMAC, 1995). Besides, little is known about the photosynthetic physiology of the dominant *Ulva* species found in eutrophicated waters of southern Spain. Therefore, the aim of this study was to assess the simultaneous effects of the photoacclimation process on: (1) growth, (2) photosynthetic performance, (3) pigment content and thallus absorptance, and (4) chemical composition (total tissue carbon and nitrogen) of *U. curvata* and *U. rotundata*.

Materials and methods

Plant material and experimental design

Field material of *Ulva curvata* and *U. rotundata* was collected in September 1994 from the intertidal mudflats of the Palmones river estuary (southern Spain). These two species cover a broad area of the mud. In this period of the year, mean atomic C : N : P ratio for *Ulva* species was 420 : 42 : 1, which indicates no N or P limitation, in comparison with mean values for different *Ulva* species taken from the literature (575 : 54 : 1) (Hernández *et al.*, unpublished). Tissue N and chlorophyll concentrations were at moderate levels, higher than those recorded in late spring–early summer for these species.

Once in the laboratory *Ulva* blades were preincubated for 2 days in dim light with artificial seawater (Woelkerling *et al.*, 1983) enriched with 26·5 μM of NH_4^+ and 2 μM of phosphate. NH_4^+ is an abundant source of N in this estuary, reaching transient peaks much higher than NO_3^--N. Discs (10 mm diameter) of the two *Ulva* species were cultured in a light- and temperature-controlled growth chamber (Koxka, EC 540F) (12 : 12 h light : dark cycle, 15 °C) for 6 days in aerated 1 dm^3 flasks with artificial seawater (as above) at different photon fluence rates (PFR): 0, 2, 11, 30, 60 and 200 $\mu mol\, m^{-2}\, s^{-1}$ (λ = 400–700 nm) (measured with a LiCor LI-1000 radiometer, with a 2π collector LiCor LI 193 SA). The range of incident light levels was obtained by covering the fluorescent tubes (Philips TLD 36W/54) with a variable number of layers of neutral black nylon net. Each flask initially contained 20 discs of *U. curvata* or *U. rotundata*. Culture medium was renewed every 2 days, and the remaining NH_4^+ present in the seawater measured according to Slawyck & MacIsaac (1972). At this point, 10 random discs per flask were taken for measurements of surface area and mass, and were returned to the corresponding flasks. Two of these discs (three at the beginning and at the end of the experiment) were used for absorptance measurements and pigment determinations. For simplicity, only initial and final results are shown. Data from discs (3–6) were pooled for determination of tissue C and N content (Perkin-Elmer C-N-H 240-C analyser). Samples for estimation of tissue C and N were taken at the beginning and end of the experiments.

Pigments were extracted from fresh discs (10 mm diameter) in 4 ml of *N*,*N*-dimethyl formamide, kept in darkness overnight at 4 °C and determined according to Porra *et al.* (1989). Absorptance (A) was estimated as: $A = 1-10^{-OD}$, where OD is the optical density (absorbance) of the discs at 678 nm measured in a spectrophotometer (Hitachi U-1100) with the opal-glass technique (Shibata, 1959). Calibration with a LiCor-spectroradiometer (model LI-1800 UW) was carried out to ensure the accuracy of the absorptance estimates by using *Ulva* blades with a wide range of chlorophyll contents. Calibration functions were:

$$A_{678\ \text{opal-glass}} = -0{\cdot}039 + 1{\cdot}036\, A_{678\ \text{spectroradiometer}}$$

$$r = 0{\cdot}98$$

$$A_{400\text{–}700(\text{wide band})} = 0{\cdot}007 + 0{\cdot}657\, A_{678\ \text{spectroradiometer}}$$

$$r = 0{\cdot}99$$

These linear functions were used to estimate wide-band (400–700 nm) absorptance values.

Cell size was measured with a Leitz LABORLUX S inverted microscope.

Growth was assumed to be exponential with time. Thus, growth rates (d^{-1}) were calculated from linear regression of ln(surface area, fresh mass) on time. For tissue C and N, specific growth rates were determined between the final (6 days) and initial values.

The photon yield for net growth was determined according to:

$$\phi_n = \frac{\Delta A}{B \cdot C \cdot D \cdot E} \qquad (1)$$

where ΔA is the net variation of C per disc during the experiment ($\mu mol\ C\ disc^{-1}\, s^{-1}$), B is the PFR of the cultures ($\mu mol\, m^{-2}\, s^{-1}$), C is the arithmetic mean surface of the discs during the experiment (m^2), D is the mean absorptance of the discs during the experiment and E is the light period (0·5, at 12 h L : D cycle).

The potential μ based on photosynthetic performance at each growth PFR was determined, and compared with the observed μ on C basis, according to:

$$\text{Potential } \mu = \text{diurnal } \mu_C - \text{nocturnal losses}$$

$$= \left(\frac{A \cdot B}{C \cdot D \cdot E}\right) - \tfrac{1}{2}F \qquad (2)$$

where A is the net photosynthesis rate at each growth-PFR ($\mu mol\ O_2\, m^{-2}\, s^{-1}$), derived from the photosynthetic parameters (Jassby & Platt, 1976), B is a conversion factor ($12\ h\ d^{-1} \times 3600\ s\ h^{-1}$), C is the photosynthetic quotient

Table 1. Linear regression for fitting theoretical photosynthetic parameters (predicted from three P–PFR models) to experimental values

Photosynthetic parameters	Models		
	Jassby & Platt (1976)	Bannister (1979)	Edwards & Walker (1983)
P_{max} (μmol O_2 m^{-2} s^{-1})	$y = -0{\cdot}01 + 1{\cdot}01x$ ($r^2 = 0{\cdot}99$) (1·00–1·02)[a]	$y = -0{\cdot}02 + 1{\cdot}04x$ ($r^2 = 0{\cdot}99$) (1·03–1·06)	$y = -0{\cdot}06 + 1{\cdot}17x$ ($r^2 = 0{\cdot}99$) (1·10–1·23)
α (mmol O_2 mol photons^{-1})	$y = -0{\cdot}05 + 1{\cdot}04x$ ($r^2 = 0{\cdot}98$) (0·94–1·14)[a]	$y = 0{\cdot}06 + 1{\cdot}13x$ ($r^2 = 0{\cdot}97$) (1·02–1·25)	–
LCP (μmol photons m^{-2} s^{-1})	–	–	$y = 2{\cdot}3 + 0{\cdot}68x$ ($r^2 = 0{\cdot}90$) (0·54–0·83)

Confidence limits (95%) shown in parentheses ($n = 14$).
[a] Slope not significantly different from 1.

(1·12 μmol O_2 μmol^{-1} C), D is the conversion factor (10^6 μmol C mol^{-1} C), E is the thallus-specific C (mol C m^{-2}) and F is the maintenance respiratory rate (0·017 d^{-1} for *U. curvata* and 0·023 d^{-1} for *U. rotundata*).

Oxygen exchange rates

Photosynthesis–PFR (P–PFR) curves were determined at the beginning (initial conditions) and the end of the experiments. The O_2 exchange of 10 mm diameter discs was measured at 12 PFRs from 0 to 1750 μmol m^{-2} s^{-1}, with a Hansatech polarographic O_2 electrode (Walker, 1989). *Ulva* discs were held perpendicular to the light field (high-intensity light source LS2, Hansatech) with a nylon hook through the capillary of the plunger of the 3 cm^3 reaction vessel. The O_2 electrode system was calibrated daily with N_2-purged seawater and O_2-saturated seawater. Measurements started at 20% O_2 saturation by bubbling with N_2. After 5 min of dark O_2 uptake discs were exposed to each PFR for 4 min. During the P–PFR measurements, O_2 concentration never exceeded 90% saturation. The temperature was maintained at the value (15 ± 0·05 °C) used for the growing cultures. Two replicates were measured per light treatment. Absorptance measurements and chlorophyll determinations were carried out after each P–PFR curve using the same discs.

Photosynthetic parameters, predicted from some theoretical P–PFR models that do not include photoinhibition (Jassby & Platt, 1976; Bannister, 1979 ($c = 2$); Edwards & Walker, 1983) were compared with the experimental values to obtain increased accuracy (Table 1). None of the P–PFR measurements showed photoinhibition (curves not shown). Experimental P_{max} values, estimated as the mean of four or five measurements at saturating PFRs, fitted better to the values predicted by Jassby & Platt (1976). P_{max} derived from Bannister (1979) and, most especially, from Edwards & Walker (1983) overestimated experimental mean values. Observed values of the initial slope of the P–PFR curves (α), were also computed by linear regression using the lowest four or five PFRs. They also fitted better to those predicted by Jassby & Platt (1976). For the light compensation points (LCP), observed LCP($LCP = R_d/\alpha$) deviated markedly from those predicted by the model of Edwards & Walker (1983), particularly at higher values. Hence, observed LCP values were used. Photon yield for O_2 evolution (ϕ) was calculated as α/absorptance.

Statistics

Experimental photosynthetic parameters were compared with the values predicted by theoretical P–PFR models by simple linear regression. The 95% confidence interval for the slope was set to test whether the line fitted included the predicted slope equal to unity. The responses of the variables tested at several PFRs were compared for significant differences between the two *Ulva* species. These comparisons were carried out using a simple linear correlation (Zar, 1984). The equality of the linear relationship between R_d and μ_c found in the two *Ulva* species was tested by a test of equality of slopes (Zar, 1984). Statistical significance was set at $p < 0{\cdot}05$.

Results

Growth

Specific growth rates based on fresh mass (μ_w), area (μ_A) or tissue C (μ_C) were linear with incident light (note the log scale for growth-PFR) and not saturated in the range of incident light applied (Fig. 1). Both species reached a growth rate close to 0·2 d^{-1} at 200 μmol m^{-2} s^{-1}. However, in darkness, thallus expansion occurred (Fig. 1*B*), despite the reduction in mass (Fig. 1*A*) and tissue carbon (Fig. 1*C*), (negative μ_w and μ_C values). At very low light levels (2 μmol m^{-2} s^{-1}), thallus expansion in *U. rotundata* took place without parallel growth in mass or C content, whereas *U. curvata* grew in area and C content but did not increase in mass. Cell size was not significantly affected by growth-PFR (data not shown), and cells

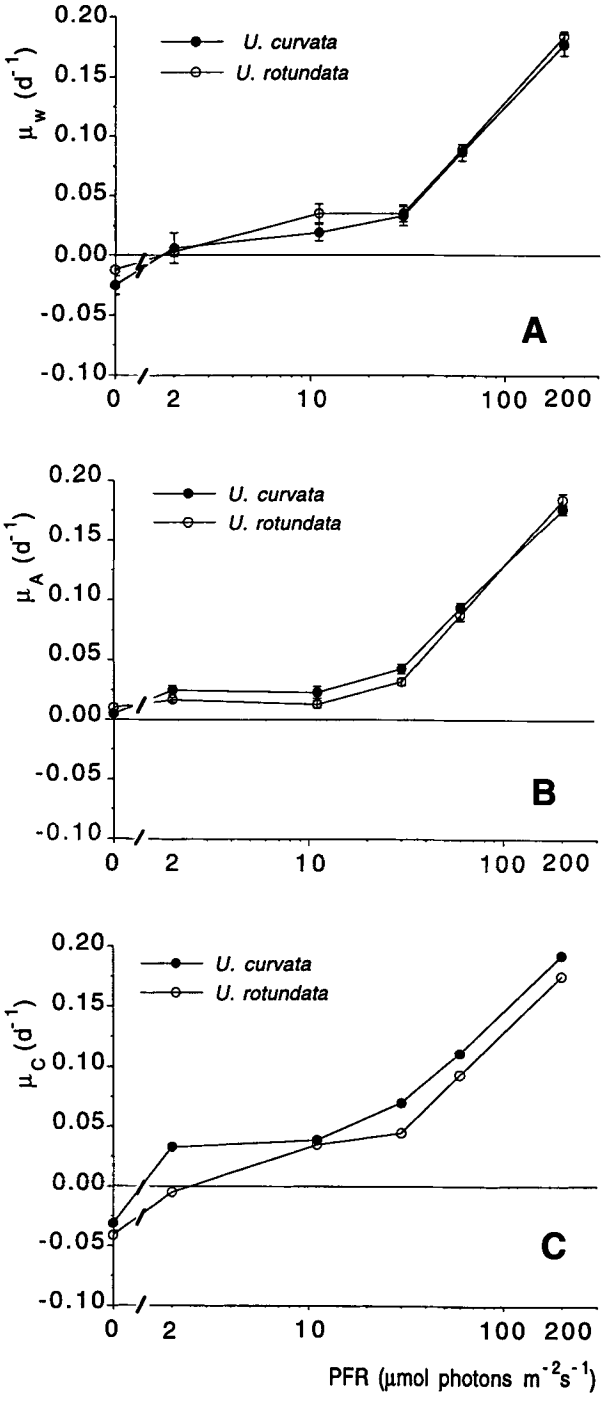

Fig. 1. Specific growth rates calculated on the basis of fresh mass (μ_w; *A*), surface area (μ_A; *B*) and carbon (μ_C; *C*) for *Ulva curvata* and *U. rotundata* discs incubated at several PFRs for 6 days at 15 °C in a 12 : 12 h LD cycle ($n = 10 \pm$ SE).

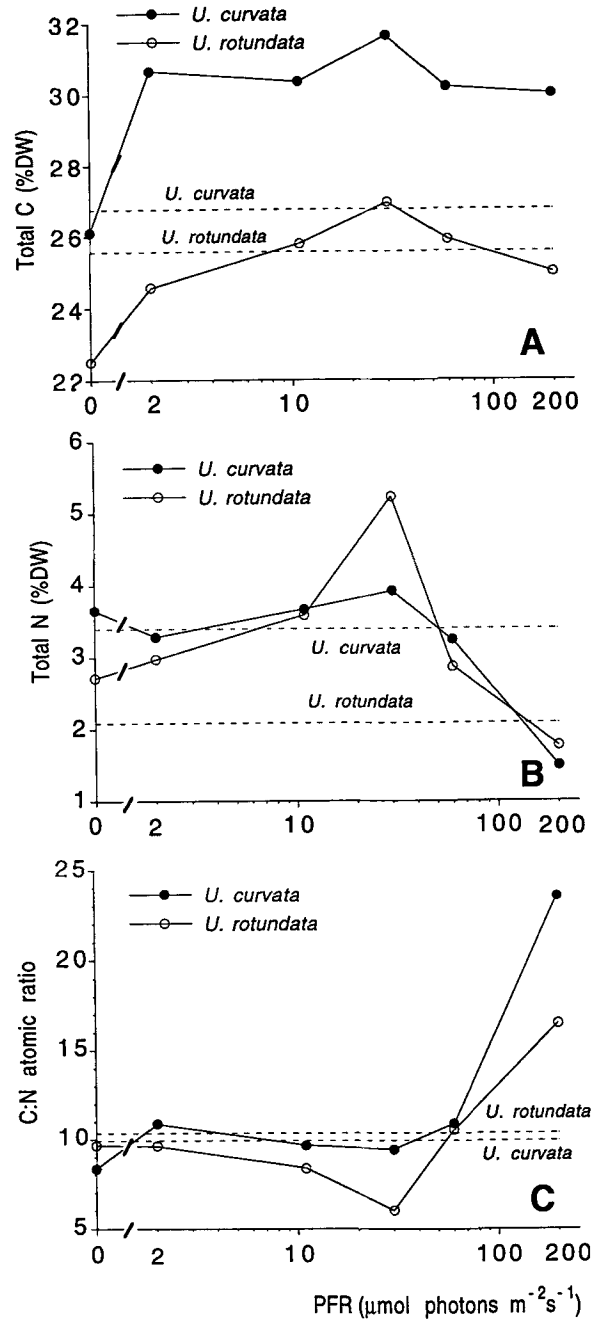

Fig. 2. Total carbon (*A*), nitrogen (*B*) and C : N atomic ratio (*C*) for *Ulva curvata* and *U. rotundata* discs incubated at several PFRs for 6 days at 15 °C in a 12 : 12 h LD cycle. Horizontal broken lines are initial values for each species before incubation.

were always smaller in *U. curvata* than in *U. rotundata* (Table 2).

Elemental composition

Tissue C concentration was always higher in *U. curvata* than in *U. rotundata* (Fig. 2*A*). In *U. curvata* tissue C concentration increased by a similar extent relative to the initial value at all PFRs except in darkness. In *U. rotundata*, tissue C followed a saturation curve, with lower values than the initial ones in darkness and 2 μmol m^{-2} s^{-1}. Pre-experimental samples of *U. curvata* also had a higher tissue N concentration than *U. rotundata* (Fig. 2*B*). *U. rotundata* had a higher N content at moderate light levels. For both species there was a marked decrease in tissue N at higher PFR levels, with values below the initial content. Initial C : N atomic ratios were similar in the two species, and remained around initial values after 6 days of culture, with the exception of the 200 μmol m^{-2} s^{-1} treatment, where the C : N ratio increased sharply, as a result of the drop in N on a dry mass basis (Fig. 2*C*).

Table 2. Biometry of *Ulva curvata* and *U. rotundata* cells (SE, $n = 120$)

	U. curvata	*U. rotundata*	Ratio *U. rotundata*/*U. curvata*
Cell length (μm)	11·1 (0·16)	18·8 (0·30)	
Cell width (μm)	7·2 (0·14)	12·4 (0·26)	
Cell height (μm)	22·9	23·6	
Cell surface (μm^2)	477·0 (9·0)	958·0 (21·0)	2·01
Cell volume (μm^3)	994·0 (28·0)	2851·0 (92·0)	2·87
Plasmalemma/cell volume (%)[a]	0·48	0·34	0·71

[a] Membrane 10 nm thick.

We calculated the N-specific growth rate, to combine the effect of N assimilation and growth. Growth rates based on tissue N (μ_N) were light-saturated, with a slight decrease in high PFRs. *U. rotundata* exhibited higher rates (Fig. 3), with positive values even at low light levels. By contrast with μ_N, the net NH_4^+ incorporation was not affected by growth-PFR (Fig. 3). *U. curvata* exhibited higher net NH_4^+ uptake rates than *U. rotundata*.

Photosynthetic pigments and absorptance

Initially the *U. curvata* thalli had higher concentrations of photosynthetic pigments and a higher absorptance than *U. rotundata* (Fig. 4). Pigment concentration responded similarly to PFR in both species. Lower values were recorded at very low light (2 μmol m^{-2} s^{-1} and darkness) and with high light exposure (200 μmol m^{-2} s^{-1}); maximal values occurred at intermediate PFRs, with no significant differences between the species ($p > 0·1$). At very low light levels, *U. curvata* had higher chlorophyll contents and absorptances than *U. rotundata*.

To determine whether the sharp decrease in chlorophyll at high PFR was promoted by a reduction in the net synthesis rate and/or by a dilution effect due to growth, total chlorophyll content per disc is plotted in Fig. 4D.

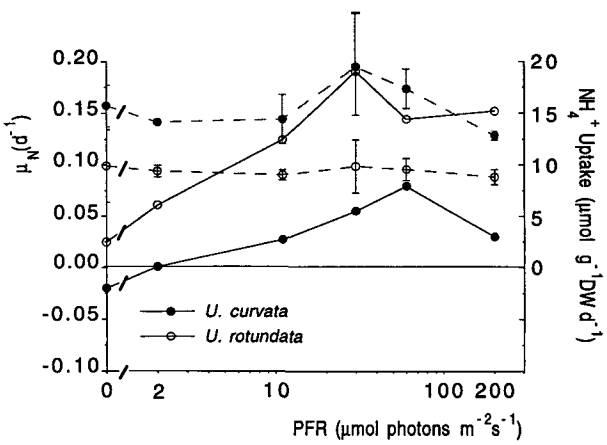

Fig. 3. Nitrogen-specific growth rate (μ_N, continuous line) and NH_4^+ uptake rate (broken line) for *Ulva curvata* and *U. rotundata* discs incubated at several PFRs for 6 days at 15 °C in a 12:12 h LD cycle ($n = 3 \pm$ SE).

At 200 μmol m^{-2} s^{-1}, the net chlorophyll variation per disc was higher than at very low light levels and positive with respect to initial conditions. In comparison with the net synthesis rate at 60 μmol m^{-2} s^{-1}, the dilution effect accounted for *c.* 30% of the variation, whereas the remaining 70% was due to a diminished net synthesis rate.

Photosynthesis

Both species displayed a similar variation of the light-saturated photosynthesis rate (P_{max}) with respect to growth-PFR. *U. curvata* showed higher values than *U. rotundata*. However, this variation depended on the way P_{max} was expressed. Thus, an optimum at intermediate PFRs was observed when P_{max} was expressed on area basis (Fig. 5*A*). When P_{max} was expressed on a tissue N basis, a linear phase was followed by a plateau at PFRs higher than 11 μmol m^{-2} s^{-1} (Fig. 5*B*). P_{max} scaled to chlorophyll displayed a complex pattern, with maxima located at 11 μmol m^{-2} s^{-1} and at 200 μmol m^{-2} s^{-1} (Fig. 5*C*). For both species, P_{max} was not enhanced with respect to the initial rates after culturing algae in darkness and at 2 μmol m^{-2} s^{-1}.

Initial values of photosynthetic efficiency (α) and photon yield for photosynthesis (ϕ) were similar for both species (Fig. 6). Growth-PFR affected these parameters differently. While *U. rotundata* reached an optimum at intermediate PFRs for both parameters, *U. curvata* showed maxima at low and high PFRs. In this species, the increase in ϕ at high PFRs was caused by a reduction in absorptance at this PFR, without a parallel decrease in α.

Pre-experimental material of *U. curvata* had a higher light compensation point for photosynthesis (LCP) than *U. rotundata* (Fig. 7). Similar figures were found after 6 days in darkness. LCP was low after culturing at low light levels, and increased with growth-PFR in both species. Dark respiration rates (R_d) were positively correlated with growth-PFR and growth rate (Fig. 8), and no significant interspecific differences ($p > 0·2$) were found.

The net growth photon yield on a C basis was negatively correlated with growth-PFR (Fig. 9). However, at the lowest PFR tested, the two *Ulva* species displayed opposite trends. Whereas *U. rotundata* showed a negative

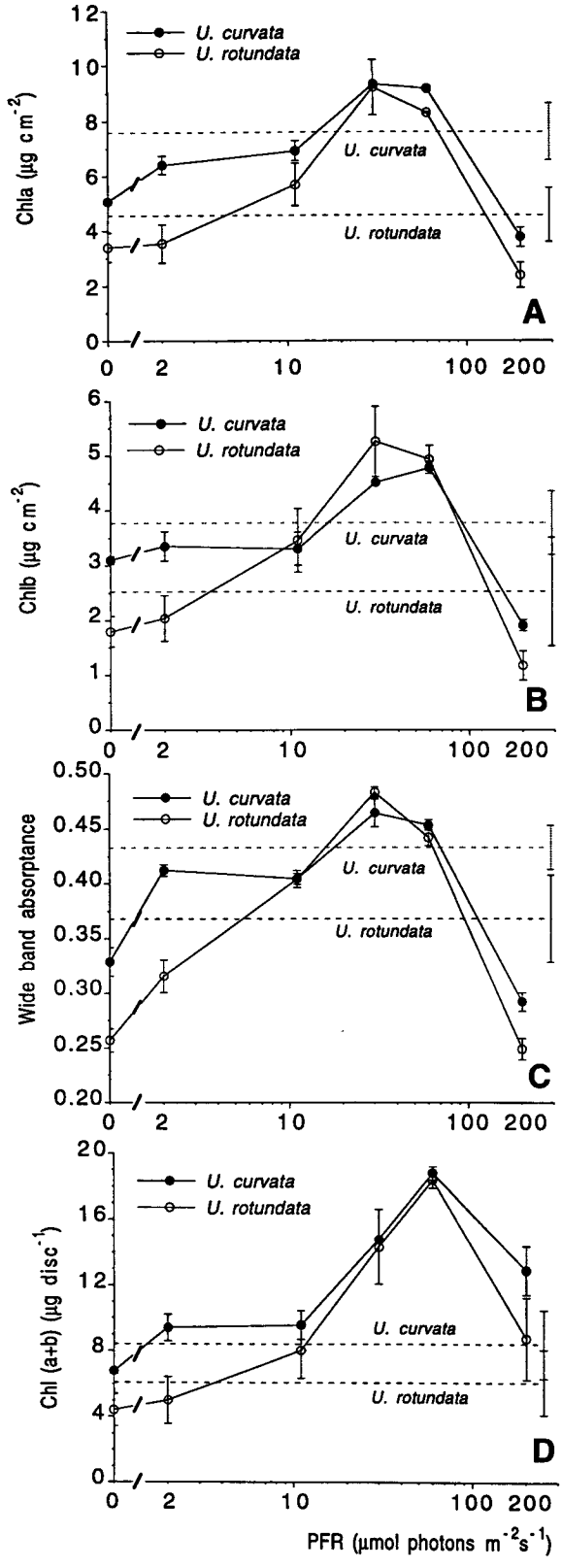

Fig. 4. Chlorophyll *a* (*A*), chlorophyll *b* (*B*), wide-band absorptance (400–700 nm) (*C*) and total chlorophyll per disc (*D*) for *Ulva curvata* and *U. rotundata* incubated at several PFRs for 6 days at 15 °C in a 12 : 12 h LD cycle. Horizontal broken lines are initial values for each species before incubation ($n = 3 \pm$ SE).

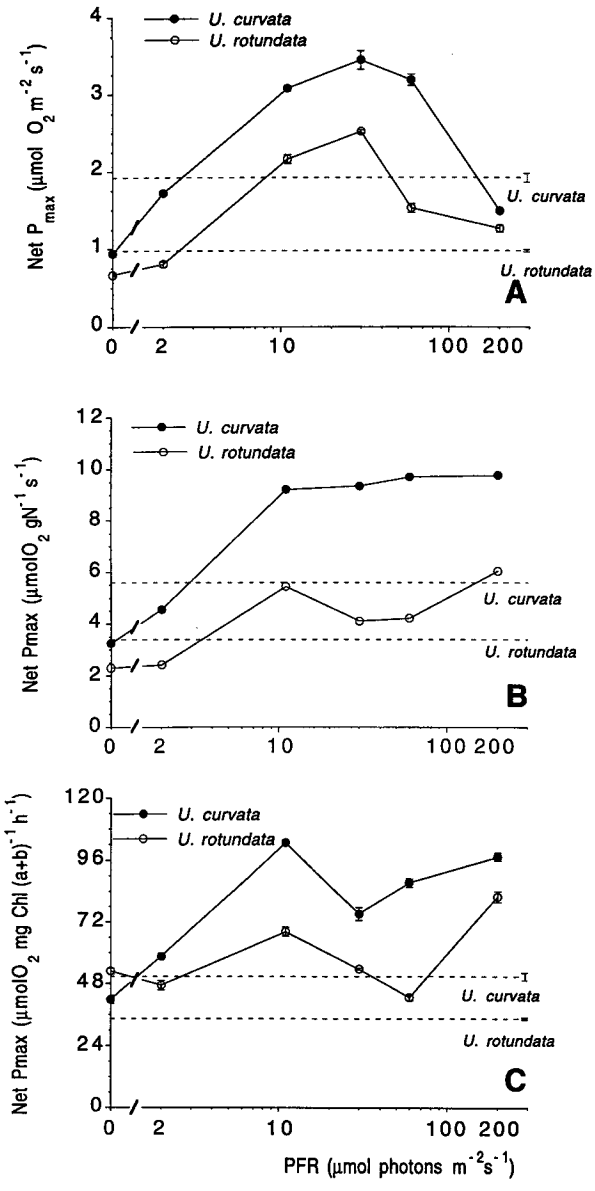

Fig. 5. Maximum net photosynthesis rates calculated on the basis of (*A*), nitrogen content (*B*) and total chlorophyll (*C*) for *Ulva curvata* and *U. rotundata* incubated at several PFRs for 6 days at 15 °C in a 12 : 12 h LD cycle. Horizontal broken lines indicate initial values for each species before incubation ($n = 2 \pm$ SE).

value (net loss of C), *U. curvata* reached an efficiency much higher than the theoretical limit (110 mmol C mol^{-1} absorbed photons, based on a photosynthetic quotient (PQ) of 1·12: Raven, (1984). Given the difficulty of estimating the incident PFR at very low light levels, especially when thalli are cultured in bubbled flasks, we must acknowledge that the actual PFR for this treatment may be somewhat higher than 2 μmol m^{-2} s^{-1} for both species (a PFR about 4–5 μmol m^{-2} s^{-1} would explain the inconsistency of our high estimate).

The potential μ values based on net photosynthesis rate measured at each growth PFR were compared with the observed μ_C (Fig. 10). These calculations were carried out using the photosynthetic parameters and thallus-specific C (*c.* 250 mmol C m^{-2}) after 6 days of culture at

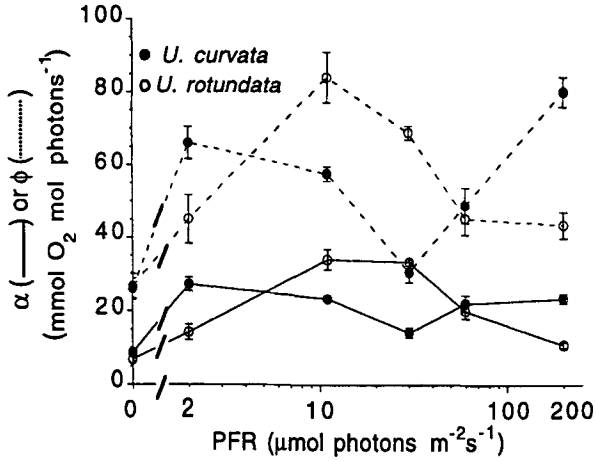

Fig. 6. Photosynthetic efficiency on an incident light basis (α) and photon yield (ϕ) for *Ulva curvata* and *U. rotundata* incubated at several PFRs for 6 days at 15 °C in a 12 : 12 h LD cycle. Initial values of α and ϕ were similar in the two species (9 mmol O_2 (mol incident photon)$^{-1}$ and 22 mmol O_2 (mol absorbed photon)$^{-1}$, respectively) ($n = 2 \pm$ SE).

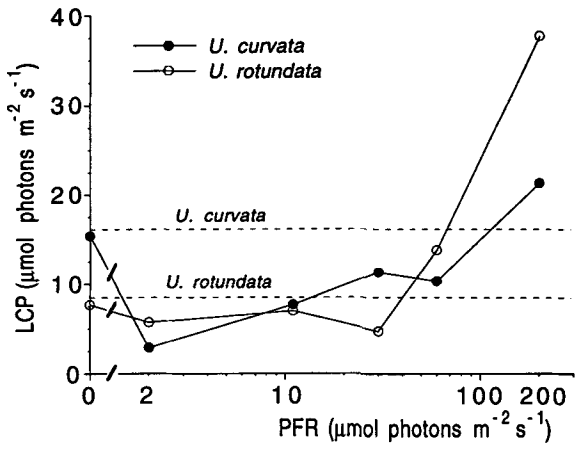

Fig. 7. Light compensation point for photosynthesis (LCP) for *Ulva curvata* and *U. rotundata* incubated at several PFRs for 6 days at 15 °C in a 12 : 12 h LD cycle. Horizontal broken lines indicate initial values for each species before incubation ($n = 2 \pm$ SE).

each growth PFR, and a PQ of 1·12. Respiration in the dark period (12 h) has been considered as maintenance respiration, which must be one order of magnitude lower than that in the light (Geider *et al.*, 1985). The estimates of potential μ_C correlate fairly well with the observed μ_C values, with only small deviations from the 1 : 1 slope.

Discussion

Growth

Growth based on mass, area or carbon content (μ_w, μ_A or μ_C) of both *Ulva* species was not light-saturated over the range of PFRs applied (up to 200 μmol m^{-2} s^{-1}). Higher light-saturation points for growth have been reported for these species: about 750 μmol m^{-2} s^{-1} for *U. rotundata* (Henley, 1992) and 500 μmol m^{-2} s^{-1} for *U. curvata* (Duke *et al.*, 1986). Growth rates (up to 200 μmol photons m^{-2} s^{-1}) were similar for both species, and comparable to those observed for the same or different *Ulva* species (Duke *et al.*, 1986, 1989; Ramus & Venable, 1987; Henley & Ramus, 1989*a*, *b*; Sand-Jenson, 1988*b*; Henley *et al.*, 1991*a*; Henley, 1992; Geertz-Hansen & Sand-Jensen, 1992). μ_w, μ_A and μ_C were equivalent measures of growth apart from values obtained at the lowest PFR applied (darkness and 2 μmol m^{-2} s^{-1}). At these very low light levels, *U. rotundata* had negative or no growth on a C or mass basis, although not on an area basis. Thus, thallus expansion took place by diluting internal C and fresh mass, that is, thallus-specific C (mol C m^{-2}) and biomass per area (g FW m^{-2}) decreased. For *U. curvata*,

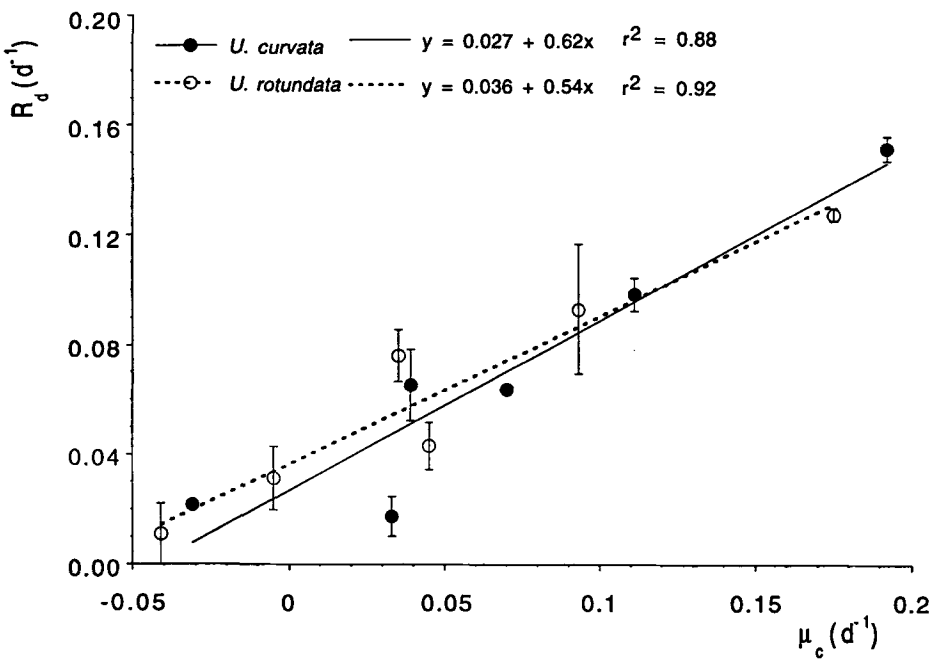

Fig. 8. Relationship between dark respiration rate (R_d) and carbon-specific growth rate (μ_C) for *Ulva curvata* and *U. rotundata*. R_d values were obtained from P–PFR curves.

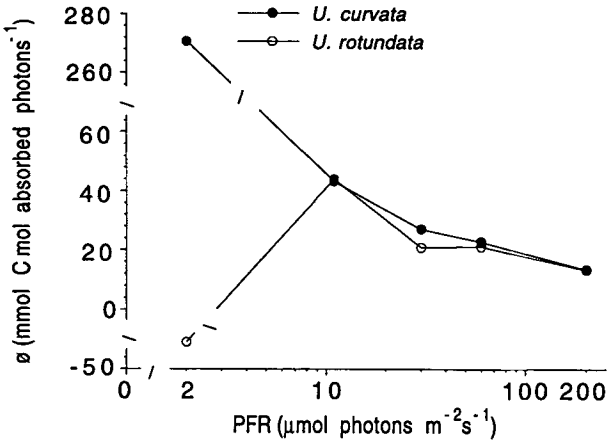

Fig. 9. Photon yield for net carbon growth on an absorbed light basis for *Ulva curvata* and *U. rotundata* incubated at several PFRs for 6 days at 15 °C in a 12 : 12 h LD cycle. Note that the value for *U. curvata* at 2 μmol m^{-2} s^{-1} is above the theoretical limit (see text for further explanation).

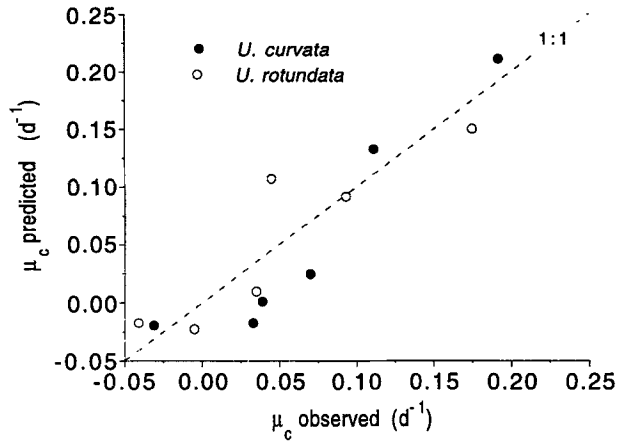

Fig. 10. Relationship between potential μ_C predicted from photosynthesis measurements using Eq. 2 and μ_C measured for *Ulva curvata* and *U. rotundata* incubated at several PFRs for 6 days at 15 °C in a 12 : 12 h LD cycle. Dotted line represents the equality between the two estimates (slope 1 : 1; see text for further explanation).

growth was maintained on an area or C basis but not on a mass basis. These observations are consistent with the response of *Ulva lactuca* photoacclimated at very low PFRs (Sand-Jensen, 1988*b*), and of *U. rotundata* after a large decrease in irradiance (Henley, 1990). Given that there was no significant change in cell number per unit area, thallus expansion must be supported by cell division (Henley, 1990).

Photosynthetic pigments

Chlorophyll (*a* and *b*) and absorptance exhibited bell-shaped response curves with respect to log PFR. Thus, there was an optimum at intermediate PFRs for chlorophyll synthesis. The lack of pigment synthesis at very low light levels, around or below the LCP, has been observed in other *Ulva* species (Sand-Jensen, 1988*b*), green microalgae (Thielmann *et al.*, 1991) and red macroalgae (Vergara & Niell, 1995; Kübler & Raven, 1995). As a result of photosynthetic limitation, protein synthesis must be restricted (Vergara & Niell, 1995). In our study, although NH_4^+ uptake was not affected by light limitation, there was an inefficient assimilation of inorganic N into biomass (low values of μ_N at low light levels and darkness), which may limit chlorophyll and overall protein and membrane synthesis at extremely low PFRs. The data obtained on NH_4^+ uptake represent the net balance over a 2 day period, and are not indicative of a surge uptake process. Rather, they represent the result of internally controlled uptake (Pedersen, 1994). Membrane transport appears to be inhibited when NH_4^+ accumulates in the tissue, as large internal NH_4^+ pools are difficult to maintain in *Ulva* (Fujita *et al.*, 1988). Added to that, some NH_4^+ efflux may occur. NH_4^+ incorporation was not limited by external N supply (mean NH_4^+ concentration remaining in seawater every 2 days was 13·7 ± 3·7 μM). Also, NH_4^+ taken up was sufficient to balance the N accumulated in the tissue (data not shown).

At light levels above the saturation point for photosynthesis, the sharp decrease in chlorophyll was promoted by a reduced net synthetic rate and by a dilution effect. In *Dunaliella tertiolecta* (Woods Hole clone DUN) each of these effects is equally responsible for the chlorophyll variation (Falkowski, 1984). In this microalga, the redox status of the plastoquinone pool is thought to act as a 'light sensor', regulating *cab* gene transcription (Escoubas *et al.*, 1995).

Changes in absorptance paralleled variations in chlorophyll, indicating that pigment concentration is the main factor responsible for the optical properties of thin *Ulva* thalli. The Chl *a*/Chl *b* ratio was not affected by PFR (data not shown). Henley & Ramus (1989*b*) observed the increase in this ratio, but at lower chlorophyll contents than those observed in the current study.

Photosynthesis

The photosynthetic acclimation of *Ulva* can be considered as adaptive, at least at low and intermediate PFR levels (*sensu* Givnish, 1988). Algae acclimated to low light levels improve the net photosynthesis rate at low light, in comparison with high-light-acclimated algae (Fig. 11). The adjustment of R_d makes the net photosynthetic rate at 2 and 11 μmol m^{-2} s^{-1} inversely related to growth-PFR. In contrast, low-light-acclimated algae show a reduced photosynthetic rate under high PFR in comparison with algae grown under higher PFRs (parabolic shape at light saturation, 200 μmol m^{-2} s^{-1}, Fig. 11). High-light-acclimated algae exhibit lower photosynthesis rates at light saturation (200 μmol m^{-2} s^{-1}) than those acclimated to intermediate light levels, with a higher chlorophyll and N content. However, the photosynthesis rates achieved at 200 μmol m^{-2} s^{-1} are sufficient to maintain a higher growth rate than in algae cultured under intermediate PFRs (Fig. 10).

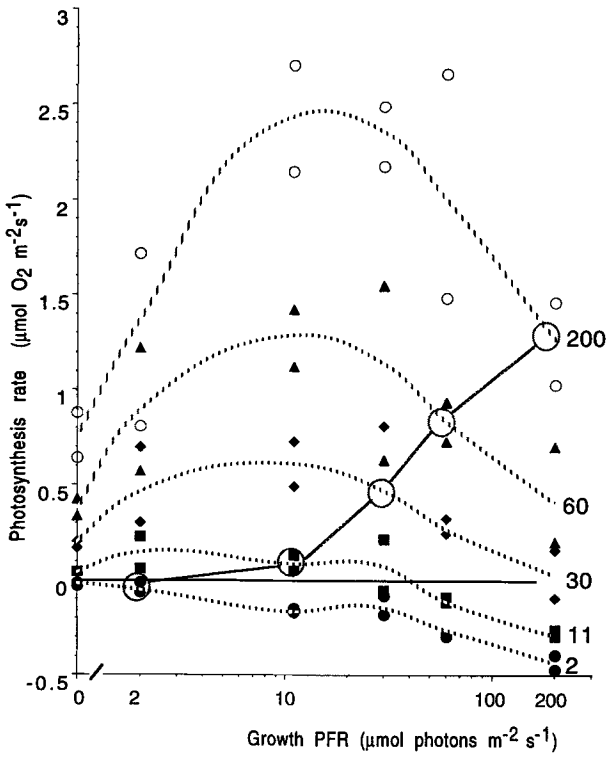

Fig. 11. Dotted lines, fitted by eye, show the net photosynthesis rate (derived from Jassby & Platt model) as a function of growth-PFR for the two *Ulva* species at 2 (●), 11 (■), 30 (◆), 60 (▲) and 200 (○) μmol m^{-2} s^{-1}. Circles connected by continuous lines indicate the photosynthetic rates for PFR equal to the growth PFR.

The P_{max} decreased on an area basis for high-light-grown algae, as had been reported by Sand-Jensen (1988*b*) for *U. lactuca* cultured in N-sufficient conditions. This response also occurs in *U. rotundata* under N limitation, but not in N-sufficient cultures (Henley *et al.*, 1991*b*). Our results suggest that there was incipient N limitation under high light conditions, i.e. a drop in tissue N for both species. This limitation was not due to external NH_4^+ depletion, since external NH_4^+ was not totally exhausted (about 50% of the initial concentration remained in the flasks at the end of 2 days). Instead assimilation of internal NH_4^+ into soluble N compounds and proteins must be the rate-limiting step, in comparison with an unsaturated growth rate on a C basis. Actually, P_{max} did not decrease on a N basis for high-light-grown algae, suggesting a limitation by C fixation due to N deficiency (assuming a correlation between Rubisco and internal N content). P_{max} is independent of growth-PFR when scaled to protein, but inversely related to growth-PFR on a dry mass basis (Foy & Gibson, 1982), given the correlation between Rubisco and total cell protein (Falkowski *et al.*, 1989).

At light limitation, P_{max} decreased not only on an area basis but also on a N basis. This may indicate that electron transport chain elements (lower catalytic membrane concentration and/or electron transport chain density) limit the light-saturated photosynthesis rate. The chlorophyll-specific light-saturated photosynthesis rate tends to increase with growth-PFR in microalgae (reviewed by Geider & Osborne, 1992). In the present study, a more complex pattern was observed. However, the use of very low light levels that are limiting for chlorophyll synthesis will tend to result in a higher P_{max} when scaled to this variable.

Photon yields for photosynthesis (ϕ) decreased with increasing growth-PFR (Dubinsky *et al.*, 1986). This pattern has been explained, in part, as a consequence of an increase in cyclic photophosphorylation with respect to non-cyclic photophosphorylation under high light (Herzig & Dubinsky, 1993). In *U. rotundata* ϕ decreased as algae acclimated to high light, but not in *U. curvata*, where ϕ was even higher than at lower light levels.

Dark respiration and growth

Thallus-specific C and dark respiration are key factors for seaweed growth in low light climates, since they affect the light compensation point for growth (Markager & Sand-Jensen, 1994). Both species have low thallus-specific C values (about 0·25 mol C m^{-2}) which are typical of foliose species. Light compensation points for growth on a mass basis were similar for the two species (*c.* 2 μmol m^{-2} s^{-1}), and slightly lower for *U. curvata* (1 μmol m^{-2} s^{-1}) than for *U. rotundata* (2·5 μmol m^{-2} s^{-1}) on a C basis. These values are close to those reported by Sand-Jensen (1988*b*) and Markager & Sand-Jensen (1992) (0·33–2·5 μmol m^{-2} s^{-1}) for *U. lactuca* acclimated to low PFRs. Dark respiration rates were positively related to growth-PFR, indicating a tight coupling between growth and metabolic activity.

Maintenance respiratory rates (R_m) obtained after 6 days of culture in darkness were 0·031 d^{-1} for *U. curvata* and 0·041 d^{-1} for *U. rotundata* (data derived from the μ_C–PFR relationship, Fig. 1C). These values for R_m were higher than those estimated according to the model proposed by Markager & Sand-Jensen (1994). (R_m = 0·017 d^{-1} for *U. curvata* and 0·023 d^{-1} for *U. rotundata*.) However, organic C losses are included in the former but not in the latter estimation of R_m. R_0, the respiration rate at $\mu_C = 0$, was 0·027 d^{-1} and 0·036 d^{-1}, respectively (y-axis intercept at $\mu_C = 0$, Fig. 8). The slope (k) of the R_d versus net growth rate relationships (Fig. 8) can be transformed into K, the slope of R_d versus gross growth rate. The fraction $(1 - K)$ represents the proportion of the gross C fixation, in excess of R_m, converted into new biomass (Markager & Sand-Jensen, 1994). No significant differences ($p > 0·2$) were found between *U. curvata* ($1 - K = 0·62$) and *U. rotundata* ($1 - K = 0·65$), in agreement with a similar μ_C for both species. Similar positive trends have been reported in *U. lactuca* and other seaweeds after prolonged incubation periods ($1 - K = 0·83$ for *U. lactuca*: Markager & Sand-Jensen, 1994). Apart from interspecific differences, the higher temperature in our experiments (15 °C vs 7 °C), and the effect of short incubation times (minutes vs

hours), resulted in lower values of $(1 - K)$ for our species.

Photon yields for net growth declined with growth-PFR in both species (Fig. 9). A similar trend has been reported by Markager (1993). *U. curvata* reached the highest values at 2 μmol protons $m^{-2} s^{-1}$, and they were much greater than the theoretical limit. As was argued previously, the actual incident light may be slightly higher for this treatment. In contrast, *U. rotundata* showed a negative value, because of a decrease in thallus-specific C. Assuming a photosynthetic quotient (PQ) of 1·12 (nitrogen source was NH_4^+), the theoretical maximum efficiency of gross C fixation is about 110 mmol C (mol absorbed photon)$^{-1}$. Given the value of K calculated above, the theoretical maximum photon yield for net growth should be about 70 mmol C mol^{-1} absorbed photon, which is slightly higher than the value recorded in this study.

The estimates of potential μ_C correlated fairly well with observed μ_C values, with small deviations from the 1 : 1 slope (Fig. 10). It might be expected that none of the points would fall below the 1 : 1 line, as it should be impossible to maintain a higher growth rate than that allowed by the photosynthetic performance. In any case, the data points should be on or above the 1 : 1 line. The slight deviation of some of our observed values from the predicted model may be explained by several reasons. First, we have chosen the end values of photosynthetic parameters and thallus-specific C, but these estimates change through the course of the experiment (6 days) at each PFR. Secondly, the PQ of 1·12 is necessarily approximate. Third, photosynthesis measurements and growth were carried out under different light sources (Björkman lamp vs fluorescent lamps). That could result in small differences in the light field, which would be especially important at subsaturating PFRs. Finally, as Falkowski *et al.* (1985) pointed out, dark respiration rate (our model includes the net photosynthesis rate) can be higher than the respiration rate in the light, and small errors in short-term photosynthetic measurements of oxygen can be amplified when extrapolated to daily rates. These authors also found that the predicted μ values were lower than the actual μ data for three species of marine phytoplankton cultured under different irradiances.

Is there a cell size effect?

Both species grew at similar rates (Fig. 1), and with similar photon yields for growth over most of the range of light levels used (Fig. 9), apart from the very low light level. However, the light-saturated rate of photosynthesis was always lower for *U. rotundata*, regardless of the basis (area, N or chlorophyll) for expressing the photosynthetic rate. *U. curvata* has smaller cells than *U. rotundata* (Table 2). Although incompletely scaleable, the proportion of volume occupied by membranes in a cell is inversely related to cell size (Raven, 1986; Table 2). In these experiments the cellular volume was 2·8 times lower for *U. curvata*, implying a higher energetic cost of maintaining a given growth rate for such a small cell, as more membrane (with a higher C content than the mean cell value) would need to be synthesized (Raven, 1986). Actually, the C concentration on a volume basis (dry mass) was always higher for *U. curvata*. Smaller cells would compensate for the extra cost with a higher photosynthetic capacity. Despite *U. curvata* having higher photosynthesis rates than *U. rotundata*, the predicted μ values fitted well with observed μ data, as previously shown (Fig. 10).

An improved photon absorption in *U. curvata* grown under very low light levels (2 μmol $m^{-2} s^{-1}$) must be due to a higher chlorophyll concentration compared with *U. rotundata*, as well as a reduced package effect. It must account for the high values of α and ϕ at very low PFRs (Fig. 6), contributing to the positive photon yield for growth in this species.

There were also differences with respect to N utilization. Whereas *U. curvata* showed higher rates of net NH_4^+ incorporation, N assimilation based on biomass (μ_N) was more efficiently achieved in *U. rotundata*. Whether differences in cell size (assuming nutrient transport and leakage processes are a function of membrane area : volume ratio) are a key factor in the incorporation and use of N by these species remains to be examined.

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