

# Consequences of light limitation for carbon acquisition in three rhodophytes

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**ABSTRACT:** We investigated the consequences of growth at limiting photon flux density (PFD) for the uptake of inorganic carbon by 2 species of red seaweed which are able to use  $\text{HCO}_3^-$  for photosynthesis [*Palmaria palmata* (Huds.) Lamour and *Laurencia pinnatifida* (L.) Kuntze] and one which is dependent on diffusive uptake of bulk  $\text{CO}_2$  [*Lomentaria articulata* (Huds.) Lyngb.] We attempted to induce energetic limitation of plants and/or reduce their demand for inorganic carbon by growing them at a low PFD and comparing their carbon use characteristics with those of plants grown at high PFD. There were significant increases in the carbon isotope discrimination (more negative delta values relative to the PDB standard) when the 3 species were grown at 25 relative to 75  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . In the 2 species which are able to use bicarbonate, the decreased  $\delta^{13}\text{C}$  was associated with lower initial slopes of inorganic carbon uptake curves. Taken together, the changes in  $\delta^{13}\text{C}$  and dissolved organic carbon uptake indicated that light-limited *P. palmata* and *L. pinnatifida* were not using low concentrations of inorganic carbon as efficiently as were plants grown at high PFD. This hypothesis was further supported by evidence that light limitation also caused a reduction in C:N ratio and in the % dry matter made up of carbon in those species. In contrast, growth of *L. articulata*, which is dependent on diffusive uptake of  $\text{CO}_2$  for photosynthesis, was inhibited by the higher PFD and there were no significant effects of growth PFD on inorganic carbon uptake efficiency, C:N ratio or % C in this species.

**KEY WORDS:** *Laurencia pinnatifida* · *Lomentaria articulata* · *Palmaria palmata* ·  $\text{CO}_2$  ·  $\text{HCO}_3^-$  · Light limitation · Photosynthesis · Stable carbon isotope ratios

## INTRODUCTION

Understanding the uptake of dissolved inorganic carbon (DIC) by marine macrophytes is more difficult than in the case of terrestrial plants, which have their leaves in the air. All gas exchanges between the media and submerged plants are complicated by the slower diffusion in water, which is not completely offset by the thinner diffusion boundary layers in water under comparable fluid-flow/plant-size combinations, chemical equilibria of inorganic carbon species in large volumes of external solution and the possible biological interaction with these processes (e.g. the production of carbonic anhydrase). Algae which are able to photosynthesize at high rates in seawater have evolved means of using bicarbonate (which makes up about 90% of the total inorganic carbon pool in seawater at the typical pH of around 8.0) (Bidwell & MacLachlan 1985, Beer & Israel 1990, Maberly 1990) thereby raising the

inorganic carbon concentration at the site of carboxylation, and hence the photosynthetic rate, above that possible if carbon uptake occurred only via passive diffusion (Maberly et al. 1992, Raven et al. 1993). Any means of increasing the concentration of  $\text{CO}_2$  in the chloroplasts (where carbon fixation occurs) above the equilibrium concentration achieved by diffusion can be thought of as a carbon concentrating mechanism (CCM). There are, however, species of red algae which are unable to utilize  $\text{HCO}_3^-$  as a photosynthetic carbon source (Johnston et al. 1992).

The mechanistic specifics of the CCM have only recently been partially elucidated for some green microalgae (Rotatore et al. 1992, Ramazanov et al. 1993) and remain largely undescribed for rhodophytes and phaeophytes (but see Cook & Colman 1987, Cook et al. 1988, Smith & Bidwell 1989). However, the characteristics of carbon use have made it possible to identify algae in which a CCM is or is not operating, as they

have photosynthetic rates and inorganic carbon affinities which are too great to be supported by diffusive entry of dissolved  $\text{CO}_2$  alone (Maberly 1990, Maberly et al. 1992). There are indications that the CCM is inducible by carbon limitation in several species of macroalgae (see Surif & Raven 1990, Levavasseur et al. 1991 and Maberly et al. 1992 for discussion of the degree of suppression of the CCM); that is, plants growing in adequate carbon concentrations do not operate the CCM. The strongly facultative nature of the CCM suggests that there is considerable energetic cost to its operation, indeed, strong enough to outweigh the potential benefit of enhanced productivity under conditions where carbon concentration may be temporarily limiting the rate of photosynthesis, for example during light flecks. There have been attempts to determine the quantum cost of operating an active inorganic carbon uptake mechanism. In general there appeared to be a decrease in quantum yield for photosynthesis under conditions where a CCM would be operating (Raven & Lucas 1985 and references therein, Beardall 1991).

Here, we attempted to make use of this energetic cost to repress/derepress the CCM by manipulating a factor other than the inorganic carbon concentration or the pH. That is, we used a demand side scheme to lower the need for carbon uptake (the photosynthetic rate) by lowering the photon flux density (PFD) at which the plants were grown. We used 3 species of red macrophyte, 2 of which have been previously shown to possess a CCM (*Palmaria palmata* and *Laurencia pinnatifida*) and 1 which does not (*Lomentaria articulata*) (Maberly 1990, Johnston et al. 1992). We were able to compare the photosynthetic characteristics of a given species with its CCM functioning at different levels of efficiency, under the same external carbon concentrations, and also to compare the effect of growth conditions on species which do and do not possess a CCM.

## METHODS

**Collection and culture conditions.** *Palmaria palmata* (L.) Kuntze, *Laurencia pinnatifida* (Huds.) Lamour and *Lomentaria articulata* (Huds.) Lyngb. were collected from Castle Rocks, St. Andrews, UK ( $2^\circ 7' \text{ W}$ ,  $56^\circ 20' \text{ N}$ ). Plants were cleaned of any visible epiphytes and grown in the laboratory at  $10^\circ \text{C}$  in Provasoli enriched seawater media (PES; Provasoli 1968) at either  $75 \pm 5$  or  $25 \pm 5 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  in a 12 h : 12 h light: dark cycle. All cultures were vigorously aerated, which maintained air equilibrium levels of DIC (Kübler unpubl. data). The media were changed every 5 to 6 d, at which time plants were cleaned with paper towels and the biomass density maintained at 1.0 to  $1.5 \text{ g l}^{-1}$ .

Growth rates were determined by reweighing 10 blotted, tagged individuals in each culture at each media change and calculating the specific growth rate per day. Plants grown under these conditions were used for all further measurements. The growth experiment was repeated and data from the 2 repetitions were pooled.

**Carbon and nitrogen content and carbon stable isotope ratio.** Subsamples of each culture were blotted to remove surface water, weighed, dried overnight at  $80^\circ \text{C}$ , reweighed and ground to powder. Subsamples consisted of whole individual plants or, in the case of *Lomentaria articulata*, 2 or 3 whole individuals. C:N ratios and  $\delta^{13}\text{C}$  values (relative to the PDB standard) were determined on  $\sim 1 \text{ mg}$  of the dried, powdered subsamples in a combined Carlo-Erba elemental analyzer/VG SIRA II mass spectrophotometer. The  $\delta^{13}\text{C}$  value of organic carbon has been previously shown to be a sensitive indicator of the inorganic carbon source for photosynthesis in macroalgae, with plants restricted to  $\text{CO}_2$  use having values in the range of  $-35$  to  $-30\text{‰}$ , while those which also use  $\text{HCO}_3^-$  have values  $> -25\text{‰}$  (Maberly et al. 1992). These different stable carbon isotope signals reflect the discrimination of ribulose-1,5-bisphosphate carboxylase acting on intracellular carbon pools derived from  $\text{HCO}_3^-$  and/or dissolved  $\text{CO}_2$ , with the  $\text{CO}_2$  in seawater being  $\sim 10\text{‰}$  more negative than the  $\text{HCO}_3^-$  (Mook et al. 1974, O'Leary 1992). Carbon fixation rates per unit tissue nitrogen were calculated according to Surif & Raven (1990) from the organic nitrogen content and the maximum photosynthetic rate (see below).

**Inorganic carbon dose response curves.** The response of net photosynthetic rate to inorganic carbon concentration was determined at  $10^\circ \text{C}$  in a Hansatech  $\text{O}_2$  electrode.  $\text{CO}_2$ -free seawater was prepared by acidifying Millipore-filtered seawater to  $\text{pH} \leq 4.0$  and sparging with  $\text{N}_2$  for at least 5 h. Tris-base buffer (25 mM) was then added and the pH adjusted to 8.0 with carbonate-free NaOH prepared as described in Johnston et al. (1992). An apical section of plant material ( $\sim 30 \text{ mg}$ ) was placed in the electrode chamber with 2 ml of  $\text{CO}_2$ -free buffered seawater and  $\text{O}_2$  exchange measured.  $\text{NaHCO}_3$  solution was injected and photosynthesis measured over a range of concentrations up to 10.0 mM. The initial slopes of DIC response curves were calculated by linear regression over the range of 0.0 to 0.8 mM  $\text{HCO}_3^-$ . The slope is taken to indicate how effectively plants use low concentrations of inorganic carbon, as discussed by Johnston et al. (1992). The mean maximum rate of net photosynthesis ( $P_{\text{max}}$ ) was calculated as the mean highest rate of oxygen evolution recorded in the course of each response curve.

Chlorophyll a content was subsequently determined for each apex. Chlorophyll a was extracted by sequen-

Table 1. *Palmaria palmata*, *Laurencia pinnatifida* and *Lomentaria articulata*. Characteristics of seaweeds grown at  $75 \pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (high PFD) or  $25 \pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (low PFD). Values are given as mean (SE) for  $n = 5$  to 10

	<i>P. palmata</i>		<i>L. pinnatifida</i>		<i>L. articulata</i>	
	High PFD	Low PFD	High PFD	Low PFD	High PFD	Low PFD
Growth (% d <sup>-1</sup> )	7.0 (1.2)	3.7 (0.8)	3.0 (0.5)	1.2 (0.2)	2.0 (1.1)	3.2 (1.0)
% Dry biomass	18.1 (0.7)	16.5 (0.5)	14.2 (0.8)	12.9 (0.4)	17.6 (0.3)	17.1 (0.5)
% C (of dry wt)	34.7 (0.7)	30.6 (0.6)	32.4 (0.6)	30.1 (0.4)	29.9 (0.6)	30.3 (0.5)
% N (of dry wt)	3.06 (0.63)	3.82 (0.04)	3.21 (0.04)	3.34 (0.14)	3.51 (0.08)	3.75 (0.09)
Chlorophyll a ( $\mu\text{g g}^{-1}$ )	0.12 (0.02)	0.19 (0.01)	0.17 (0.04)	0.15 (0.01)	0.17 (0.20)	0.19 (0.2)
$P_{\text{max}}$ ( $\mu\text{mol O}_2 \text{g}^{-1} \text{h}^{-1}$ )	9.26 (0.36)	3.82 (0.52)	6.98 (0.93)	2.66 (0.88)	0.47 (0.07)	4.40 (0.76)

tial addition of dimethyl sulfoxide (DMSO) and methanol (MeOH) and quantified spectrophotometrically as described previously (Kübler & Davison 1993).

**Statistical analyses.** All within-species comparisons were made using the Mann-Whitney *U*-test for comparing 2 means. Unless otherwise noted, 'significant' implies  $p \leq 0.05$ .

## RESULTS

Growth rates (biomass specific) were significantly reduced in *Palmaria palmata* ( $p = 0.05$ ) and *Laurencia pinnatifida* ( $p = 0.05$ ) when grown at 25 relative to  $75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , while the growth rate of *Lomentaria articulata* was not significantly affected by PFD over this range (Table 1). This suggests that the growth rate of *L. articulata* is limited by some other factor even at the low PFD used here. The biomass produced by *P. palmata* and *L. pinnatifida* grown at

$75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  had a significantly higher dry:wet mass ratio than that grown at  $25 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  ( $p = 0.05$ ,  $p \leq 0.002$  for *P. palmata* and *L. pinnatifida* respectively).

$\delta^{13}\text{C}$  values of high- and low-PFD-grown plants are given in Fig. 1. All species exhibited increased fractionation of stable carbon isotopes at low PFD. The increase was greatest in *Palmaria palmata* (30.1% or 5.6%,  $p \leq 0.001$ ), intermediate in *Laurencia pinnatifida* (17.9% or 4.1%,  $p < 0.002$ ) and least in *Lomentaria articulata* (11.5% or 3.9%,  $p \leq 0.001$ ). The stable carbon isotope ratios of the source DIC pool in the media were not found to differ at the 2 light levels (Kübler unpubl.).

The mean initial slopes of DIC uptake curves are shown in Fig. 2. The initial slope was significantly greater in high- relative to low-PFD-grown *Palmaria palmata* ( $p < 0.001$ ) and arguably greater in high- relative to low-PFD-grown *Laurencia pinnatifida* ( $p \leq 0.10$ ). The initial slope of carbon uptake in *Lomentaria*

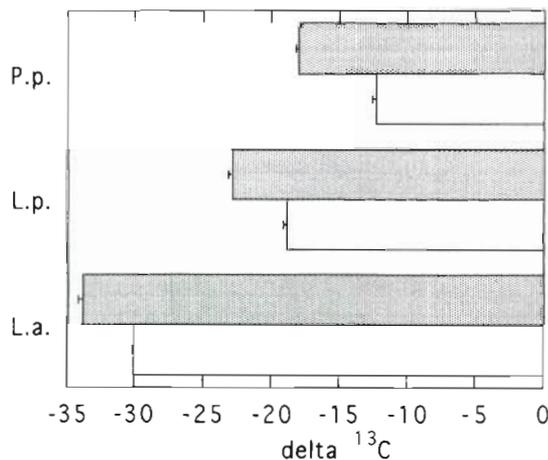


Fig. 1.  $\delta^{13}\text{C}$  values for organic carbon of *Palmaria palmata* (P.p.), *Laurencia pinnatifida* (L.p.) and *Lomentaria articulata* (L.a.) grown under PFD of  $25 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (shaded bars) or  $75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (open bars) ( $n = 5$  to 10). Error bars = 1 SE

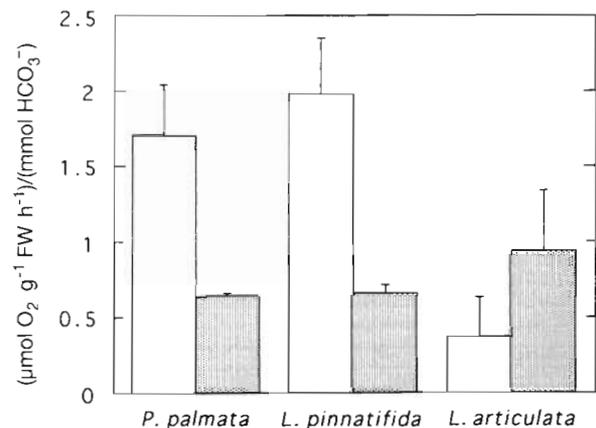


Fig. 2. *Palmaria palmata*, *Laurencia pinnatifida* and *Lomentaria articulata*. Initial slopes of the response of photosynthesis to DIC concentration for seaweeds grown under PFD of  $25 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (shaded bars) or  $75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (open bars) ( $n = 3$ ). Error bars = 1 SE

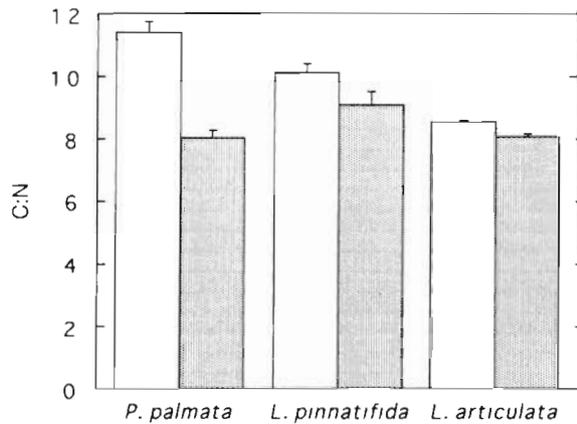


Fig. 3 *Palmaria palmata*, *Laurencia pinnatifida* and *Lomentaria articulata* C:N ratios for seaweeds grown under PFD of 25  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (shaded bars) or 75  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (open bars) ( $n = 10$ ). Error bars = 1 SE

*articulata* was unaffected by growth light level and was comparable to those of low-PFD-grown *P. palmata* and *L. pinnatifida*.  $P_{\text{max}}$  values of *P. palmata* and *L. pinnatifida* (Table 1) were greater for plants grown at high PFD relative to those grown at low PFD ( $p \leq 0.05$  in both cases).  $P_{\text{max}}$  of *L. articulata* grown at high PFD was significantly lower than that of low-PFD-grown plants ( $p \leq 0.05$ ), suggesting photoinhibition and cumulative damage to the photosynthetic apparatus due to carbon limitation at high PFD.

The chlorophyll *a* concentration on a fresh biomass basis was greater in *Palmaria palmata* and *Lomentaria articulata* grown at low relative to high PFD ( $p < 0.005$  for *P. palmata*;  $p \leq 0.05$  for *L. articulata*) but was not significantly different for *Laurencia pinnatifida* at the 2 PFDs ( $p \geq 0.05$ ) (Table 1).

The C:N ratio (on a mass basis) of *Palmaria palmata* was 11.40 (SE = 0.35) when grown at 75  $\pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and 8.04 (SE = 0.24) when grown at 25  $\pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  ( $p \leq 0.001$ ; Fig. 3). Similarly, *Laurencia pinnatifida* had C:N ratios of 10.11 (SE =

0.29) and 9.08 (SE = 0.43) when grown at the high and low PFD, respectively ( $p = 0.05$ ). The C:N ratio of *Lomentaria articulata* was not significantly different at the 2 light levels (Fig. 3) and was comparable to that of *P. palmata* grown at low PFD (8.31, SE = 0.08). Light limitation of the growth of *P. palmata* and *L. pinnatifida* at PFD = 25  $\pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  was associated with a decrease in the % of dry biomass composed of carbon (from 34.7% to 30.6% for *P. palmata*,  $p = 0.002$ ; from 32.4% to 30.1% for *L. pinnatifida*,  $p = 0.01$ ). The % carbon of *L. articulata* was not different (30.0%) at the 2 growth PFDs (Table 1) and was similar to that of the other species at 25  $\pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . All 3 species showed some indication of photoacclimation as significant increases in % nitrogen (Table 1) at the lower PFD. These data were suggestive of carbon limitation in high- and low-PFD-grown *L. articulata* and low-PFD-grown *P. palmata* and *L. pinnatifida*.

Carbon fixation rates per unit tissue nitrogen calculated for *Palmaria palmata* and *Laurencia pinnatifida* were significantly greater for high- than for low-PFD-grown plants ( $p \leq 0.05$  in both cases; Table 2). *Lomentaria articulata* fixed carbon faster per unit tissue N when grown at low PFD ( $p \leq 0.05$ ).

## DISCUSSION

The light level at which plants were grown in the laboratory had significant effects on the  $\delta^{13}\text{C}$  values of the organic matter and carbon use characteristics of all 3 species (Figs. 1 & 2). Growth at 25 relative to 75  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  had contrasting effects on the carbon use characteristics of species which were previously shown to be able to use  $\text{HCO}_3^-$  for photosynthesis (*Palmaria palmata* and *Laurencia pinnatifida*) and the one which was not (*Lomentaria articulata*) (Johnston et al. 1992). Recent analysis of a large number of macroalgae (including those used here) has shown a clear connection between  $\delta^{13}\text{C}$  values and pH compensation points (Maberly 1990, Maberly et al. 1992). More negative values of  $\delta^{13}\text{C}$  in plants grown at low PFD could result from several factors including lower photosynthetic rates and therefore a relative decrease in photosynthetic limitation due to DIC supply, and/or decreased usage of the  $\text{HCO}_3^-$  pool. The latter of these possibilities would also be associated with a decrease in the pH compensation point. We hypothesize that the decrease of  $\delta^{13}\text{C}$  of *P. palmata* and *L. pinnatifida* grown at low PFD was due to a change in the characteristics of inorganic carbon uptake, such that a smaller proportion of the carbon fixed during growth

Table 2. *Palmaria palmata*, *Laurencia pinnatifida* and *Lomentaria articulata* Carbon fixation rate per unit tissue N of cultured and freshly collected seaweed. Values (mean and SE) for cultured plants grown at 25  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (low PFD) or 75  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (high PFD) were calculated from  $P_{\text{max}}$  ( $n = 3$ ) and nitrogen content ( $n = 10$ ); values for freshly collected plants from the same location were taken from Johnston et al. (1992)

	Carbon fixation rate ( $\mu\text{mol O}_2 \text{mmol}^{-1} \text{N h}^{-1}$ )		
	<i>P. palmata</i>	<i>L. pinnatifida</i>	<i>L. articulata</i>
Cultured, high PFD	8.50 (1.16)	10.50 (2.04)	9.58 (1.54)
Cultured, low PFD	23.16 (0.90)	21.11 (2.83)	3.34 (1.78)
Freshly collected	16.81	9.22	14.55

was actively taken up from the  $\text{HCO}_3^-$  pool. This hypothesis was supported by the fact that the initial slope of the DIC uptake curves was significantly greater in high-PFD-grown *P. palmata* ( $p \leq 0.001$ ), and marginally greater in high-PFD-grown *L. pinnatifida* ( $p \leq 0.10$ ). The initial slope of the DIC uptake curve for *L. articulata* was not significantly affected by growth PFD (Fig. 2) which is consistent with this species' being dependent on diffusive uptake of DIC at both PFDs.

There are several examples of decreasing  $\delta^{13}\text{C}$  values with increasing depth for both seagrasses and macroalgae (Wefer & Killingley 1986, Cooper & McRoy 1988, Cooper & DeNiro 1989, Durako & Hall 1992). This pattern is generally attributed to increasing light limitation and decreasing photosynthetic rate at depth. However, controlled experiments in which light level is the only manipulated variable are few and there are numerous other factors which would also correlate with depth including temperature, duration of exposure to air, and flow rates. An exception is found in the work of Wienke & Fischer (1990), who identified trends of decreasing (more negative)  $\delta^{13}\text{C}$  with decreasing growth rate and light limitation in 8 of 12 polar or cold-temperate macroalgal species. The range of light effects, from no response to a net difference in  $\delta^{13}\text{C}$  of ca +20‰ in a period of 5 wk (for *Desmarestia antarctica*; Wienke & Fischer 1990), illustrates the huge variability in the means of carbon acquisition, and its potential for acclimation in seaweeds. The implications of decreased photosynthetic rate for carbon isotope fractionation in aquatic plants are discussed in Raven (1992). In this case, we demonstrated that a decrease in growth light level resulted in a small decrease in  $\delta^{13}\text{C}$  values in diffusion-limited plants (*Lomentaria articulata*; Fig. 1) but a large difference in plants which facultatively operate a CCM (*Laurencia pinnatifida* and *Palmaria palmata*; Fig. 1). Raven et al. (1993) proposed the poleward trend of decreasing carbon fixation rates as a potential explanation for the trend of more negative  $\delta^{13}\text{C}$  values in plants at higher latitudes. The relative repression of CCMs at low light level in high latitudes would also contribute to this trend. This possibility could be tested by comparing the delta values of perennials to those of summer annuals which would only be exposed to high light levels and have fully induced CCMs.

Results for *Lomentaria articulata* were quite different from those that would be predicted by a direct relationship between light level and inorganic carbon demand. *L. articulata* grown at low PFD had more negative  $\delta^{13}\text{C}$  than plants grown at high PFD, but this decrease was associated with greater rates of net photosynthesis and growth (Table 1). However, it seems likely that the high-PFD-grown *L. articulata* were experiencing photoinhibition and subsequent damage.

If this were the case, it is possible that the absolute rate of carbon fixation was greater at the higher light level but was accompanied by a greater requirement for maintenance respiration and, thereby, lower net photosynthetic and growth rates. It should be noted that the high PFD used in the laboratory ( $75 \pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ), although growth-saturating for many marine macroalgae, is only ~5% of full sunlight and even shaded microhabitats in the intertidal are likely to experience PFDs  $>75 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  at low tide. Partial photoinhibition may serve as a photoprotective mechanism, allowing *L. articulata* to survive and grow at light levels in excess of those it can use for carbon fixation. A mechanism by which photoinhibited PSII reaction centers serve to nonphotochemically dissipate excess excitation energy has been described for the terrestrial shade plant *Tradescantia albiflora* (Adamson et al. 1991, Öquist et al. 1992a, b) and may also operate in *L. articulata*.

Based on the response of these species to growth light level in the laboratory, it is possible to speculate about the light environments the freshly collected plants used by Johnston et al. (1992) and Maberly et al. (1992) may have experienced. Comparing the  $\delta^{13}\text{C}$  values for the organic carbon of lab-grown (Fig. 1) and freshly collected plants (Maberly et al. 1992) we see that for *Lomentaria articulata* and *Laurencia pinnatifida*, high-PFD-grown lab plants ( $\delta^{13}\text{C} = -30.0\text{‰}$  and  $-18.83\text{‰}$  respectively) were similar to the freshly collected plants ( $-30.0\text{‰}$  and  $-17.3\text{‰}$  respectively) while low-PFD-grown plants were more negative ( $-33.9\text{‰}$  and  $-22.9\text{‰}$  respectively). In the case of *Palmaria palmata*, the high-PFD-grown plants ( $\delta^{13}\text{C} = -12.3\text{‰}$ ) were similar to the 'drift' population of Maberly et al. (1992) ( $-14.5\text{‰}$ ) while the low-PFD-grown plants were more like those of the attached population of Maberly et al. (1992) ( $\delta^{13}\text{C} = -17.9$  and  $-18.5\text{‰}$  respectively). Such comparisons suggest that *L. articulata* and *L. pinnatifida* were not extremely light limited in the field and that *P. palmata* collected as drift were exposed to higher PFD than the attached population. However, our initial attempts to correlate the degree of natural shading in an attached population of *P. palmata* with the  $\delta^{13}\text{C}$  values of the organic carbon have been unsuccessful (Kübler unpubl.).

Taken together, the data for  $\delta^{13}\text{C}$  of organic carbon and the initial slopes of inorganic carbon uptake curves suggest the presence of an inducible CCM in *Palmaria palmata* and potentially in *Laurencia pinnatifida* but not in *Lomentaria articulata*. This is consistent with the hypothesis proposed by Johnston et al. (1992) that plants without an evident CCM in field collections are restricted to shaded environments because they are limited by the rate of diffusive uptake of  $\text{CO}_2$ . It does not appear that *L. articulata* can be induced to

express a CCM if grown at higher light levels. In this respect it is interesting to note that in fresh water the plants found at the depth limit, which are probably light-limited, are usually facultative or obligate non-users of  $\text{HCO}_3^-$  (Boston et al. 1989).

The fact that the ability to use  $\text{HCO}_3^-$  for photosynthesis appears to be facultative suggests that it is energetically expensive or, assuming optimal functioning, that the advantage of being able to use  $\text{HCO}_3^-$  at any time carbon becomes limiting is outweighed by the cost of maintaining the ability. It is not clear from the results given here whether the derepression of  $\text{HCO}_3^-$  use at low light is cued by the alleviation of carbon limitation at low light or by the diversion of energetic resources to photoacclimation. The chlorophyll *a* content of *Palmaria palmata* was significantly greater for plants grown at low relative to high PFD (Table 1), indicating photoacclimation of this species. In contrast, the chlorophyll *a* content of *Laurencia pinnatifida* was not significantly affected by the growth light level. The carbon fixation rates per unit tissue N of *P. palmata* and *L. pinnatifida* were significantly lower when grown at low PFD relative to high PFD (Table 2). Comparing these values to those for freshly collected plants which were able to use  $\text{HCO}_3^-$  (Johnston et al. 1992), the value in high-PFD-grown *L. pinnatifida* was greater than that of the freshly collected *L. pinnatifida*. In contrast, the value in the freshly collected *P. palmata* was intermediate between the high- and low-PFD-grown *P. palmata*. Taken together, these results indicate that *P. palmata* and *L. pinnatifida* have a greater nitrogen requirement at lower PFD, which is consistent with photoacclimation increasing light harvesting, and that growth at the high PFD in the laboratory resulted in a lower N cost of growth (more effective use of N) than occurred in the field population. The lower N cost for growth of *Lomentaria articulata* at high PFD is consistent with its low photosynthetic rate and probable photoinhibition.

Summarizing, we have illustrated the repression of an inorganic carbon uptake mechanism in 2 species of red macroalgae by growth at low PFD. *Palmaria palmata* and *Laurencia pinnatifida* exhibited more efficient uptake of limiting concentrations of inorganic carbon, less negative  $\delta^{13}\text{C}$  values and greater nitrogen use efficiency when grown at a growth-saturating PFD compared to plants grown at a limiting PFD. In contrast, *Lomentaria articulata*, which is unable to utilize the bicarbonate pool for photosynthesis, did not gain any advantage in terms of photosynthetic or growth rate when grown at increased PFD.

**Acknowledgements.** This work was supported by the Natural Environment Research Council (UK) under Grant Number GR3/8463. We thank Dr A. M. Johnston for many useful discussions and technical assistance. Dr C. M. Scrimgeour and

the staff of the Department of Anatomy and Physiology, University of Dundee, provided invaluable support for the mass spectrometric measurements.

#### LITERATURE CITED

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*This article was submitted to the editor*

*Manuscript first received: January 25, 1994*

*Revised version accepted: April 11, 1994*