The acquisition of inorganic carbon by four red macroalgae

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Abstract. Photosynthesis was studied in four species of red marine macroalgae: Palmaria palmata, Laurencia pinnatifida, Lomentaria articulata and Delesseria sanguinea. The rate of O2 evolution for submerged photosynthesis was measured as a function of incident photon flux density at normal pH and inorganic carbon concentration (pH 8.0, 2 mol m-3), and as a function of inorganic carbon concentration at pH 8.0 at saturating and at limiting photon flux density. The rate of CO2 uptake was measured for emersed photosynthesis as a function of CO2 partial pressure at saturating photon flux density. Previous pH-drift results suggest that Palmaria and Laurencia are able to use HCO3- as well as CO2 whereas Lomentaria and Delesseria are restricted to CO2. None of the algae are saturated by 2 mol m-3 inorganic carbon at high light (400 µmol m-2 s-1) but are saturated at low light (35 µmol m-2 s-1). The inorganic C concentration at which half the light-saturated rate of O2 evolution is achieved is higher for Palmaria and Laurencia (1.51 and 1.85 mol m-3) than for Lomentaria and Delesseria (0.772 and 0.841 mol m-3). The lower values for the latter two species could reflect their putative restriction to CO2. If expressed in terms of CO2, the half-saturation values yield 7.2 and 7.8 mmol m-3 respectively, which are very similar to values obtained previously during pH-drift experiments but at lower concentrations of HCO3-, consistent with restriction to CO2. The photosynthetic conductance (m s-1), calculated from the initial slope for photosynthesis at low concentrations of inorganic carbon, correlates with the suggested ability to extract inorganic carbon based on pH-drift results. Calculations made assuming that CO2 is the only species diffusing across the boundary layer are consistent with boundary layer thicknesses of 20 and 19 µm for Lomentaria and Delesseria respectively, which is feasible given the rapid water movement in the experiments. For Laurencia however, an unreasonably small boundary layer thickness of 6 µm is necessary to explain the flux, which indicates co-diffusion by HCO3-. In the apparent absence of external carbonic anhydrase, direct uptake of HCO3- rather than external conversion to CO2 is indicated in this species. In air, the CO2 concentration at which photosynthesis is half-maximal increases in the same order as the ability to raise pH in drift experiments. At 21 kPa the CO2 compensation partial pressures for Palmaria and Laurencia at 0.56 and 1.3 Pa are low enough to suggest a carbon-concentrating mechanism is operating, while those of Lomentaria at 1.8 Pa and particularly that of Delesseria at 4.5 Pa could be explained without a carbon-concentrating mechanism. The algae tested (all except Delesseria) showed more O2 evolution than could be accounted for with a photosynthetic quotient of 1.0 and uncatalysed conversion of HCO3- to CO2 outside the cell in high light at pH 8.0 when high algal fresh weight per unit medium was used. These results are concordant with other data suggesting use of HCO3- by Palmaria and Laurencia, but discordant with the rest of the available information in indicating use of HCO3- by Lomentaria. The reason for this is unclear. The light-saturated rate of O2 evolution on an algal area basis and the photon flux density needed to saturate photosynthesis were related partly to the habitat from which the seaweeds were collected, but more strongly to the ability to use HCO3-. Values for the two users of HCO3-, Palmaria (population used was intertidal; also occurs subtidally) and Laurencia (intertidal/shaded intertidal), were greater than for Lomentaria (shaded intertidal), which was greater than Delesseria (subtidal), both of which are believed to be restricted to CO2. In accordance with earlier δ13C data and, for Delesseria, estimates of the achieved growth rates in situ, carbon is likely to be saturating and use of HCO3- is unlikely to occur in the normal low-light habitats of Lomentaria and Delesseria. Analysis of N-use efficiencies show that they are closer to the low-CO2-affinity Laminariales than the high-CO2-affinity Fucaceae.

Key words: Bicarbonate – Diffusion – Inorganic carbon – Light – Rhodophytes
Many macroalgae, including some of the red macroalgae, show low rates of photorespiration (Johnston and Raven 1986a; Johnston 1991). Since, all RUBISCOs studied so far, including those from two species of red algae, show oxygenase activity (Hilditch et al. 1991), the suppression of photorespiration is likely to be caused by the ability to accumulate inorganic carbon (Ci). The relatively high activities of β-carboxylating enzymes in some macroalgae, most notably the Phaeophyceae and the chlorophyte *Udotea flabellum* (Reiskind and Bowes 1991) has suggested that the mechanism of Ci accumulation may be biochemical as in terrestrial C₄ plants. However, ¹⁴CO₂ labelling patterns and the *in vivo* kinetics of phosphoenolpyruvate carboxykinase (PEPCK), the major β-carboxylating enzyme of many macroalgae, show that β-carboxylation is almost invariably a secondary reaction following the fixation of CO₂ by RUBISCO in the Phaeophyceae (Kremer 1981; Johnston and Raven 1989; Johnston 1991) although in *U. flabellum* a C₄-like fixation pattern may be operating.

An alternative way of raising internal concentrations of Ci is to transport Ci actively via a biophysical mechanism. A number of papers imply active use of HCO₃⁻ using methods of varying rigour (Raven 1970; references in Maberly 1990: Johnston 1991). In 2 species of chlorophyte (Maberly 1990), 15 species of rhodophyte (Cook and Colman 1987; Cook et al. 1986; Cook et al. 1988) and 2 species of phaeophyte (Cook et al. 1986) rates of carbon uptake have exceeded the theoretical maximum uncatalysed rate of CO₂ supply from the dehydration of HCO₃⁻, implying direct use of HCO₃⁻. Recent work by Beer et al. (1990) has demonstrated directly that the chlorophyte *Ulva fasciata* is able to accumulate Ci by a biophysical mechanism.

There is a large range in ability of different species to extract Ci from seawater and air (Sand-Jensen and Gor 1984; Axelsson and Uusitalo 1988; Maberly 1990) in terms of apparent compensation point for CO₂, final pH attained in a closed system, apparent half-saturation concentrations for Ci and the extent of oxygen inhibition of net photosynthesis. The most effective species tested are green species from rockpools such as *Ulva* sp. and *Enteromorpha* sp. (Maberly 1990). The intertidal brown algae are less effective and the subtidal brown algae less effective still, although apparently able to use HCO₃⁻ (Surif and Raven 1990; Maberly 1990; Johnston 1991). Six species were found, out of 35 species tested, which appeared to be unable to use HCO₃⁻ since in pH-drift experiments the final pH did not exceed pH 9, equivalent to a final CO₂ concentration of more than 1 mmol m⁻³ and so greater than concentrations at which a concentrating mechanism needs to be invoked (Maberly 1990). Furthermore, in one of these species the maximum rate of CO₂ production appeared to impose a ceiling on carbon uptake which again implies a restriction on CO₂. All six species were rhodophytes and five grow subtidally, while the sixth grows intertidally, but in shady sites.

The range of uptake ability found can be rationalised to some extent in terms of habitat. The most efficient green algae from rockpools may experience substantial carbon depletion when the tide is low (Maberly 1992). The intertidal brown algae photosynthesise in water and also in air until desiccation intervenes (Johnston and Raven 1986a; Madsen and Maberly 1990). Rapid photosynthesis in air would require the ability to use HCO₅⁻ since, although Ci is supplied from atmospheric CO₂, concentrations of CO₂ in the film are likely to be low as a result of photosynthetic uptake. Species growing in low light, such as the subtidal red algae, may not be substantially carbon-limited, reducing the benefit of a concentrating mechanism, particularly if the energy required to operate the active uptake process exceeds that saved by restricting photorespiration.

The photosynthetic characteristics related to inorganic C acquisition of brown macroalgae, as conspicuous members of the intertidal and subtidal communities, have been relatively well studied (e.g. Johnston and Raven 1986a, b, 1987; Madsen and Maberly 1990; Surif and Raven 1990), but, in contrast, equivalent data for red algae are relatively sparse (see, however, Oates 1986; Smith and Bidwell 1989a, b; Raven et al. 1990a).

One aim of this work was to provide data for intertidal and subtidal red algae which could be compared with those for brown algae. A second aim was to compare the physiology of two species that apparently were able to use HCO₅⁻ with two species that apparently were not.

**Material and methods**

**Material**

*Laurencia pinnatifida* (Huds.) Lamour. *Lomentaria articulata* (Huds.) Lyngb. and *Palmaria palmata* (L.) O Kuntze, were collected from the Castle Rocks, St. Andrews (2° 47' W, 56° 20' N). *Delesseria sanguinea* (Huds.) Lamour was collected off the East Neuk at Crail (2° 37' W, 56° 15' N) from a depth of 9 m. They were either used straight away or kept in a cold room at 5 °C for not more than 48 h. The intertidal species were kept in a tray lined with damp blue paper towel at 50 gmol photon m⁻² s⁻¹ (PAR, 400-700 nm) and used straight away or kept in a cold room at 5 °C for not more than 48 h. The intertidal brown algae were kept in a tray lined with damp blue paper towel at 50 μmol photon m⁻² s⁻¹ (PAR, 400-700 mm) and not allowed to desiccate. *Delesseria* was kept in aerated filtered seawater under the same light regime. Only healthy material was selected for experimental use.

**Thallus characteristics**

To aid comparison between reports in this work and elsewhere, morphometric and constitutive characteristics of the thalli were measured. Fresh weight was measured after hydration in seawater for 15 min, blotting with a paper towel until no surface water was visible and weighing. Dry weight was measured after drying overnight at 80 °C. Chlorophyll *a* was measured on material of known fresh weight as described by Johnston and Raven (1987). Surface area was determined by photocopying the flattened thallus, cutting out the shape, weighing the paper and relating this to a standard piece of paper of known weight and area. C and N content of dried plant material was determined using a Carlo-Erba C,H,N analyzer.

**Light dose response curves**

The response of apparent photosynthesis to light quantity was measured as change in oxygen concentration. A Rank Oxygen Electrode (Rank Brothers, Bottisham, Cambridge, UK) was illuminated by a slide projector (Prestinox 680, Auto), and kept at 10 °C.