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Exogenous inorganic carbon sources for photosynthesis in seawater by members of the Fucales and the Laminariales (Phaeophyta): ecological and taxonomic implications

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Summary. Characteristics of inorganic carbon assimilation by photosynthesis in seawater were investigated in six species of the Fucales (five Fucaceae, one Cystoseiraceae) and four species of the Laminariales (three Laminariaceae, one Alariaceae) from Arbroath, Scotland. All of the algae tested could photosynthesise faster at high external pH values than the uncatalysed conversion of HCO_3^- to CO_2 can occur, i.e. can "use" external HCO_3^-. They all had detectable extracellular carbonic anhydrase activity, suggesting that HCO_3^- use could involve catalysis of external CO_2 production, a view supported to some extent by experiments with an inhibitor of carbonic anhydrase. All of the algae tested had CO_2 compensation concentrations at pH 8 which were lower than would be expected from diffusive entry of CO_2 supplying RUBISCO as the initial carboxylase, consistent with the operation of energized entry of HCO_3^- and / or CO_2 acting as a "CO_2 concentrating mechanism". Quantitative differences among the algae examined were noted with respect to characteristics of inorganic C assimilation. The most obvious distinction was between the eulittoral Fucaceae, which are emersed for part of, or most of, the tidal cycle, and the other three families (Cystoseiraceae, Laminariaceae, Alariaceae) whose representatives are essentially continually submersed. The Fucaceae examined are able to photosynthesise at high pH values, and have lower CO_2 compensation concentrations, and lower $K_{1/2}$ values for inorganic C use in photosynthesis, at pH 8, than the other algae tested. Furthermore, the Fucaceae are essentially saturated with inorganic C for photosynthesis at the normal seawater concentration at pH 8 and 10° C. These characteristics are consistent with the dominant role of a "CO_2 concentrating mechanism" in CO_2 acquisition by these plants. Other species tested have characteristcs which suggest a less effective HCO_3^- use and "CO_2 concentrating mechanism", with the Laminariaceae being the least effective; unlike the Fucaceae, photosynthesis by these algae is not saturated with inorganic C in normal seawater. Taxonomic and ecological implications of these results are considered in relation to related data in the literature.

Key words: Phaeophyta – Inorganic carbon assimilation – Carbonic anhydrase – Carbon dioxide compensation concentration – Ecology – Taxonomy

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The Phaeophyta are typically marine or estuarine organisms. Two major orders of brown algae, the Fucales and the Laminariales, have members which can grow at salinities as low as 2–4‰, i.e. some 10% or less of normal seawater salinity. The minimum concentration of HCO_3^- in such waters is ≈ 1 mol m^{-3} as compared to the ≈ 2 mol m^{-3} of full-salinity seawater (see Raven and Samuelsson 1988). The free CO_2 concentration in equilibrium with air in such solutions increases with decreasing temperature, and with decreasing salinity at a given temperature (Skirrow 1975). The Laminariales are essentially cold-sea organisms, and the Fucales are centered in cooler marine environments. The upshot of these considerations is that the Fucales and Laminariales are generally exposed to 2 (rarely as low as 1) mol m^{-3} HCO_3^-, and CO_2 at 10–20 mmol m^{-3}, at air-equilibrium in their natural (submersed) environment. An additional consideration, not examined in detail in this paper, is the assimilation of CO_2 from the atmosphere while eulittoral plants are emersed; this consideration applies more to (certain) members of the Fucales than to the Laminariales.

The options for inorganic C acquisition by submersed members of the Phaeophyta, in terms of plasmalemma transport, are diffusive (lipid-solution) entry of CO_2; mediated (including active) entry of CO_2; and mediated (including acitve) entry of HCO_3^-. The approaches taken here are as follows. The capacity to "use" HCO_3^- is indicated by a rate of photosynthetic inorganic C assimilation in excess of that which can be supplied by the *uncatalysed* rate of conversion of exogenous HCO_3^- to CO_2 in the medium. The capacity to "use" HCO_3^-, when indicated by this test, can be analysed by the presence of extracellular carbonic anhydrase. The occurrence of a significant (in the quantitative context of the inorganic C assimilation rate) extracellular carbonic anhydrase activity permits the "use" of exogenous HCO_3^- in inorganic C assimilation to be considered in the context of catalysed, external conversion of HCO_3^- to CO_2, with the subsequent possibility of CO_2 being the form of inorganic C which enters the cell under conditions of HCO_3^- "use". The occurrence of purely diffusive entry of CO_2, with or without external carbonic anhydrase, when considered in the context of the quantity and kinetics of RUBISCO in the cell, imposes constraints on the in vivo rate of photosynthesis as a function of external CO_2 concentration. These relate to the "carboxylation efficiency"

($\Delta Ps/\Delta CO_2$) and the CO_2 compensation concentration. Most immediately interpretable is CO_2 compensation concentration, in that the ratio of carboxylase to oxygenase activity in air-equilibrated solution is, for all known RUBISCOs, such as to yield a CO_2 compensation concentration of ≥ 3 Pa at 25° C. Any lower CO_2 compensation concentration than this suggests that there is a mechanism concentrating CO_2 at the site of RUBISCO. The operation of a C_4-type biochemical CO_2 concentrating in the Phaeophyta is unlikely (Kerby and Raven 1985; Raven 1984), and a lower CO_2 compensation concentration in the Phaeophyta is likely to be the result of active transport (influx) of some inorganic C species at some membrane(s) between the medium and the site of RUBISCO activity. It is important to note that, with four membranes *inside* the plasmalemma separating RUBISCO from the environment, any such transport may not necessarily be related to any mediated (active) inorganic C influx at the plasmalemma.

However, relatively few brown algae have been tested for exogenous C assimilation using the "hard" test of photosynthetic use of HCO_3^- as the excess of the rate of inorganic C assimilation over that of the rate of uncatalysed conversion of HCO_3^- to CO_2 (Cook et al. 1986; Johnston and Raven 1986a).

Our objective here is to investigate the carbon acquisition mechanism of some macroscopic Phaeophyta from Arbroath, Scotland. The results obtained are considered in relation to the taxonomy of the organism and to their habitat in the hope of distinguishing the influence of these two factors on any differences in carbon acquisition which are uncovered. The organisms used, and some information on their taxonomy and habitat, are listed in Table 1.

Materials and methods

Plant collection

10 species of brown alga were investigated (see Table 1). The algae were collected at Arbroath, Scotland (Ordnance Survey grid reference NO 659 412) at low tide. The material was maintained in a cool room (10° C) in a perspex aquarium tank with fluorescent illumination (30 μmol photon $m^{-2} s^{-1}$, 400–700 nm) supplied for 12 h in each 24 h. No reproductive material was used and all plants were free of macroscopic epiphytes and were apparently healthy. Only apical portions were used in experiments on the Fucales and basal portions of the blades in those on the Laminariales, i.e. growing regions of the thalli in both orders.

Preparation of buffered seawater with lower total inorganic carbon concentration

Sea water (35‰ salinity) from the site of algal collection was filtered (Whatman no. 1 filter paper). Inorganic carbon was removed by adding 1.0 kmol m^{-3} HCl to lower the pH to less than 4 before sparging for at least 1 h with high purity N_2 to remove CO_2 (as well as much O_2). A known amount of solid buffer with a pK_a appropriate to the pH to be used was added to give the concentration needed and the pH was then adjusted as desired with freshly prepared NaOH (1.0 kmol m^{-3}); all manipulations were under nitrogen. pH was measured with a Pye Unicam pH meter (model 293) fitted with a glass/calomel combination electrode (Russell pH Ltd.).

Determination of CO_2 compensation concentration

A closed system IRGA (ADC Type 225 Mk3) was used to measure the CO_2 compensation concentration (expressed as partial pressure). The IRGA was calibrated with CO_2-free air and high purity standard CO_2 (359 ppm: P.K. Morgan Ltd. Kent, England). The assimilation chamber was a 20×10^{-6} m^3 test tube. Three separate media were used, media "a", "b" and "c" (see caption to Table 5 for details).

The alga (0.3–0.5 g) was placed in the assimilation chamber containing 7.0×10^{-6} m^{-3} medium (aerated over night prior to use) and aerating by means of the built-in pump in the IRGA at a flow rate of 2×10^{-4} m^3 min^{-1} in a closed system. The CO_2 compensation concentration was achieved within 3 h. The experimental temperature was kept constant at 10° C by circulating water from a constant temperature water bath through a jacket surrounding the assimilation chamber. Samples were illuminated by a 500 W lamp (Thorn 35M F500) giving 500 μmol photon (400–700 nm) $m^{-2} s^{-1}$. For every run, samples were pre-

Table 1. Orders, families and habitats of the species of brown algae used in the work reported here

Species	Order	Family	Habitat from which plant was obtained at Arbroath
Pelvetia canaliculata (L.) Dcne. and Thur.	Fucales	Fucaceae	Upper littoral zone, exposed at low tide and almost dry, especially in summer.
Fucus spiralis (L.)	–do–	–do–	Upper littoral zone. Lower than *P. canaliculata*. Exposed at low tide and almost dry in summer.
Ascophyllum nodosum (L.) Le Jolis	–do–	–do–	Upper littoral zone. Exposed at low tide. Grows mainly on sheltered rocks.
Fucus vesiculosus (L.)	–do–	–do–	Upper littoral zone. Exposed at low tide. Dominant on more exposed shores.
Fucus serratus (L.)	–do–	–do–	Lower littoral zone. Exposed at low spring tide; does not dry out much, even in summer. Not exposed at neap tide.
Halidrys siliquosa Lyngb.	–do–	Cystoseiraceae	In rock pool, permanently submersed. Same level as *F. vesiculosus* and *F. serratus*.
Laminaria digitata (Huds.) Lamour.	Laminariales	Laminariaceae	In rock pool, permanently submersed.
Laminaria hyperborea (Gunn.) Fosl.	–do–	–do–	In rock pool, permanently submersed.
Laminaria saccharina (L.) Lamour.	–do–	–do–	In rock pool, permanently submersed.
Alaria esculenta (L.) Grev.	–do–	Alariaceae	In littoral and sublittoral region. Exposed at low tide but constantly moist in exposed condition.

treated with the medium used for 1 h with illumination at 500 μmol photon $m^{-2} s^{-1}$. Preliminary experiments with the oxygen electrode showed that 500 μmol photon $m^{-2} s^{-1}$ was saturating for photosynthesis and no photoinhibition was detected.

The pH compensation point, and the CO_2 compensation concentration (expressed as partial pressure) at the pH compensation point were determined in the same experiment. The pH compensation point was measured by running the experiment in unbuffered seawater at 10° C and illuminating with 500 μmol photon $m^{-2} s^{-1}$ in the assimilation chamber as previously described, for up to 6 h; the CO_2 compensation concentration was usually achieved within less than 3 h. After the experiment the pH of the bathing medium was measured.

Inorganic C-dependent photosynthetic O_2 evolution

The apparent rate of photosynthetic oxygen evolution over a range of pH values was measured in buffered seawater at a constant total inorganic carbon concentration of 2 mol m^{-3}, supplied by $NaHCO_3$ (see Captions to Fig. 1a, b for the details of buffer and concentration). Samples (≈0.1 g) were placed in 4.0×10^{-6} m^3 low total inorganic carbon concentration buffered seawater in the oxygen electrode chamber (Rank Bros., Bottisham, Cambridge, England). The experimental temperature was maintained at 10° C by circulating water from a thermostatically controlled water bath through the water jacket surrounding the chamber. The electrode was calibrated with air saturated distilled water (21 kPa O_2) and for zero O_2 after the addition of sodium dithionite. Salinity effects on solubility were calibrated using the table of Riley and Skirrow (1975). Saturating light [335 μmol photon (400–700 nm) $m^{-2} s^{-1}$], was supplied from a slide projector fitted with a 150 W quartz iodide bulb. The algae were preilluminated with saturating light for 30 min prior to the experiment. The initial rate of O_2 evolution was measured before $NaHCO_3$ was added (see MacFarlane and Raven 1985 for further discussion of this O_2 evolution independent of exogenous inorganic carbon, or, more accurately, at the inorganic C compensation concentration). The measured rate of O_2 evolution (inorganic C-dependent) was the rate after 2.0 mol m^{-3} inorganic carbon was added. The actual apparent rate of photosynthetic O_2 evolution was estimated by the difference between the measured and the initial rate of O_2 evolution.

Determination of $K_{1/2}$ $[HCO_3^- + CO_2]$ values

A known (≈0.1 g) fresh weight of alga was placed in 4.0×10^{-6} m^3 of buffered seawater with low total inorganic carbon concentration (pH 8.0, 30 mol m^{-3} HEPES) in an O_2 electrode chamber and illuminated by saturating light. The alga was preilluminated with saturating light for 30 min prior to the experiment. The HCO_3^- concentration was increased stepwise during the experiment by injecting a known amount of $NaHCO_3$-enriched distilled water (200 mol m^{-3} HCO_3^-) to give the desired inorganic carbon concentration. Photosynthetic rates were expressed as μmol O_2 evolved per unit of total surface area (m^2) per s. Surface area was determined by placing the alga between two sheets of glass and tracing with a fine marker pen. The image was then transferred to graph paper and the area measured. The total surface area (m^2) was determined by doubling the measured area. The concentration of CO_2 species when the photosynthetic rate was half-maximum was calculated as a function of pH at 10° C using the equation of Park (1969). The equilibrium constant was taken from Mehrbach et al. (1973).

Determination of carbonic anhydrase activity

The Wilbur-Anderson electrometric method (Wilbur and Anderson 1948) was used to assay carbonic anhydrase activity by measuring the rate of hydration of CO_2 (the forward direction of the reversible reaction $CO_2 + H_2O \leftrightharpoons HCO_3^- + H^+$). The enzyme activity was expressed in enzyme units (E.U.) based on chlorophyll $a+c$ content using the formula:–

$$\text{E.U.} = 10(t_b/t_e - 1)/\text{mg chlorophyll}$$

where,

t_b = the time in seconds for a pH change from 8.2 to 6.3 with boiled plant or boiled plant extract.
t_e = the time in seconds for a pH change from 8.2 to 6.3 with plant or plant extract.

An estimation of the chlorophyll $a+c$ content was as described by Seeley et al. (1972) with slight modifications. 4.0 g fresh weight of algal thallus was cut into small pieces and treating them with 16.0×10^{-6} m^3 DMSO (dimethyl sulfoxide) for 5 min. A modification to the methods of Seeley et al. (1972) involved grinding with acid-washed sand and the addition of a sufficient volume of 100% acetone to extract the pigments. This mixture was centrifuged with a bench centrifuge. Normally this step involved two sequential centrifugations to ensure there were no pigments left in the extracted material (rendering it colourless). The specific absorptivities ($E_{1cm}^{0.1}$%) for chlorophyll *a* and *c* used by Seely et al. (1972) were used.

CO_2-saturated water was prepared by bubbling pure CO_2 from a CO_2 cylinder into 2×10^{-4} m^3 of double distilled water. The distilled water container was kept on ice and was bubbled with pure CO_2 for at least one hour before use and continuously throughout the experiment, thus insuring that the aliquots removed for each assay were CO_2-saturated.

The extraction-buffer for total cabonic anhydrase activity consisted of 300 mol m^{-3} tris-borate, pH 8.2, 25 mol m^{-3} dithiothreitol, 5 mol m^{-3} EDTA, 2% (w/v) Polyclar AT, 0.5% (w/v) bovine serum albumin (Fraction V, Sigma), and 0.1% Triton X-100 (see Graham and Smillie 1976). 2.0 g fresh material was rinsed with distilled water and chilled to ≈3° C before grinding with acid-washed sand in a chilled mortar and pestle with 12.0×10^{-6} m^3 extraction medium (ratio of 1:6). The extract was then centrifuged in a bench centrifuge (5000 g for 5 min) to sediment sand and cell debris. The supernatant was then stored on ice until the carbonic anhydrase activity was assayed; this interval was not normally longer than 30 min.

For total carbonic anhydrase activity determination, 3.0 cm^3 of 25 mol m^{-3} veronal buffer (Barbital; 5,5-diethyl barbituric acid purchased from SIGMA) pH 8.2 and 0.1×10^{-6} m^3 supernatant (boiled or unboiled) were introduced sequentially from a syringe with a long needle into an 18.0×10^{-6} m^3 glass tube diameter of 1.8 cm and 7.5 cm

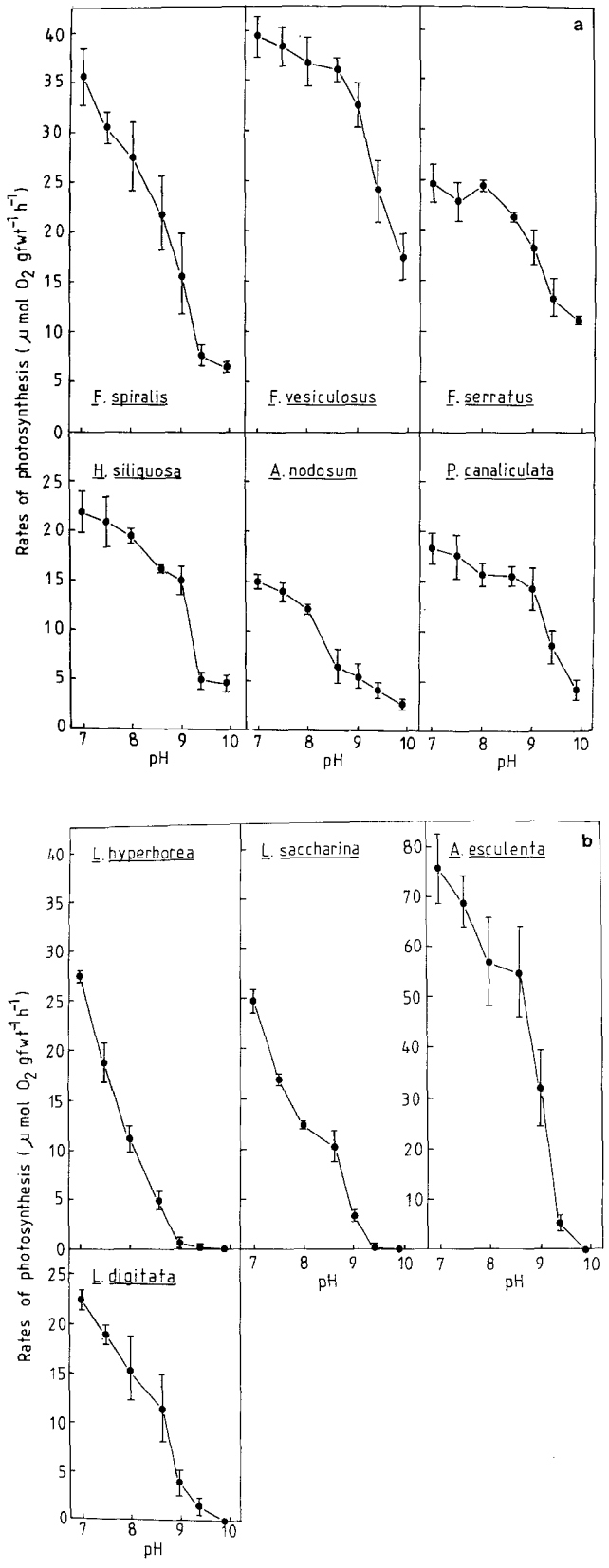

Fig. 1. The apparent rate of photosynthetic oxygen evolution as a function of external pH for the Fucales (**a**) and Laminariales (**b**). The experimental medium was 2.0 mol m^{-3} total inorganic carbon concentration buffered seawater with 10 mol m^{-3} N-2-Hydroxyethalpiperazine-N, -2-ethanesulfonic acid at pH 7.0, 7.5, 8.0, 10 mol m^{-3} 2[N-cyclohexylamino] ethanesulfonic acid at pH 8.6, 9.0, 9.4, or 10 mol m^{-3} Cyclohexylaminopropane sulfonic acid at pH 9.9. Errors quoted are standard errors of the mean of two or three experiments at each pH value. At pH 9.9 the O_2 evolution was measured between 5–10 min for the Laminariales and it is likely that gross photosynthesis would decline with time so that the value might be negative (i.e. respiration exceeding photosynthesis) if the experiments were prolonged

length containing a glass/calomel combination electrode (Russell pH Ltd.); pH was monitored with a Unicam pH meter (model 293). The tube was cooled in an ice bath. When the temperature of the mixture reached ≈3° C, 3.5×10^{-6} m^3 of cooled CO_2-saturated water was injected within one second, with the needle of the syringe below the surface of the buffer solution. The time taken for the pH to drop from pH 8.0 to 6.3 for an extract which had been boiled for 5 min was between 100–105 s. The assay was performed in triplicate for every sample of supernatant fraction.

For external carbonic anhydrase activity determination, 0.3 g fresh alga was washed with chilled distilled water, blotted dry, cut into small pieces (≈50 mm^2) and introduced into the mixture of 3.0×10^{-6} m^3 25 mol m^{-3} veronal buffer pH 8.2 and 1.0×10^{-6} m^3 of double distilled water in the glass tube previously described. 2.0×10^{-6} m^3 of CO_2-saturated water was then introduced and the time taken for the pH to drop to 6.3 was recorded. A control experiment was carried out in an analogous manner with material equal in weight to unboiled material which had been boiled for 5 min. The time taken for a pH drop from 8.2 to 6.3 in the control experiment was about 125 s.

Results

The data obtained in this study is briefly discussed here in relation to relevant data on the same organisms obtained by others. A more wide-ranging taxonomic context for the data is employed in the discussion.

Figure 1 shows the apparent rate of photosynthetic O_2 evolution as a function of pH in six species of Fucales and four of Laminariales in the presence of 2 mol m^{-3} total inorganic carbon. The Fucales, and *Alaria esculenta*, show a smaller decrease in photosynthetic rate with increasing pH than do the three *Laminaria* spp. This is consistent with (but does not prove) a greater capacity for HCO_3^- use by members of the Fucales, and *Alaria esculenta*, than the *Laminaria* spp. tested.

As was found for *Ascophyllum nodosum* by Johnston and Raven (1986a), the rate of photosynthesis by 0.1 gfw of alga was, in all cases, less than the rate of uncatalysed conversion of HCO_3^- to CO_2 in 4 cm^3 of medium at pH 8.0 and at pH 9.4. However, as was also found for *Ascophyllum nodosum* (Johnston and Raven, 1986a), the rate of CO_2 diffusion from a bulk phase (with CO_2 and HCO_3^- at equilibrium) through the 30 μm unstirred layer is inadequate to account for the photosynthetic rate at either pH 8.0 or pH 9.4, even if it is assumed that the plant surface maintains $[CO_2]=0$, so that the *bulk phase* HCO_3^- to CO_2 conversion cannot support photosynthesis. The possibility that uncatalysed HCO_3^- to CO_2 conversion *within the unstirred layer* is adequate to supply CO_2 for photosynthesis is disposed of by the results in Table 2. The ratio of photosynthetic rate to the rate of uncatalysed conversion of HCO_3^- to

Table 2. The ratio of the apparent rate of photosynthesis at pH 8.0 and pH 9.4 to the production of CO_2 by the uncatalysed reaction $HCO_3^- \rightarrow CO_2 + OH^-$, in the 30 μm thick unstirred layer (volume calculated *via* area per gfw). Maximum CO_2 supply rate (nmol $gfw^{-1}\ s^{-1}$) was calculated by using equation (11) of Miller and Colman (1980). $K_1 = 7.97 \times 10^{-7}$ and $K_2 = 4.72 \times 10^{-10}$ were from Mehrbach et al. (1973) (values at 10° C). Values of k_1, k_3 and K_1 of equation (9) of Miller and Colman (1980) is the value at 25° C (i.e. the values presented here might be underestimates the ratio because of overestimation of uncatalysed rate). Values of the rates of photosynthetic O_2 evolution (nmol $gfw^{-1}\ s^{-1}$) are from Fig. 1(a) and 1(b)

Species	Ratios of photosynthetic O_2 evolution to uncatalysed CO_2 production in the unstirred layer	
	pH 8.0	pH 9.4
P. canaliculata	22	122
F. spiralis	32	98
A. nodosum	36	128
F. vesiculosus	52	366
F. serratus	32	205
H. siliquosa	47	132
L. digitata	32	36
L. hyperborea	27	8
L. saccharina	14	2
A. esculenta	14	14

CO_2 always exceeds one, and is higher for the Fucales than the Laminariales, especially at pH 9.4. The downward correction of the photosynthetic rate to take into account the occurrence of a photosynthetic quotient in excess of unity (Axelsson 1988), would only reduce the quoted ratios by $\approx$1.17-fold and thus not alter the conclusions that the Fucales have a quantitatively greater capacity for HCO_3^- use than do the Laminariales. The data showing a rate of photosynthesis in excess of the uncatalysed rate of CO_2 production from HCO_3^- in *Ascophyllum nodosum* agrees with the data of Johnston and Raven (1986a).

Table 3 shows the half-saturation values of inorganic carbon-dependent O_2 evolution expressed in terms of $HCO_3^- + CO_2$ concentration and of free CO_2 concentration, and also of the inorganic C-saturated rate of O_2 evolution. The inorganic C-saturated rates, expressed on an area basis, are higher for the eulittoral members of the Fucales than for *Halidrys siliquosa* or for members of the Laminariales.

An even more impressive correlation between photosynthetic performance in the Fucaceae and the other algae examined here relates to the half-saturation values for inorganic C ($HCO_3^- + CO_2$) and, necessarily correlated at the constant pH of 8.0, the half-saturation value for CO_2. The significantly higher affinity (lower half-saturation concentration) of the Fucaceae for inorganic C than of other algae examined is very unlikely to be a result of differences between organisms of water flow over thallus segments in the O_2 electrode chamber, and thus of boundary layer thickness; the difference in affinity is a result of phenomena within the thallus.

The data in Table 3 suggest that photosynthesis by the Fucaceae is close to inorganic C saturation under submersed, light-saturated conditions. Where data from the same species are available in the literature there is good agreement with the values in Table 3. Examples are from the work of Johnston and Raven (1986b) on *Ascophyllum*

Table 3. The external carbon concentration for half-maximum CO_2-dependent O_2 evolution ($K_{1/2}[HCO_3^- + CO_2]$) at pH 8.0, the CO_2 concentration at $K_{1/2}[HCO_3^- + CO_2]$ was calculated using equation of Park (1969) and K_1, K_2 values were from Mehrback et al. (1973). Temperature was 10° C

Species	$K_{1/2}$ $[HCO_3^- + CO_2]$ (mol m^{-3})	$K_{1/2}$ $[CO_2]$ at $[HCO_3^- + CO_2]$ (mmol m^{-3})	V_{max} μmol O_2 $m^{-2}\ s^{-1}$
P. canaliculata	0.65±0.18	7.7	3.91±0.65
F. spiralis	0.70±0.10	8.3	4.76±1.08
A. nodosum	0.72±0.08	8.5	3.70±0.25
F. vesiculosus	0.63±0.16	7.5	5.40±1.47
F. serratus	0.78±0.06	9.2	5.57±2.06
H. siliquosa	1.47±0.25	17.4	2.53±0.06
L. digitata	2.15±0.56	25.5	3.50±0.96
L. hyperborea	2.08±0.34	24.6	2.30±0.46
L. saccharina	2.17±0.03	25.7	2.90±0.60
A. esculenta	2.02±0.45	23.9	2.60±0.61

nodosum from the Tay estuary, Scotland (15–30‰ salinity) and of Sand-Jensen and Gordon (1984) on *Fucus vesiculosus* from Roskillfjord, Eastern Denmark (15‰ salinity). These data on eulittoral populations which are exposed, when submersed, to close to 2.0 mol m^{-3} of $CO_2 + HCO_3^-$, are similar to those of Raven and Samuelsson (1988) for a submersed population of *Fucus vesiculosus* from the Gulf of Bothnia whose habitat has an essentially constant 2–4‰ salinity and 1 mol $CO_2 + HCO_3^-$ m^{-3}. This population is almost certainly derived from eulittoral populations living at higher mean salinities and inorganic C concentration (Raven and Samuelsson 1988). By maintaining its ancestral affinity for inorganic C as the external dissolved inorganic C concentration declined, the Gulf of Bothnia population forms an interesting link with the other non-Fucaceous algae examined in this investigation (Table 3). *Halidrys siliquosa* and the four Laminalarialian species spend very little of their time exposed to air in their natural habitat, and, although the populations we examined were exposed to the full sea-water concentration of inorganic C, they are only about half-saturated with inorganic C in their natural environment.

The eulittoral Fucaceae examined here are thus inorganic C-saturated in their natural habitat, when the tide is in, while the normally more completely submersed *Halidrys siliquosa*, *Alaria esculenta* and *Laminaria* spp. are only about half saturated. This difference is similar to that found in terrestrial plants when C_4 and C_3 plants are compared at light saturation at ambient atmospheric CO_2 and O_2 partial pressures. Terrestrial C_4 plants are like the Fucaceae in being essentially inorganic C-saturated under normal conditions, while terrestrial C_3 plants resemble *Halidrys siliquosa* and the members of the Laminariales in not being saturated with inorganic C under natural conditions. As with terrestrial C_4 plants, the intertidal fucoids may obtain benefits in terms of N use efficiency by their inorganic C acquisition strategy (Raven et al. 1987). It is also possible (but untested) that the intertidal fucoid could, as is known to occur for terrestrial C_4 plants, have benefits in terms of water use efficiency (Raven et al. 1987). For the fucoid this would relate to C acquired *from air* per unit of H_2O evaporated from plant water when the tide is out.

Some data are available from which it is possible to assess the extent to which the observed $K_{1/2}$ in terms of CO_2 for the brown algae at 10° C is compatible with diffusive entry of CO_2. The data of Davison (1987) and of Davison and Davison (1987) on *Laminaria saccharina* RUBISCO activity and photosynthesis as a function of temperature of growth and measurement suggests that the $K_{1/2}$ (CO_2) for RUBISCO assayed at 10° C is ≈ 20 mmol m^{-3} (using appropriate pKa′ for CO_2/HCO_3^- at that temperature and ionic strength). Furthermore, the extractable RUBISCO activity (assayed at inorganic C saturation) is certainly not more than is required to account for in vivo rates of photosynthesis (assayed at normal seawater inorganic C concentration of 2.0 mol m^{-3} at pH 8.0) at 10° C. These enzymic data thus indicate that there is certainly no excess of RUBISCO carboxylase relative to that needed for C-saturated photosynthesis which is about twice that found in normal seawater concentration of inorganic C in *Laminaria saccharina* (Table 3). We would, then, expect a $K_{1/2}$ in terms of CO_2 of more than the in vitro value, since there is diffusive resistance to CO_2 entry even if external carbonic anhydrase (demonstrated later: Table 4) maintains free CO_2 close to the equilibrium value in the cell wall. However, with a diffusion distance of 5 μm from the outside of the plasmalemma to the site of RUBISCO with an effective diffusion coefficient of 10^{-9} m^2 s^{-1}, then the flux of 1.45 μmol m^{-2} s^{-1} at $K_{1/2}$ (Table 3) corresponds to a concentration difference of 7.25 mmol m^{-3} (RUBISCO site relative to that outside the plasmalemma), so that the external 25.7 mmol m^{-3} becomes 18.45 mmol m^{-3} inside. This calculation suggests that, on the basis of RUBISCO kinetics in vitro and inorganic C fixation kinetics in vivo, diffusive CO_2 entry (with external carbonic anhydrase: Table 4) could account for the observed inorganic C fixation rate in seawater. It is worth noting that, with adequate cell wall carbonic anhydrase activity to permit all of the inorganic C flux to the plant surface to occur as HCO_3^-, and even with an unstirred layer 7 μm thick, and a $D_{HCO_3^-}$ of 10^{-9} m s^{-1}, the flux of 1.45 μmol m^{-2} s^{-1} could be obtained with a gradient of 0.1 mol m^{-3}, i.e. 1.9 mol m^{-3} at the surface with 2 mol m^{-3} in the bulk phase.

In *Ascophyllum nodosum*, by contrast, the much higher affinity (Table 3) for inorganic carbon (as total inorganic C at pH 8, better expressed as CO_2) is not correlated with higher CO_2 affinity, or CO_2-saturated capacity, of RUBISCO (Johnston unpublished) so that active inorganic C influx at the plasmalemma or some internal membrane(s) is required.

Table 4 shows the carbonic anhydrase activity of the algae, measured in the direction of hydration of CO_2, in plant extracts and the external carbonic anhydrase measured in plant segments. The members of the Fucaceae have a higher total activity in extracts (internal and external) than do *Halidrys siliquosa* and the Laminariales, and a smaller fraction of the activity, and generally a lower absolute activity, is external. These data show that external carbonic anhydrase activity is generally lower in those algae (the Fucaceae) with a greater capacity for use of HCO_3^- (Table 2). The findings for *Ascophyllum nodosum* contrast with earlier preliminary data on carbonic anhydrase location (Raven et al. 1987).

The CO_2 compensation concentration in buffered seawater at pH 8, expressed as the CO_2 partial pressure in the gas-stream (21 kPa O_2) in equilibrium with the seawater, is given in Table 5. The values for *Ascophyllum nodosum* are comparable with those of Johnston and Raven (1986a). The CO_2 compensation concentration values are lower than those found for C_3 terrestrial plants with diffusive CO_2 entry, where the balance between CO_2 fixation and CO_2 evolution by photorespiration and "dark" respiration occurs at ≥ 2 Pa CO_2 at 10° C (see Heath and Orchard 1957; Björkman et al. 1969; Brooks and Farquhar 1985). It is very unlikely that the selectivity factor (*sensu* Jordan and Ogren 1981; 1984) of Phaeophyta RUBISCO would be such as to yield a lower CO_2 compensation partial pressure than that of C_3 terrestrial plants, granted diffusive CO_2 and O_2 exchange. Accordingly, the values for the Fucaceae are much below the C_3 with diffusive CO_2 entry expectation, while *Halidrys siliquosa* and the Laminariales show values which are, perhaps, somewhat less than expected for C_3

Table 4. External, total and the percentage (%) of external carbonic anhydrase activity. Enzyme activity was expressed in enzyme units (E.U.)/mg chlorophyll (a+c). Number of replications are shown in parentheses

Species	CA activity (E.U.)		External CA as a percentage of total CA
	External	Total	
P. canaliculata	21.0±10.0 (3)	742±202 (3)	2.8
F. spiralis	30.8± 6.0 (4)	1407±267 (3)	2.2
A. nodosum	27.5± 5.0 (5)	979±114 (3)	2.9
F. vesiculosus	55.0±10.0 (3)	765±188 (4)	7.2
F. serratus	26.0± 7.0 (4)	490±193 (4)	5.3
H. siliquosa	31.0± 4.0 (4)	114± 25 (3)	27.0
L. digitata	69.0± 8.0 (3)	229±68 (4)	30.0
L. hyperborea	65.0±31.0 (3)	334±124 (4)	20.0
L. saccharina	87.0±22.0 (3)	141± 98 (4)	62.0
A. esculenta	95.0±19.0 (3)	315±126 (4)	30.0

Table 5. CO_2 compensation partial pressure (Pa) at different media. "a" – Buffered seawater pH 8.0 [30 mol m^{-3} HEPES(N-2-hydroxyethyl piperazine-N′-2-ethanesulfonic acid] "b" – Buffered seawater pH 5.5 [20 mol m^{-3} MES(2[N-morpholino] ethanesulfonic acid) for the Fucales; 10 mol m^{-3} MES for the Laminariales. "c" Buffered seawater pH 8.0 [30 mol m^{-3} HEPES+10 mmol m^{-3} 5-acetamido-1,3,4-thiadiazole-2-sulphonamide (diamox). All data obtained at 10° C and PFD 500 μmol photon m^{-2} s^{-1}. Number in the brackets = no. of samples used. The values in "c" are based on single determination

Species	CO_2 compensation partial pressure (Pa)		
	"a" – pH 8.0	"b" – pH 5.5	"c" – pH 8.0 +10 mmol m^{-3} diamox
P. canaliculata	0.33±0.15 (3)	2.0±0.5 (3)	2.0
F. spiralis	0.20±0.20 (3)	2.0±0.5 (3)	2.0
A. nodosum	0.32±0.07 (3)	3.0±0.2 (3)	2.3
F. vesiculosus	0.40±0.10 (3)	3.0±0.6 (3)	1.5
F. serratus	0.23±0.21 (2)	3.0±0.0 (2)	1.7
H. siliquosa	1.85±0.21 (2)	5.9±0.6 (3)	7.5
L. digitata	1.85±0.21 (2)	4.5 (1)	8.5
L. hyperborea	0.60±0.14 (2)	5.5 (1)	8.0
L. saccharina	1.73±0.51 (3)	6.6±1.5 (4)	8.7
A. esculenta	1.95±0.28 (3)	3.0±0.0 (4)	7.5

plants with diffusive CO_2 entry. This is consistent with the occurrence of a mechanism which concentrates CO_2 to a level higher than that in the external medium at the site of RUBISCO activity. Since the marine Phaeophytes do not seem to use C_4-type biochemistry to act in CO_2 accumulation in the light (Kerby and Raven 1985, Johnston unpublished work) such a CO_2 accumulation probably involves transmembrane active influx of HCO_3^- and CO_2 (Johnston unpublished work). Table 5 also shows the CO_2 compensation concentration (again expressed as CO_2 compensation partial pressure in equilibrium with seawater) at an external pH of 5.5. The five members of the Fucaceae tested showed a substantial (up to 10-fold) increase in CO_2 compensation concentration at pH 5.5 relative to pH 8.0. *Halidrys siliquosa* and members of the Laminariales generally showed smaller relative increases, although, starting from a higher value at pH 8.0 than in the Fucaceae, higher CO_2-compensation concentration at pH 5.5 are found. The CO_2 compensation concentration at low pH in the Fucaceae is little lower than would be expected at 10° C for a plant with C_3 biochemistry and diffusive CO_2 entry (see Heath and Orchard 1957; Björkman et al. 1969; Brooks and Farquhar 1985). The CO_2 compensation concentration of the Laminariales and *Halidrys siliquosa* are well within the range expected at 10° C for a plant with C_3 biochemistry and diffusive CO_2 entry (see Heath and Orchard 1957; Björkman et al. 1969; Brooks and Farquhar 1985). It would thus appear that the suppression of RuBPo activity in seawater at pH 8.0 is much less marked, or is absent, at pH 5.5, suggesting that HCO_3^- entry at the plasmalemma is involved in this suppression.

Table 5 also shows the effect of diamox (acetazolamide) at 10 mmol m^{-3} on the CO_2 compensation concentration at pH 8.0. For the Fucaceae the low values at pH 8.0 in the absence of diamox are increased by diamox to values closer to those at pH 5.5 in the absence of diamox. For the Laminariales and *Halidrys siliquosa*. The values in the absence of diamox at pH 8.0 are increased by diamox at pH 8.0 to values higher than those found without diamox at pH 5.5. These results are consistent with an involvement of carbonic anhydrase activity in the suppression of RuBPo in seawater at pH 8.0. Whether this relates to catalysis of an extracellular conversion of HCO_3^- to CO_2, prior to CO_2 entry across the plasmalemma, or to some intracellular function of the enzyme (e.g. in catalysing transport through an aqueous phase: Raven and Glidewell 1981; Cowan 1986), is not clear. We note that either of these proposed mechanisms of carbonic anhydrase involvement could yield an increase in CO_2 compensation concentration, but that the available data do not permit these two (or any other) possibilities to be distinguished.

Table 6 shows pH compensation points and the CO_2 compensation partial pressure (Pa) at the pH compensation point in unbuffered seawater. It will be seen that members of the Fucaceae can increase the pH of seawater to pH 9.7 or above, where the CO_2 concentration is equivalent to a CO_2 partial pressure of ≤ 0.1 Pa. The Laminariales cannot raise the pH above pH 9.27 (i.e. 8.9–9.27), corresponding to a CO_2 partial pressure of 0.3–1.0 Pa. *Halidrys siliquosa* is intermediate, with a compensation pH of 9.45 and equivalent CO_2 partial pressure of ≈ 0.3 Pa. These findings are consistent with a greater capacity to "use" exogenous HCO_3^- in the Fucaceae than in *Halidrys* and, especially, in the Laminariales. However, we must remember that the capacity to extract inorganic C from solution of high pH values depends not only on the affinity for HCO_3^- and/or CO_2 and compensation concentrations for the solutes, but also on the tolerance of high pH by the organisms. It is of interest that, in shorter-terms experiments, O_2 evolution independent of exogenous inorganic C occurs (Fig. 1) in Laminariales at pH values in excess of the long-term pH compensation concentration.

Table 6. pH compensation point and CO_2 compensation partial pressure at pH compensation point. All data at 10°C and a PFD of 500 μmol photon m^{-2} s^{-1}

Species	pH compensation point	CO_2 compensation partial pressure (Pa) at pH compensation point
P. canaliculata	9.7 ; 9.75	0 ; 0
F. spiralis	9.7 ; 9.75	0 ; 0
A. nodosum	9.75	
F. vesiculosus	9.88; 9.75	0 ; 0
F. serratus	9.7 ; 9.83	0 ; 0.2
H. siliquosa	9.4 ; 9.5	0.2; 0.4
L. digitata	9.0 ; 8.9	0.7; 1.0
L. hyperborea	9.05; 9.05	0.8; 0.7
L. saccharina	9.0 ; 9.1	0.5; 0.7
A. esculenta	9.1 ; 9.27	0.6; 0.3

Some of the species listed in Table 6 were also used by Axelsson and Uusitalo (1988) in their investigation of "pH drift" in seaweeds at 20° C (their Fig. 5B). They, too, found lower final pH values for a *Laminaria* species (*L. saccharina*) than for two members of the Fucaceae (*Fucus serratus* and *Ascophyllum nodosum*); however, they found similar final pH values for *Halidrys siliquosa* as for the Fucaceae, rather than the intermediate value found in Table 6.

Discussion

The data presented in this paper on the characteristics of inorganic C assimilation under submersed conditions in the large Phaeophyte algae examined suggest that both taxonomic and ecological factors are involved in accounting for their attributes. The capacity to use HCO_3^- in photosynthesis, and the extent of the operation of CO_2 concentrating mechanism, seem to decrease as follows. The Fucales: (Fucaceae; eulittoral) shows the greatest capacity to use HCO_3^- and accumulate CO_2. The Fucalean *Halidrys siliquosa* (Cystoseiraceae; sublittoral and rock pools) and to a lesser extent the Laminarian *Alaria esculenta* (Alariaceae; lowermost eulittoral and sublittoral) are intermediate between the Fucaceae and the Laminariaceae. The Laminariaceae (Laminariales living in the sublittoral and in rock pools) are, of the organisms examined, least able to use HCO_3^- and accumulate CO_2.

Data from the literature which test the validity of these general conclusions are relatively scarce. We consider only data from experiments testing similar attributes to those investigated in the work reported in this paper.

Dealing first with other species from genera used in the present work, Brown and Tregunna (1967) found that CO_2 compensation concentration in seawater adjusted to

pH 5.0–6.0 corresponds to an equilibrium partial pressure in the gas-phase of ≤ 0.3 Pa in *Fucus gardneri* Silva. While this is lower than the values for *Fucus* spp. at pH 5.5 in Table 5, the value for *F. gardneri* is, as with other members of the Fucaceae, in the category with a CO_2 compensation partial pressure in equilibrium with seawater at pH ≤ 6.0 of less than 3 Pa CO_2. Another *Fucus* species which has been used in investigations analogous to those described here is *F. distichus* spp. *edentatus* (de la Pylaie) Powell. Cook et al. (1986) did not detect external carbonic anhydrase in *F. distichus*, contrasting with the data in Table 4 for three other *Fucus* species; the techniques used were similar. Cook et al. (1986) did not apparently test for total carbonic anhydrase activity in homogenates. The cause of this discrepancy is unknown, although it should be noted that only a small percentage of total carbonic anhydrase activity was extracellular in the Fucaceae. However, despite the small size of algal segments used in the assay, it is unlikely that the extracellular activity measured is a result of loss from cut cells; the extracellular activity in *Pelvetia canaliculata* is not significantly lower than that of larger species whose thalli were damaged more in preparation.

Turning to data for other members of families some of whose members have been investigated in this paper, we deal first with the Fucaceae, and specifically with carbonic anhydrase activity measurements on *Pelvetiopsis limitata* Gardner by Cook et al. (1986). These workers found, as with the other fucoid (*F. distichus*) which they examined, no external carbonic anhydrase activity; our comments in the last paragraph cover *Pelvetiopsis limitata* as well as *F. distichus*.

For the Cystoseiraceae there is data from the Mediterranean species *Cystoseira mediterranea* Sauv.. This sublittoral species has $K_{1/2}$ in terms of free CO_2 in seawater pH at 20° C of less than 1 mmol m^{-3} (Brechignac et al. 1987). This contrasts with the value for another sublittoral (and rock pool) member of the Cystoseiraceae, *Halidrys siliquosa* (17.4 mmol CO_2 m^{-3} at 10° C: Table 3). This difference of more than an order of magnitude in the values would repay investigation of the two algae using the same techniques, i.e. the O_2 electrode method used in this work, and the C and O mass spectrometry used by the French workers. It seems very unlikely that the differences in stirring conditions between the two sets of experiments could explain the differences.

Turning to comparisons over a greater taxonomic range, i.e. between families in an order, there are relevant data available for members of the family Sargassaceae (Fucales). Treating the results chronologically, Thomas and Tregunna (1968) found that *Sargassum muticum* (Yendo) Fensholt could increase the pH of seawater to 9.57 at temperature between 12° C and 16° C, although the extent to which this related to inorganic carbon depletion or some other pH-altering reaction was variable. Dromgoole (1978) reported experimentation on the lowermost eulittoral/sublittoral *Carpophyllum maschalocarpum* (Turn.) Grev. and *Carpophyllum flexuosum* (Esp.) Grev. in seawater at 20° C which yielded a capacity to raise the pH to 9.2–9.3. Finally, Holbrook et al. (1988) found half-saturation for total inorganic carbon at pH 8.0 and 27° C of 1.9–4.3 mol m^{-3} for the sublittoral *Turbinaria turbinata* (L.) Küntze. These values appear, by comparison with the data presented in this paper, to be consistent with the values for the Cystoseiraceae, as represented by *Halidrys siliquosa*, although temperature differences for both growth and experimentation must not be ignored.

For the Laminariales, data on the half-saturation value of photosynthetic O_2 evolution for inorganic C in seawater are available for *Macrocystis pyrifera* (L.) C. Ag. (Lessoniaceae). Wheeler (1980) found a value of 1.4–3.4 mol m^{-3} total inorganic C at 15° C depending on stirring conditions. This value accords reasonably with those (Table 3) for other families of the Laminariales. Similar agreement within the Laminariales is found for the final pH in pH-drift experiments using *Chorda filum* (L.) Stackh. (Chordaceae), *Alaria esculenta* (Alariaceae) and three *Laminaria* species (Laminariaceae): Axelsson and Uusitalo (1988), and Table 6. All of those members of the Laminariaceae are sublittoral/rock pool or (*Alaria esculenta*) sometimes lowest eulittoral.

This comparison of the results reported in this paper with literature data available for brown algal inorganic C acquisition shows that the data reported here are in *general* agreement with the more fragmentary data on related algae. Major exceptions involve the occurrence of carbonic anhydrase in the Fucaceae, and the inorganic C concentration which half-saturates photosynthesis in the Cystoseiraceae.

The eulittoral brown algae (including those, like some *Fucus vesiculosus* populations, which have returned to completely submersed life) have a very "C_4-like" physiology. Like the terrestrial C_4 plants, they have low CO_2 compensation concentrations, and the inorganic C in their natural enviroment is sufficient to saturate photosynthesis. While only data for submersed photosynthesis are reported here, these conclusions also apply to emersed photosynthesis (Johnston and Raven 1986b; bin Surif and Raven unpublished work). The eulittoral Fucaceae, like terrestrial C_4 plants, have no capacity for photosynthetic C assimilation which is not used at light saturation with their natural inorganic C concentration. As indicated earlier, the eulittoral Fucaceae may have advantages in terms of nitrogen and (when emersed) water use efficiency, analogous to those found in C_4 plants. However, while the terrestrial C_4 plants operate their CO_2 concentrating mechanisms by a mixture of enzymology and anatomy, the Fucaceae have C_3-like biochemistry plus active transmembrane transport of CO_2 and/or HCO_3^-; they are clearly good users of external HCO_3^-. The other brown macroalgae examined (Laminariales, some Fucales) have some C_4-like characteristics, although the CO_2 compensation concentration is higher than in the Fucaceae, and they have a light-saturated photosynthetic capacity which is not saturated by the seawater inorganic C concentration. These organisms are generally less avid users of HCO_3^- than are the Fucaceae, although such C_4-like (C_3–C_4 intermediate) physiological characteristics as they possess are probably based on active influx of CO_2 and/or HCO_3^- as part of a CO_2 concentrating mechanism.

The differences noted in the handling of inorganic C correlate with the habitat of the organisms. No attempts were made to break the correlation by transplant experiments, so the extent to which the differences are genetic in origin has not been established. The taxonomic (phyletic) validity of the differences in photosynthetic behavior, aside from their correlation with the habitats of the various families examined, remains to be established.

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References

Axelsson L (1988) Change in pH as a measure of photosynthesis by marine macroalgae. Mar Biol 97:287–294

Axelsson L, Uusitalo J (1988) Carbon acquisition strategies for marine macroalgae. I. Utilization of proton exchanges visualized during photosynthesis in closed system. Mar Biol 97:295–300

Björkman O, Gauhl E, Nobs MA (1969) Comparative studies of *Atriplex* species with and without β-carboxylation photosynthesis and their first-generation hybrid. Yearbook of the Carnegie Institution of Washington 68:620–633

Brechignac F, Ranger C, Andre M, Dagnemet A, Massimino D (1987) Oxygen exchange in marine algae. In: Biggins J (ed) Progress in Photosynthesis Research, vol 3. Nijhoff, Dordrecht, pp 657–660

Brooks A, Farquhar GD (1985) Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Estimates from gas exchange measurements on spinach. Planta 165:397–406

Brown DL, Tregunna EB (1967) Inhibition of respiration during photosynthesis by some algae. Can J Bot 45:1135–1143

Cook CM, Lanaras T, Colman B (1986) Evidence for bicarbonate transport in species of red and brown macrophytic marine algae. J. Exp Bot 37:977–984

Cowan IR (1986) Economics of carbon fixation in higher plants. In: Givnish TJ (ed) On the economy of plant form and function. Cambridge University Press, New York, pp 133–170

Davison IR (1987) Adaptation of photosynthesis in *Laminaria saccharina* (Phaeophyta) to change in growth temperature. J Phycol 23:273–283

Davison IR, Davison JO (1987) The effect of growth temperature on enzyme activities in the brown alga *Laminaria saccharina*. Br Phycol J 22:77–87

Dromgoole FI (1978) The effect of pH and inorganic carbon on photosynthesis and dark respiration of Carpophyllum (Fucales, Phaeophyceae). Aquatic Bot 4:11–22

Graham D, Smillie RM (1976) Carbonate dehydratase in marine organisms of the Great Barrier Reef. Aust J Plant Physiol 3:113–119

Heath OVS, Orchard B (1957) Temperature effects on the minimum intercellular space carbon dioxide concentration. Nature 180:180–181

Holbrook GP, Beer S, Spencer E, Reiskind JB, Davis JS, Bowes G (1988) Photosynthesis in marine macroalgae: Evidence for carbon limitation. Can J Bot 66:577–582

Johnston AM, Raven JA (1986a) The utilization of bicarbonate ions by the macroalgae *Ascophyllum nodosum* (L.) Le Jolis. Plant Cell Envir 9:175–184

Johnston AM, Raven JA (1986b) The analysis of photosynthesis in air and water by *Ascophyllum nodosum* (L.) Le Jolis. Oecologia 69:288–295

Jolliffe EA, Tregunna EB (1970) Studies on HCO_3^- ion during photosynthesis in benthic marine algae. Phycologia 9:293–303

Jordan DB, Ogren WC (1981) Species variation in the specificity of ribulose bisphosphate carboxylase-oxygenase. Nature 291:513–515

Jordan DB, Ogren WL (1984) Species variation in the specificity of ribulose bisphosphate carboxylase-oxygenase. Arch Biochem Biophys 227:425–433

Kerby NW, Raven JA (1985) Transport and fixation of inorganic carbon by marine algae. Adv Bot Res 11:71–123

MacFarlane JJ, Raven JA (1985) External and internal CO_2 transport in *Lemanea*: interaction with the kinetics of ribulose bisphosphate carboxylase. J Exp Bot 36:610–622

Mehrback C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constant of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18:897–907

Miller AG, Colman B (1980) Active transport and accumulation of bicarbonate by a unicellular cyanobacterium. J Bacteriol 143:1253–1259

Park PK (1969) Oceanic CO_2 system: An evaluation of ten methods of investigation. Limnol Oceanogr 14:179–186

Raven JA (1984) Energetics and transport in aquatic plants. MBL Lectures in Biology. Alan R Liss Inc, New York

Raven JA, Glidewell SM (1978) C_4 characteristics of photosynthesis in the C_3 alga *Hydrodictyon africanum*. Plant Cell Envir 1:185–197

Raven JA, Glidewell SM (1981) Processes limiting photosynthetic conductance. In: Johnson CB (ed) Physiological Processes Limiting Plant Productivity. Butterworths, London, pp 109–136

Raven JA, Samuelson G (1988) Ecophysiology of *Fucus vesiculosus* L. close to its northern limit in the Gulf of Bothnia. Bot Mar (in press)

Raven JA, Johnston AM, MacFarlane JJ, bin Surif M, McInroy S (1987) Diffusion and active transport of inorganic carbon species in freshwater and marine macroalgae. In: Biggins J (ed) Progress in Photosynthesis Research vol 4. Nijhoff, Dordrecht, pp 333–340

Riley JP, Skirrow G (1975) (eds) Chemical Oceanography vol 1. 2nd Edn. Academic Press New York

Sand-Jensen K, Gordon DM (1984) Differential ability of marine and freshwater macrophytes to utilize HCO_3^- and CO_2. Mar Biol 80:247–253

Seeley RR, Duncan MJ, Vidaver WE (1972) Preparative and analytical extraction of pigments from brown algae with dimethyl sulfoxide. Mar Biol 12:184–188

Skirrow G (1975) The dissolved gases-carbon dioxide. In: Riley JP, Skirrow G (eds) Chemical Oceanography vol 2. 2nd Edn, Academic Press, New York, pp 1–192

Thomas EA, Tregunna EB (1968) Bicarbonate ion assimilation in photosynthesis by *Sargassum muticum*. Can J Bot 46:411–415

Wheeler WN (1980) Effect of boundary layer transport on the fixation of carbon by the giant kelp *Macrocystis pyrifera*. Mar Biol 56:103–110

Wilbur KM, Anderson NG (1948) Electrometric and colorimetric determination of carbonic anhydrase. J Biol Chem 176:147–154

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