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The analysis of photosynthesis in air and water of *Ascophyllum nodosum* (L.) Le Jol.

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Summary. The photosynthetic characteristics for the intertidal macroalga *Ascophyllum nodosum* were examined in air and water. Under ambient conditions of temperature (10° C) inorganic carbon concentrations (15.63 mmol CO_2 m^{-3} or 2.0 mol TIC m^{-3}) and light (500 μmol photons m^{-2} s^{-1}) photosynthesis was slightly greater by the exposed alga than by the submerged alga. In both environments photosynthesis was light saturated at 200 μmol photons m^{-2} s^{-1}. The relationship between CO_2 concentration and photosynthesis in air could be accurately analysed using Michaelis-Menten kinetics, although the range of concentrations used were not saturating. In contrast the application of the Lineweaver-Burk and Woolf plots to aquatic photosynthesis was not suitable as the experimental data was similar to the Blackman type curves and not rectangular hyperbolae. This was reflected by the applicability of the Hill-Whittingham equation to describe the photosynthesis curves. The effect of unstirred layers and other limiting factors is discussed in relation to the kinetic parameters, V_{max} and K_m.

Inter-tidal macroalgae differ from land plants in numerous ways some of which are important when considering their photosynthetic capabilities. They are exposed of two different environments, air and water, to varying degrees depending on their bathometric location. As macroalgae do not possess xylem or stomata they are unable to regulate their water status (Schonbeck and Norton 1979) and so following a short period of exposure to air they suffer from the effects of desiccation. Their lack of rigidity results in the thallus lying flat on top of other plants when exposed; this may reduce the effects of desiccation but also causes self shading and reduction of CO_2 diffusion. The submerged alga, although it is in a medium which has a total inorganic carbon concentration (TIC) of 2.0 mol m^{-3} TIC, 125 times that found in air, 14.39 mmol m^{-3}, is often limited photosynthetically by the depth of water as the availability of light is rapidly attenuated with depth. Investigators of the ability of inter-tidal algae to photosynthesise in air and water have often been restricted to a simple comparison of the photosynthetic rates observed under ambient conditions in the two environments (Johnson et al. 1974 and Quadir et al. 1979). However, a better and more ecologically relevant understanding of photosynthesis will be gained if the response of different macroalgae to changes in inorganic carbon concentration and photon flux density is compared.

Characterising photosynthesis in short term studies usually consists of observing the relationship between photosynthesis, inorganic carbon concentration and photon flux density. Three ways in which such relationships are often described are:

1) Michaelis-Menten kinetics; this derives two parameters, the V_{max} is defined as the maximum rate at which the system can function (e.g. μmol C m^{-2} s^{-1}) and the K_m the concentration (mol m^{-3}), at which the uptake rate is half the maximum rate.

$$v=\frac{V_{max}\cdot c}{K_m+c}. \tag{1}$$

Then for a given concentration of inorganic carbon, c, the photosynthetic rate, v, is given by Eq. (1). This enables the comparison between different types of plants and is often related to the primary carboxylating enzyme involved in CO_2 assimilation: ribulose bisphosphate carboxylase (RuBPc). Yet there are examples when this equation does not describe the relationship between the apparent photosynthetic rate (APS) and [TIC] (Lilly and Walker 1975; Smith and Walker 1980; Farquhar et al. 1980).

2) The Hill-Whittingham equation; incorporates a permeability constant to account for the diffusion resistance imposed by the unstirred layer (Hill and Whittingham 1955; MacFarlane and Raven 1985).

$$v=0.5\{(K_m\cdot P_u+c\cdot P_u+V_{max})-[(K_m\cdot P_u+c\cdot P_u+V_{max})^2-4\cdot c\cdot P_u\cdot V_{max}]^{0.5}\} \tag{2}$$

where the permeability of the unstirred layer (P_u, m s^{-1}) is defined as the uptake rate divided by the corresponding inorganic carbon concentration difference across the unstirred layer, (Δc),

$$P_u=\frac{v}{\Delta c}. \tag{3}$$

The thickness of the unstirred layer for carbon dioxide (d, m) is given by Eq. (4) at low pH,

$$d=\frac{D}{P_u} \tag{4}$$

where D is the diffusion coefficient of CO_2 (m^2 s^{-1}).

3) Theoretical models; photosynthesis is summarised by a few simple equations which represent the ratio of photorespiration to photosynthesis, the rates of electron transport,

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the rate of "dark" respiration in the light and the rate of ribulose bisphosphate (RuBP) carboxylation and regeneration (Farquhar and von Caemmerer 1982).

This study characterized the photosynthetic properties of *Ascophyllum nodosum* (L.) Le Jol. and compares them with data from other macroalgae and terrestial vascular plants. This is part of a general investigation into the carbon physiology of *A. nodosum*, which includes determining whether it can utilize HCO_3^- ions as a source of inorganic carbon, and whether this alga exhibits the characteristics of photorespiration.

Materials and methods

Seawater (18%. Cl) was collected from the mouth of the Tay estuary at high tide. The water was filtered through Whatman GF/C filters using a vacuum pump then aerated with laboratory air in the dark to re-establish air equilibrium. Samples of *A. nodosum* were collected from the north side of the estuary, O.S. Grid Reference NO 392 292, and were used within 24 h of collection. The surface area/fresh weight was determined by measuring the average circumference, length and weight of the fresh thallus. The fresh to dry weight ratio was obtained by weighing the thallus before and after it had been placed in an oven (60° C). Chlorophylls *a* and *c* were extracted in 90.0% methanol with a morter and pestle and recovered by centrifugation. Pigment concentrations were determined using the equations of Jeffery and Humphrey (1975).

Measurement of photosynthesis by the exposed alga

The APS in air was determined with an Infra-Red Gas Analyser (IRGA, Grubb Parsons model IR 120) calibrated at 0 and 17.86 mmol CO_2 m^{-3}. The APS at air CO_2 levels was obtained by measuring the time taken to reduce the $[CO_2]$ from 15.63 to 13.39 mmol CO_2 m^{-3}. The alga was suspended in a water-jacketed glass chamber illuminated by a 500 W lamp. The effect of desiccation on the APS was determined by observing the APS over 180 min with repeated additions of pure CO_2 to the chamber to maintain the $[CO_2]$. The alga was weighed before and after each experiment so that the rate of water loss from the alga while it was suspended in air could be calculated. The photon flux density (PFD) was 500 μmol m^{-2} s^{-1} (400–700 nm), a density generally found to be saturating for inter-tidal algae (Lüning 1981). In separate experiments after 30–40 min of exposure to air the $[CO_2]$ was allowed to fall to observe the relationship between the $[CO_2]$ and the APS and the lowest concentration attainable, the CO_2 compensation point. To observe the effect light has on the photosynthetic rate, the incident PFD was varied from 0 to 600 μmol m^{-2} s^{-1} and the APS determined at 15.63 mmol CO_2 m^{-3}.

Determining the effect total inorganic carbon concentration has on submerged photosynthesis

O_2 dependent rates of APS in water were obtained using an oxygen electrode (Rank Bros., Cambridge, England) and Carbon 14 bicarbonate ($H^{14}CO_3^-$). For oxygen electrode measurements, 0.1 g fresh weight (Fwt) of alga was placed in the oxygen electrode chamber with 4.0×10^{-6} m^3 unbuffered seawater. The rates of oxygen evolution around air-equilibrium O_2 concentrations were used as a measure of APS. The PFD incident on the outer casing of the oxygen electrode was altered to investigate the relationship between PFD and APS in water.

CO_2-free seawater was prepared by acidifying seawater with HCl to a pH less than 4.5 and then bubbling with CO_2-free nitrogen (passed through a soda lime trap) for 1 h. The pH of the solution was then slowly altered with NaOH to the required pH during which time buffers, 30 mol m^{-3} Tris(hydroxymethyl)aminomethane (TRIS) pH 8.0, or 30 mol m^{-3} 2-[N-Morpholino]ethanesulfonic acid (MES) pH 5.2, were added if needed. Algae which had been pre-incubated in the light in CO_2-free seawater for 1 h were placed in an oxygen electrode with 4–5×10^{-6} m^3 CO_2-free seawater (pH 8.0). When there was no further change in the $[O_2]$, $NaHCO_3$ was added to make up final TIC concentrations of 0.2–10.0 mol m^{-3}. This was repeated with seawater buffered at pH 5.20 except that the largest [TIC] was 0.6 mol m^{-3}. At the low pH, unlike that found at the higher pH, oxygen evolution continued after the alga had been placed in the CO_2-free seawater, often for as long as three hours. The photosynthetic rate was taken as the maximum rate of oxygen evolution following the addition inorganic carbon less the rate of oxygen evolution at the time the carbon was added.

The uptake of $H^{14}CO_3$ was also used to investigate the relationship between photosynthesis and [TIC] at pH 8.0. Apices (0.1 g fwt) of *A. nodosum* were added to 20×10^{-6} m^3 CO_2-free seawater in a 25×10^{-6} m^3 oxygen demand bottle (BOD). $NaHCO_3$ was injected (final [TIC] of 0.2–10.0 mol m^{-3}), followed by 10×10^{-9} m^3 $NaH^{14}CO_3$ (specific activity 2.4×10^3 kBq/10^{-6} m^3 seawater). Small aliquots (0.2×10^{-6} m^3) were taken to obtain the specific activity of the media. The algae were exposed to the ^{14}C for 15 min and then they were removed, rinsed, and blotted dry. The ethanol soluble ^{14}C fraction was extracted by grinding the thallus with a pestle and mortar with the addition of quartz sand and 90% ethanol. This was followed by repeated ethanol extraction until no further radioactivity was recovered. The radioactivity of the pooled extracts was measured by counting an aliquot in a Packard Instruments scintillation counter.

Analysis of the response of photosynthesis to the inorganic carbon concentration

The relationship between $[CO_2]$ and APS was analysed using the Lineweaver-Burk (reciprocal of uptake rate against reciprocal of concentration) and Woolf (quotient of concentration and uptake rate, against concentration) methods, to obtain values of V_{max} and K_m.

Results

The fresh weight, dry weight, surface area and pigment relationships of *A. nodosum* are given in Table 1. In general they are in reasonable agreement with previously published values (Seybold and Egle 1937; Baardseth 1970; Ramus et al. 1977).

Upon exposure to air, the APS of the alga first increases, then levels off and finally decreases (Fig. 1). This response is similar to those reported by Brinkhuis et al. (1976) and Quadir et al. (1979). It was during the time when APS is at its maximum that further physiological experiments were

Table 1. The relationships between fresh weight, dry weight, chlorophyll *a* and *c*, and thallus surface area for *Ascophyllum nodosum*. The numbers in brackets are standard deviation ($n = 4$)

Fresh weight / Dry weight	4.39 (0.16)
Chloro *a* / Dry weight	1.254 (0.096) mg g^{-1} Dwt
Chloro *c* / Dry weight	0.284 (0.038) mg g^{-1} Dwt
Surface area / Fresh weight	15.02 cm^2 g^{-1} Fwt

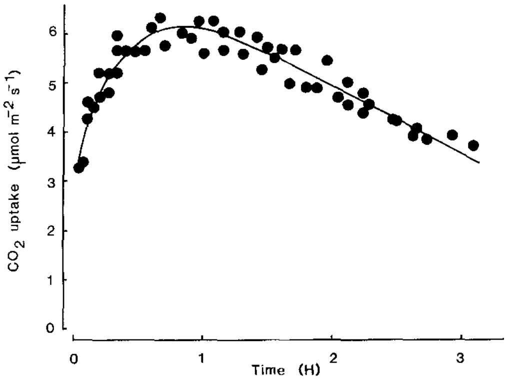

Fig. 1. The rate of apparent photosynthetic CO_2 assimilation by *Ascophyllum nodosum* following exposure to air, temperature, 10° C; PFD, 500 μmol m^{-2} s^{-1}. Results of two experiments

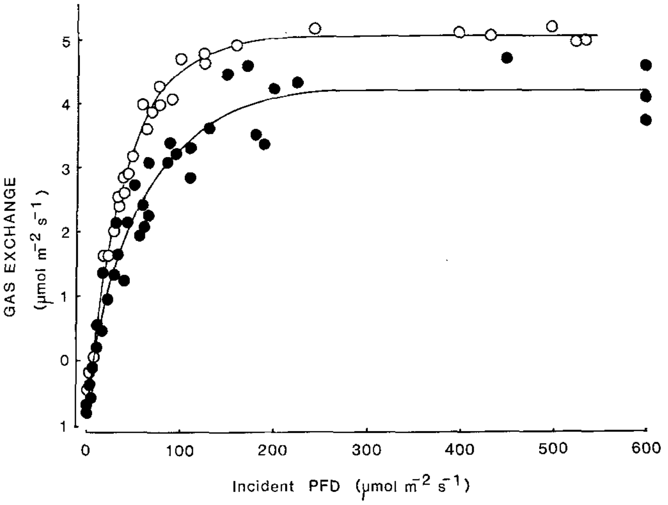

Fig. 2. Light saturation curves of *Ascophyllum nodosum* in air (open circles, μmol CO_2 m^{-2} s^{-1}) and water (closed circles, μmol O_2 m^{-2} s^{-1}), temperature, 10° C. Results of three and five experiments respectively

carried out (20–60 min after the onset of exposure to air). All rates are expressed as (mol C m^{-2} s^{-1}) and refer to the initial biomass of the alga before any water loss occurs.

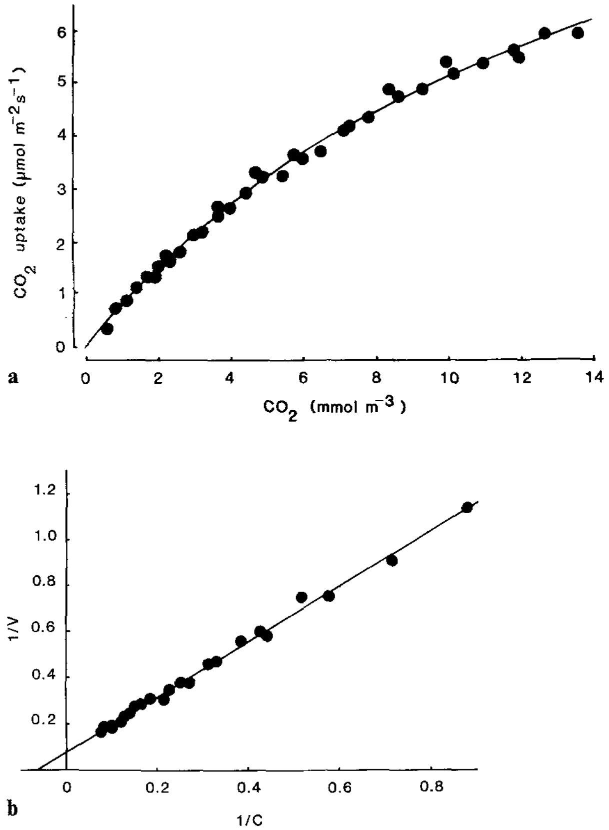

Fig. 3a. The rate of apparent photosynthetic CO_2 assimilation by *Ascophyllum nodosum* in air as a function of CO_2 concentration, temperature, 10° C; PFD, 500 μmol m^{-2} s^{-1}. Results of five experiments. Solid line represents the predicted line using Eq. (1), V_{max} 13.00 μmol m^{-2} s^{-1}, K_m 15.50 mmol m^{-3}; **b** The Lineweaver-Burk plot of **a**. $V_{max} = 13.00$ μmol m^{-2} s^{-1}, $K_m = 15.50$ mmol m^{-3}, $r^2 = 0.997$

Figure 2 shows the response of APS to different photon flux densities in air ($[CO_2] = 14.51$ mmol m^{-3}), and in water ($[TIC] = 1.8$ mol m^{-3} (pH 8.0)) respectively. Both show that photosynthesis is saturated at 200 μmol photon m^{-2} s^{-1}. Atmospheric CO_2 concentrations do not saturate photosynthesis when the alga is exposed (Fig. 3a). This Fig. also shows that the alga exhibits a low CO_2 compensation point (<0.045 mmol CO_2 m^{-3}) in air, like the C_4 higher plants. Figure 3b shows the data in Fig. 3a in a Lineweaver-Burk plot. The linear transformation gives a good regression coefficient ($r^2 = 0.997$) and predicts the kinetic parameters $V_{max} = 13.00$ μmol CO_2 m^{-2} s^{-1} and $K_m =$ 15.50 mmol CO_2 m^{-3}.

The relationship between [TIC] and APS in seawater departs from the relationship shown in Fig. 3b (Fig. 4). Inorganic carbon saturation is achieved at TIC concentrations of 1.0–2.0 mol m^{-3} at pH 8.0 (Fig. 4) and only 0.2 mol m^{-3} at pH 5.2 (Fig. 4). Also the maximum rates at the high pH are only comparable with the unsaturated atmospheric rates. Lineweaver-Burk (Fig. 5a) and Woolf (Fig. 5b) plots of the data in Fig. 4, for the uptake curve pH 8.0 measured with ^{14}C, are biphasic rather than linear. The flat portion of the curve (Fig. 5b, low [TIC]) indicates that the rate of ^{14}C uptake is proportional to the concentration. The other section (high [TIC]) has the characteristics of an enzymic system obeying Michaelis-Menten kinetics (Eq. (1)). Similar biphasic curves were also found when the data of oxygen evolution at pH 8.0 and 5.2 were analysed. The values of V_{max} and K_m are shown in Table 2. Substituting the respective values obtained from both methods into Eq. (1) for different concentrations of TIC, it is

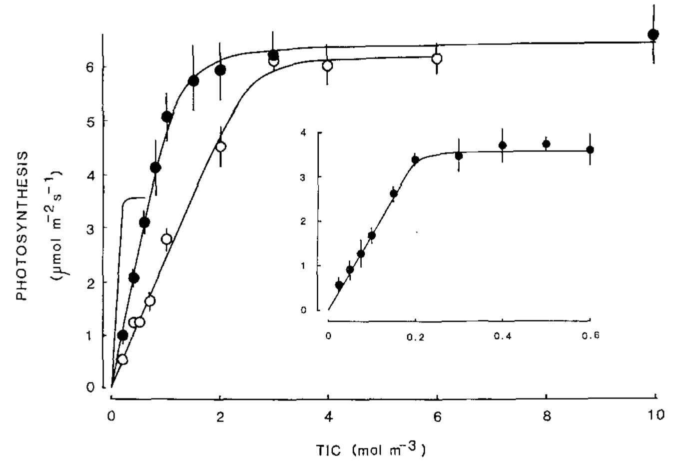

Fig. 4. The rate of photosynthesis as a function of the total inorganic carbon concentration for *Ascophyllum nodosum*. Experimental methods: pH 8.0 ^{14}C uptake (closed circles), pH 8.0 O_2 evolution (open circles) and inset pH 5.2 O_2 evolution (also shown in the main figure as line with out symbols), temperature, 10° C; PFD, 500 μmol m^{-2} s^{-1}. The solid lines are those predicted by the NAG routine for the Hill-Whittingham equation, $V_{max}=6.466$ μmol m^{-2} s^{-1}, $K_m=57.1$ mmol m^{-3} and $P_u=5.829 \times 10^{-6}$ m s^{-1}; $V_{max}=6.215$ μmol m^{-2} s^{-1}, $K_m=28.0$ mmol m^{-3} and $P_u=2.488 \times 10^{-6}$ m s^{-1}; $V_{max}=3.575$ μmol m^{-2} s^{-1}, $K_m=0.57$ mmol m^{-3} and $P_u=17.64 \times 10^{-6}$ m s^{-1} respectively. The vertical lines denote the standard deviation of uptake at each concentration ($n=4$)

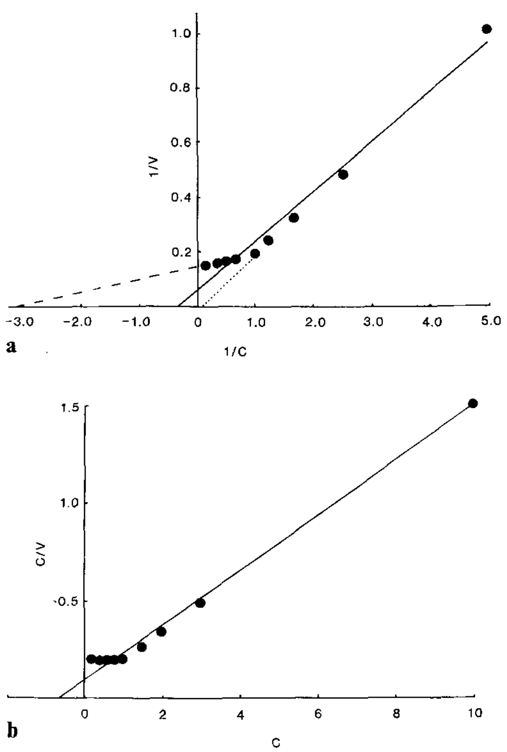

Fig. 5a. The Lineweaver-Burk plot of ^{14}C uptake pH 8.0, Fig. 4. The biphasic nature of the data when plotted are shown by the dotted line (high concentrations) and the dashed line (low concentrations). The solid line is fitted through all of the points. Each point represents the mean of four plants. The over all V_{max} and K_m values for the three uptake curves are given in Table 2. **b** The Woolf plot of ^{14}C uptake pH 8.0, Fig. 4. Note that this curve shows the same biphasic plot as that shown in Fig. 5a. Each point represents the mean of four plants. The over all V_{max} and K_m values for the three uptake curves are given in Table 2

Table 2. The kinetic parameters describing the relationship between photosynthetic ^{14}C uptake or oxygen evolution and the total inorganic carbon concentration. (1) Lineweaver-Burk, (2) Woolf

Method	V_{max} μmol m^{-2} s^{-1}	K_m (TIC) mol m^{-3}	Experimental V_{max}
O_2 evolution pH 8.0	(1) 18.19 (2) 6.89	6.56 0.662	6.08
^{14}C uptake pH 8.0	(1) 16.16 (2) 7.23	2.918 0.995	6.27
O_2 evolution pH 5.2	(1) 5.31 (2) 3.89	0.194 0.027	3.644
CO_2 uptake in air	(1) 13.00	0.0155	

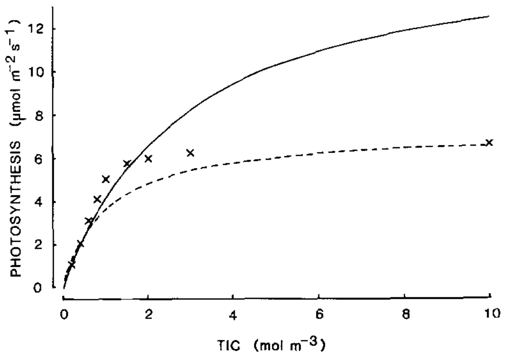

Fig. 6. The curves predicted by Michaelis-Menten kinetics (Eq. (1)) using V_{max} and K_m values obtained with the Lineweaver-Burk plot (solid line) and the Woolf plot (dashed line) of the data shown in Fig. 4. The crosses denote the experimental rates of ^{14}C uptake observed at the nine different concentrations

Table 3. The parameters obtained from the least squares curve fitting program which describe aquatic photosynthesis with the Hill-Whittingham equation and the P_u value predicted by Eq. (3)

pH	Method	Least squares program values			Eq. (3) value
		V_{max} μmol m^{-2} s^{-1}	K_m mmol m^{-3}	P_u 10^{-6} m s^{-1}	P_u 10^{-6} m s^{-1}
8.0	^{14}C uptake	6.466	57.10	5.829	5.10
8.0	O_2 evolution	6.215	28.00	2.488	2.51
5.2	O_2 evolution	3.575	0.570	17.63	17.30

apparent that neither method of Michaelis-Menten analysis properly describes aquatic photosynthesis. The Woolf parameters are influenced more by the saturated rates than the unsaturated rates whereas the reverse is true for the Lineweaver-Burk parameters, see Fig. 6.

At low [TIC] it is possible that photosynthesis is limited by diffusion across the unstirred layer accounting for the proportionality between the rate of photosynthesis and [TIC] and hence the constant P_u values. The mean P_u values obtained from Eq. (3) were 5.1×10^{-6} m s^{-1} (^{14}C pH 8.0), 2.51×10^{-6} m s^{-1} (O_2 pH 8.0) and 1.73×10^{-5} m s^{-1} (O_2

pH 5.2), assuming the [TIC] on the "plant" side of the unstirred layer is zero. Smith and Walker (1980) used a least square analysis to determine values of V_{max}, K_m and P_u (Eq. (2)) for the carbon uptake studies of Lucas (1975) on *Chara corallina.* This method was used in the present study using the NAG routine EO4FCF. The actual values for V_{max}, K_m and P_u so obtained for the three uptake curves are shown in Table 3.

Discussion

The photosynthetic rates presented here for *A. nodosum* are in general agreement with those previously reported by Kanwisher (1966) and Chock and Mathieson (1979). The response to light is typical for inter-tidal macroalgae, which do not exhibit photoinhibition of photosynthesis at high photon flux density during short term studies, and are saturated by about 200–500 μmol photon m^{-2} s^{-1} (Lüning 1981). The values reported here (200 μmol photon m^{-2} s^{-1}) are lower than many in the literature but agree well with those for *A. nodosum* reported by Chock and Mathieson (1979).

The simple comparison between the photosynthetic rates observed under ambient conditions agrees well with the suggestion that algae located towards upper reaches exhibit greater rates in air than in water, the reverse being true for algae found lower down (Johnson et al. 1974; Quadir et al. 1979). Although similar dissolved CO_2 concentrations were used for comparisons of the photosynthesis measurements in air and water, higher rates were found for *A. nodosum* when the alga was exposed to air dispite a higher [TIC] in water.

It is apparent that Michaelis-Menten kinetics can be used to describe photosynthesis of the alga exposed to air but not when applied to the submerged alga. The Lineweaver-Burk plot is dis-proportionately influenced by data points at the lower concentrations whereas the reverse is true for the Woolf plot, Fig. 6. The fact that the theoretical V_{max} obtained by the Woolf method is much closer to the experimental V_{max} than that obtained from the Lineweaver-Burk plot highlights one of the main drawbacks in using Michaelis-Menten kinetics in such a role. Originally they were defined to describe simple cell free enzyme assays which are often single step reactions, for which they are well suited. It is difficult to find an enzyme-catalysed reaction in a well-stirred, homogeneous solution which is diffusion limited because P_u values for individual enzyme molecules are large. When this approach is applied to in vivo photosynthesis certain complications arise. The uptake of inorganic carbon from the bulk phase can be separated into two parts, the transport of the carbon to the carboxylating enzyme (often considered in terms of a diffusion gradient, see Kerby and Raven 1984) and the fixation of inorganic carbon by the enzyme RUBISCO (the driving force of the diffusion process). The degree to which each part is rate controlling will influence the shape of the inorganic carbon response curve. In a totally transport controlled system the shape will be that of a Blackman-type curve, linear initially, followed by a rapid transition to saturation determined by the carboxylating enzyme or some other factor. At the other extreme when the relationship between APS and [TIC] is enzyme-controlled the curve will be the characteristic rectangular hyperbola.

Under exposed conditions there is little sign that the alga is saturated by atmospheric concentrations of CO_2. The application of the Michaelis-Menten analysis gave values of V_{max} and K_m which describe the experimental data (Fig. 3a). The predicted V_{max}, 13.00 μmol CO_2 m^{-2} s^{-1}, is much higher than the experimental submerged saturated rates. As the diffusion coefficient for CO_2 in air is 10,000 times larger than the value for water the diffusion resistance imposed by an unstirred layer will not be so significant and so the uptake curve in air primarily reflects the enzyme control. A value of 15.50 mmol CO_2 m^{-3} is towards the lower end of the in vitro K_m values for RuBPc (Jordan and Ogren 1981, 1983; Yeoh, Badger and Watson 1981). Comparisons between in vivo and in vitro studies are difficult as the conditions of the in vitro assays can greatly influence both the V_{max} and K_m. Carbonic anhydrase is frequently omitted so the kinetics of the carboxylation may be limited by the dehydration of HCO_3^- to CO_2 (Bird et al. 1980), decreasing temperature reduces both parameters (Badger 1980; Jordan and Ogren 1984), and the ionic strength of the assay mixture influences the pK_a from which the HCO_3^-/CO_2 ratio is obtained (Besford 1984). There are few estimates of Phaeophycean RuBPc activity due to the difficulty of the extraction procedure (see Kerby and Raven 1985).

When the values of V_{max}, K_m and P_u presented in Table 3 are used in the Hill-Whittingham equation for a range of carbon concentrations, the resulting curve (solid lines, Fig. 4) gives a better fit to the data than that produced by the Michaelis-Menten equation (Fig. 6) using only V_{max} and K_m, Smith and Walker (1980). The difference between the Hill-Whittingham and the Michaelis-Menten curves is much greater than the analysis by Smith and Walker (1980) for *C. corallina* because they used a $K_m = 1/2\ V_{max}$, whereas we have used values obtained from the Michaelis-Menten analysis. Dromgoole (1978) and Wheeler (1980) have both used different forms of the Hill-Whittingham equation to describe macroalgal respiration and photosynthesis respectively. Wheeler (1980) obtained the parameters V_{max} and K_m with the Woolf plot which was attempted without success in the present work.

The P_u values presented here for the different pH conditions are larger than those reported by Smith and Walker (1980) due in large part to the different stirring regimes. The *C. corallina* studies were carried out in test tubes which resulted in large unstirred layers (it should be noted that *C. corallina* and *A. nodosum* have different morphologies). Using Eq. (4) the maximum thickness of the unstirred layer was found to be 71 μm at pH 5.20 ($P_u = 17.30 \times 10^{-6}$ m s^{-1}) if the diffusion coefficient of CO_2 (D) is 1.25×10^{-9} m^2 s^{-1} (Himmelblau, 1964, extrapolated to 10° C).

The literature concerning values of K_m (TIC) for photosynthesis of macroalgae is sparse. Wheeler (1980) reported that in general K_m values ranged between 1–3 mol m^{-3} TIC citing his own work and that of Jolliffe and Tregunna (1970) and Ogata and Matsui (1965), although he alone attempted to evaluate the kinetics of his data. Recently there have been reports of lower K_m values: 0.4 mol m^{-3} for *Ulva* sp. (Beer and Eshel 1983), 0.54 mol m^{-3} for *Fucus vesiculosus* (Sand-Jensen and Gordon 1984) and 0.14 mol m^{-3} for *Ulva rigida* (MacFarlane 1985). Two species which are found towards the lower part of the tidal reach, *Chondrus crispus* and *F. vesiculosus*, exhibit high K_m values when exposed to air, 89.29 mmol m^{-3} CO_2 (Bidwell et al. 1980) and 42.41 mmol CO_2 m^{-3} (Bidwell and

McLachlan 1985) respectively, but low K_m values when submerged (1.0 mmol m^{-3} CO_2 (Brechignac and Andre 1984) and 2.36 mmol m^{-3} CO_2 (Sand-Jensen and Gordon 1984) respectively). Whether this is because these algae spend a greater proportion of the diel cycle submerged remains to be seen but it could be significant that *A. nodosum*, a mid-tidal alga, exhibits apparent K_m values in the two environments which are similar, 14.97 mmol CO_2 m^{-3} (air) and 6.70 mmol CO_3 m^{-3} (water).

There appears to be little difference between the V_{max} exhibited in air and water by *F. vesiculosus* (Bidwell and McLachlan 1985). The nature of the uptake curves is similar to those presented here; the exposed curve is a rectangular hyperbola whereas the submerged uptake curve is of the Blackman type. There are two possible explanations as to why the V_{max} predicted by Michaelis-Menten analysis from the exposed *A. nodosum* is not exhibited by the submerged alga under saturating inorganic carbon concentrations. These can be grouped together under the general heading of transport control.

The first limitation maybe the rate of electron transport which controls the rate at which RuBP is synthesized, as suggested by Farquhar et al. (1980). The V_{max} and apparent K_m values describing the curve shown in Fig. 7a are both lower than the RUBISCO values. Thus at saturating concentrations of CO_2 the experimental V_{max} will be determined by the maximum rate at which the electron transport system will operate. At sub-saturating concentrations the rate will be determined by the RUBISCO kinetics except that the diffusional limitation due to the unstirred layer will superimpose itself to produce the typical Blackman type curve. However, Dietz et al. (1984) suggest that electron transport is less important in limiting photosynthesis than previously thought.

The second limitation on in vivo V_{max} might be inorganic carbon membrane transport; using the same arguments to those used for electron transport, the same shape of curve can be obtained. Raven and Smith (1980) considered that the maximum rate of primary active transport would be within the range of 1–10 μmol solute m^{-2} s^{-1} and the V_{max} values of 6.215–6.466 μmol C m^{-2} s^{-1} are with in this suggested range. Spalding and Ogren (1983) and Kaplan et al. (1980) have published K_m values of 0.16 mol m^{-3} and 0.15 mol m^{-3} for the membrane transport of HCO_3^- by *Chlamydomonas reinhardtii* and *Anabaena variabilis* respectively. Figure 7b shows that a lower K_m value is required to give a curve which, when the diffusion limited initial slope is added, gives a rapid transition to saturation. This is a similar to the suggestion of MacFarlane and Raven (1985) who said the value of $V_{max}/2$ K_m needs to be at least 20 times the corresponding P_u value. As a comparison the V_{max} values reported here are equivalent to 225–233 μmol C dm^{-2} h^{-1} a range of rates which is at least twice the size of many rates reported for marine macroalgae (Kremer 1981). Whether or not the latter are saturated rates is difficult to determine, but as they were mostly made in natural seawater ([TIC] = 2.0 mol m^{-3}) the [TIC] was probably above the apparent K_m, and it is likely that the saturated rates reported here are amongst the highest exhibited by submerged macroalgae.

Although we have assumed that the initial linearity of the relationship between photosynthesis and [TIC] is due to diffusion limitation, there is an alternative possible explanation, namely that RUBISCO is always operating at CO_2 concentrations that are well below its V_{max}. At low [TIC], Eq. (1) approximates to the equation for the kinetics of a first order reaction. Then $v = (V_{max}/K_m)$ and as the minimum P_u is equal to v/S then P_u (minimum) = V_{max}/K_m. The increase in V_{max} results in a proportional increase in the K_m if the initial slope of v verses c remain constant. Figure 7b shows how an increase in V_{max} and K_m values produces an initial slope at low concentrations similar to that imposed by diffusional limitation in Fig. 7a and also that the RUBISCO V_{max} needs to be 60 times the in vivo experimental value to describe the data. Table 4 gives the extreme K_m values for the transport controlled (minimum values) and enzyme controlled (maximum values) systems necessary to produce Blackman-type curves together with the values obtained by the Hill-Whittingham equation.

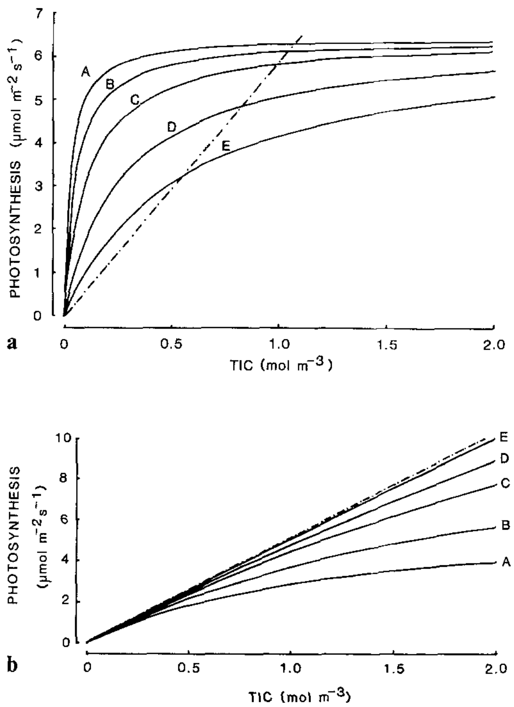

Fig. 7a. The theoretical relationship between transport control, diffusional limitation and decreased K_m required to produce a biphasic curve observed experimentally. The apparent V_{max} was equal to 6.466 μmol CO_2 m^{-2} s^{-1}. The incomplete line represents an initial slope imposed by diffusional limitation ($P_u = 5.10 \times 10^{-6}$ m s^{-1}). The Michaelis constants for each curve are A = 27.7 mmol m^{-3}, B = 55.4 mmol m^{-3}, C = 0.110 mol m^{-3}, D = 0.277 mol m^{-3} and E = 0.554 mol m^{-3}. **b** The change in V_{max} and K_m values required for the initial slope to be similar to that imposed by diffusional limitation. The values for V_{max} and K_m for each curve are: A, 6.466 μmol m^{-2} s^{-1} 1.268 mol m^{-3}; B, 12.932 μmol m^{-2} s^{-1} 2.535 mol m^{-3}; C, 32.33 μmol m^{-2} s^{-1} 6.34 mol m^{-3}; D, 64.66 μmol m^{-2} s^{-1} 12.68 mol m^{-3} and E, 387.9 μmol m^{-2} s^{-1} 76.08 mol m^{-3} respectively. The incomplete line represents an initial slope imposed by diffusional limitation ($P_u = 5.10 \times 10^{-6}$ m s^{-1})

If the results from ^{14}C uptake and O_2 evolution studies at pH 8.0 are compared then it is apparent that the method employed in measuring photosynthesis is of great importance as the initial slopes of the two curves differ considerably. This is not unexpected as the stirring was poorer in the oxygen electrode chamber than in the BOD bottles. If the stirring is improved, so reducing the unstirred layer and removing the diffusional component, this would lead

Table 4. The K_m values needed to satisfy the arguments on which Fig. 7a and b are based. (1) Transport controlled, (2) Enzyme controlled, (3) Predicted value by NAG routine

	$K_m^{(1)}$ mmol m^{-3}	$K_m^{(2)}$ mol m^{-3}	$K_m^{(3)}$ mmol m^{-3}
^{14}C uptake	27.7	76.08	57.10
O_2 evolution	62.4	149.8	28.00
O_2 evolution	3.06	12.18	0.57

to a more rectangular hyperbola-like curve. Whether this curve would reflect the carboxylation kinetics is difficult to foresee as the interaction of a "limited" V_{max} (i.e. transport control) at high [TIC] and an enzyme controlled initial slope will still remain. In support of this is that the analysis of the data presented in Fig. 4 for the uptake curve pH 8.0 measured with ^{14}C predicts a K_m value in between the minimum and maximum values presented in Table 4 and the P_u value predicted by the NAG routine is greater than the value calculated using Eq. (3), (Table 3). From the oxygen electrode studies the predicted K_m values were less than the minimum value presented in Table 4 while the P_u values were very similar. The effect of the higher K_m value than predicted for the ^{14}C uptake studies is that the transition from diffusional limitation to saturation is more gradual than that observed in the oxygen electrode studies.

In conclusion this work confirms the importance of the unstirred layer as a limiting factor for aquatic macrophytes. The use of Michaelis-Menten kinetics to describe the photosynthetic characteristics of the submerged alga is not possible but equally the V_{max} and K_m values predicted by the Hill-Whittingham equation cannot readily be related to the kinetics of the carboxylating enzyme, RuBPc. The K_m and P_u values are a reflection of the degree of stirring and the kinetics of the uptake of inorganic carbon at the plasmalemma, and carboxylation. Many factors, as yet unknown, appear to control the V_{max} (i.e. low pH results in a decrease) and if the apparent V_{max} is less than the RUBISCO V_{max} then the apparent K_m will be less than the real K_m which thus results in increased complexity when the in vivo and in vitro values are compared. The difference between the carbon assimilation curves presented here and elsewhere suggest that macroalgae exhibit a range of apparent K_m values. Further study of the relationships between in vitro studies and well stirred in vivo studies of different algae should help us to understand some of the factors which limit the rate of photosynthesis and whether this is related to their ecological distribution.

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