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Effects of O_2, pH and DIC on photosynthetic net-O_2 evolution by marine macroalgae *

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Abstract. Net photosynthetic O_2 evolution by five marine macroalgae: *Ulva lactuca* L., *Enteromorpha* sp., *Ceramium strictum* Harvey, *Fucus serratus* L., and *F. vesiculosus* L., collected from Danish waters in the summer of 1983 was followed at increasing O_2 and with pH either fixed close to pH 7, 8 or 9, or drifting upwards during photosynthesis in a closed chamber to determine the effects of changing O_2, pH and DIC (dissolved inorganic carbon) on photosynthesis. Increasing O_2, increasing pH and decreasing DIC together limited O_2 evolution. Raising the O_2 concentration with pH and DIC held constant resulted in less inhibition of net-O_2 evolution than when all three factors acted together. The O_2 inhibition of photosynthesis was similar to the reported O_2 inhibition of ribulose 1,5-bisphosphate carboxylase isolated from lower and higher plants. Net-O_2 evolution as a function of the molar ratio of O_2 to $HCO^-_3 + CO_2$ in solution provided a general, linear relationship ($r^2 = 0.72$ to 0.84), predicting inhibition of photosynthesis based on O_2, pH and DIC changing together. Slopes of this relationship, representing competition between O_2 and carbon based on external concentrations, were similar for the five taxonomically different algae, suggesting that similar processes act to reduce net-O_2 evolution.

Introduction

Photosynthesis of submerged macrophytes generates O_2, raises pH and lowers the concentration of dissolved inorganic carbon (DIC) in solution. Where photosynthesis is intense, such as in dense stands of macrophytes, physical exchange of O_2 and DIC becomes reduced and can lead to O_2 and pH attaining high levels and the depletion of DIC around the plants (Van et al. 1976, Hillebrand 1983). All three variables, separately or in combination, may reduce net-O_2 evolution and carbon fixation (Dromgoole 1978, Simpson et al. 1980, Burris, 1981). A changing ratio of $O_2 : CO_2$ combines changes in all three variables, and the two gases can compete for reaction sites on the bifunctional enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (RUBISCO; Bowes and Ogren 1972, Lorimer 1981).

Higher land plants belonging to the "C-4" group can concentrate CO_2 internally via intermediary trapping of CO_2 in C-4 acids (Bidwell 1983). These plants display low CO_2 compensation points and low sensitivity to high O_2 concentrations. Algae all appear to be ordinary C-3 plants, but there are nevertheless examples of an apparent insensitivity to O_2 in both unicellular (Badger et al. 1978) and multicellular forms (Lloyd et al. 1977, Bidwell and McLachlan 1985, Beer and Israel 1986, Beer and Shragge 1987). The common suggestion is that this O_2 insensitivity is based on elevated CO_2 concentrations at the reaction site for CO_2 fixation (Badger et al. 1980), probably achieved by efficient use of external HCO_3^- (Beer and Eshel 1983, Sand-Jensen and Gordon 1984, Bidwell and McLachlan 1985, Lucas and Berry 1985, Kerby and Raven 1985).

In this paper we examined the individual and combined effects of increasing O_2 and pH and decreasing DIC on the photosynthetic net-O_2 evolution of five taxonomically-diverse species of marine macroalgae: *Ulva lactuca*, *Enteromorpha* sp., *Ceramium strictum*, *Fucus serratus* and *F. vesiculosus*. All five algae are common in Danish coastal waters and may form dense stands where changes in chemical conditions generated by photosynthetic activity subsequently hamper their photosynthetic process.

Materials and methods

Collection of plants

The algae used in the experiments were collected during the early summer of 1983 from shallow Danish waters (0.3 to 0.5 m) with salinities between 17 and 20‰. Four of the algae, *Ulva lactuca*, *Enteromorpha* sp., *Ceramium strictum* and *Fucus vesiculosus*, were

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collected from Roskilde Fjord, an estuary subject to sewage input and nutrient enrichment from agricultural catchments (Borum 1985). The collection site was Ølsted Strand, in the outer part of the estuary, where phytoplankton biomasses are usually 2 to 4 mg chl *a* m^{-3} (Borum 1985) and dense mats of unattached *Ulva lactuca* and *Enteromorpha* sp. occasionally develop in shallow waters. *Fucus serratus* was collected from the wall of a rock jetty at Helsingør, where the water is clear and phytoplankton biomass is low (about 1 mg chl *a* m^{-3}).

Measurement of photosynthesis

On return to the laboratory algae were placed in filtered (Whatman GF/C) seawater from the collection site under low light (~100 μE m^{-2} s^{-1}) at 15 °C before use (within 48 h). The photosynthetic O_2 evolution of plants was measured in filtered, well-stirred seawater in a rectangular perspex chamber (30 × 6 × 3 cm). Water was circulated by a submersible pump (Eheim, West Germany) delivering a flow of about 1200 ml min^{-1} and providing an exchange time of 30 s and a flow velocity of 1 cm s^{-1} in the photosynthetic chamber. The chamber was linked in series to a fast Clarke-type O_2 electrode, a pH electrode (Radiometer, Copenhagen) and a temperature transducer. Signals were amplified and registered on a chart recorder as previously described (Sand-Jensen 1983). The water temperature was held at 15 °C and light was supplied at 1000 μE m^{-2} s^{-1} using quartz halogen projector lamps positioned above the chamber. This light level is saturating for photosynthesis of most sub-littoral macroalgae (Lüning 1981), but below the extreme irradiances which can cause photoinhibition (Arnold and Murray 1980).

Intact plants were used in the chamber; thalli were detached from holdfasts when the material was bulky. Plants were pre-conditioned for 1 to 2 h in the photosynthetic chamber prior to measurement. The oxygen concentration of experimental water was manipulated by bubbling with N_2-gas for short periods, by aerating the solution or by adding pre-oxygenated seawater to the chamber. The O_2 electrode was calibrated by Winkler titrations at the start, during and end of every experiment. Concentrations of DIC were measured by Gran titration with 0.1 *N* HCl (Stumm and Morgan 1970). DIC was 1.70 m*M* in seawater from Helsingør and 2.08 m*M* from Roskilde Fjord. Changes in DIC during photosynthetic experiments were calculated according to equations of Stumm and Morgan (1970). The proportions of carbon species (i.e. CO_2, HCO_3^-, CO_3^{2-}) were altered by adding small aliquots of HCl or NaOH to water recirculating in the chamber. Molar concentrations of each component were calculated from dissociation constants for brackish waters (Skirrow 1965) and carbonate alkalinity curves for the required pH range (7.0 to 9.6).

Photosynthesis was expressed as μmol O_2 evolved mg^{-1} chlorophyll min^{-1}. Oxygen inhibition was expressed as percent inhibition for every 100 μM increase of O_2 in the solution – obtained from slopes of linear regressions of net-O_2 evolution versus increasing O_2 concentrations, over the O_2 and pH ranges specified, and starting from the same DIC concentration. Chlorophyll pigments (*a* for red algae, *a* + *c* for brown algae, and *a* + *b* for green algae) were extracted on duplicate samples by homogenizing in a tissue grinder. The extract was filtered and measured spectrophotometrically according to Jeffrey and Humphrey (1975).

Manipulation of O_2, pH and DIC

The experimental conditions in the photosynthetic chamber were manipulated in two ways: (1) allowing pH to drift upwards during photosynthesis, starting with the pH of solution close to about 8; and (2) holding pH close to 7, 8 or 9 (±0.2 pH units) by adding HCl or NaOH to the reaction chamber during photosynthesis. Inhibition of photosynthesis was determined in short runs starting at the background level of DIC, then at successively higher levels of O_2 concentration. DIC was returned to the background level before each run by renewing the solution. Solution changes were performed at O_2 concentrations below, just above and well above saturation. DIC concentrations were usually reduced by 15 to 20% during photosynthesis in each experimental run before the solution was replaced and the experiments continued. Solution replacement was achieved by introducing fresh seawater (corresponding to twice the chamber volume) into the chamber while the old solution was siphoned off. This procedure prevented handling of algae and exposure to air.

Contribution of single factors (O_2, pH and DIC) to inhibition of photosynthesis

The contribution of single factors to inhibition of photosynthesis was estimated by comparing net-O_2 evolution at fixed and varied pH and DIC over similar O_2 ranges. The contribution of O_2 alone was estimated from changes of photosynthesis in fresh seawater (constant DIC) of different O_2 concentration at a given pH (Expt. 1). Comparing photosynthesis with pH drifting upwards and with it fixed over similar O_2 ranges provided an estimate of the contribution of pH (Expt. 2). Comparing the photosynthetic responses of the algae to rising O_2 and falling DIC at fixed pH with that observed with rising O_2 concentrations in fresh seawater of constant DIC and fixed pH provided an estimate of the contribution of DIC to inhibition of photosynthesis (Expt. 3).

Results

Oxygen inhibition of net-O_2 evolution

Oxygen inhibition of the five macroalgae: *Ulva lactuca*, *Enteromorpha* sp., *Ceramium strictum*, *Fucus serratus* and *F. vesiculosus*, varied depending on the O_2 and pH range used and whether DIC was being reduced in photosynthesis. The values in Table 1 show the extent of O_2 inhibition produced by the five algae combined, in seawater starting at the same DIC concentration and with O_2 increase in the chamber produced by adding O_2 to fresh seawater or by photosynthetic output.

The inhibition of photosynthesis was generally lower at low O_2 levels for a given pH (Table 1). The lowest oxygen inhibition was observed where low O_2 concentrations were accompanied by low pH (Table 1). At pH 7 with CO_2:HCO_3^- ratios in solution 10-fold higher than at pH 8, O_2 inhibition of net-O_2 evolution was 3 to 16% for each 100 μM increase of O_2 between 40 and 440 μM. At pH 8, O_2 inhibition was 10 to 24% per 100 μM increase between 50 and 420 μM O_2 (Table 1).

Oxygen inhibition was higher when pH drifted upwards in photosynthesis. Under these conditions O_2 increase was accompanied by reduced CO_2:HCO_3^- ratios in solution and declining concentrations of DIC. For example, raising pH from 8.5 to 9.3, starting at about 330 μM O_2, produced 23 to 44% inhibition per 100 μM O_2 increase, while the O_2 inhibition was only 12 to 32% when pH was fixed close to 8 (Table 1). Greater O_2 inhibition was also observed with pH drifting from 9.0 to 9.7 rather than being held constant at pH 9 (Table 1).

Raising the O_2 concentration of the solution through photosynthesis (thus including any influence of DIC depletion) rather than adding O_2 to fresh seawater resulted

Table 1. Inhibition of net-O_2 evolution by five macroalgae (*Ulva lactuca*, *Enteromorpha* sp., *Ceramium strictum*, *Fucus serratus* and *F. vesiculosus*) under different pH and O_2 conditions. Combined results for the five species are shown for O_2 increases produced: (A) by addition of O_2 to fresh seawater, and (B) by the algae during photosynthesis

pH	Expt.	O_2 range[a] (μM)	O_2 inhibition (% per 100 μM O_2 increase)
Drifting pH			
8.0 to 8.9	A	100–390	10–22
8.0 to 9.7	B	100–410	18–23
8.5 to 9.1	A	300–610	9–36
8.5 to 9.3	B	320–610	23–44
9.0 to 9.7	B	400–830	36–45
Fixed pH			
7.0	A	40–450	3– 6
	B	40–440	3–16
	A	280–680	6–15
	B	280–660	12–21
	B	540–910	19–48
8.0	A	50–580	4– 8
	B	50–420	10–24
	A	320–690	3–14
	B	340–670	12–32
	B	480–840	25–70
9.0	A	140–430	2–16
	B	140–430	14–25
	A	360–620	3
	B	360–580	22–31
	B	620–750	50[b]

[a] Air-equivalent saturation O_2 concentration was 285 μM at the experimental temperature
[b] *Fucus serratus* only

in substantially greater inhibition (Table 1). This effect was apparent at both undersaturated and saturated O_2 concentrations and with pH fixed close to 7, 8 ot 9, or with it drifting upwards from pH 8.

Contribution of single factors (O_2, pH and DIC) to inhibition of net-O_2 evolution

Because photosynthesis was not measured over identical O_2 ranges it is not possible to calculate, exactly, the relative contributions of the individual factors, O_2, pH and DIC, to the observed inhibition of photosynthesis. Only experiments in which inhibition was examined over similar O_2 ranges under different pH and DIC conditions, can be used in the analysis. The approach is outlined below in more detail for one of the algae used: *Enteromorpha* sp.

Net-O_2 evolution of *Enteromorpha* sp. declined by 17.6% per 100 μM O_2 rise between 160 and 370 μM O_2, when pH drifted upwards from 8.2 and DIC was naturally depleted by photosynthesis. This value represents, therefore, the combined effect of all three factors. The inhibition due to O_2 alone, gauged from measuring photosynthesis between 49 and 394 μM O_2 with pH fixed close to 8 and DIC maintained at 2.06 μM by water renewal, was 6.1% per 100 μM O_2 or 35% of the combined effect (Expt. 1). When pH was fixed close to 8.1 and DIC was naturally depleted, net-O_2 evolution declined by 11.5% per 100 μM O_2 rise for O_2 increases between 49 and 424 μM O_2. The relative contribution of pH was therefore estimated to be 6.1% (i.e. 17.6% less 11.5%) per 100 μM O_2 increase, or 35% of the combined effect (Expt. 2). Finally, the contribution of DIC to inhibition corresponded to 5.4% (i.e. 11.5% less 6.1%) per 100 μM O_2 increase, or 30% of the combined effect (Expt. 3). Based on the above calculations, which assume additive interaction of O_2, pH and DIC, all three factors operated similarly to reduce net-O_2 evolution of the alga.

When the same approach was used with the remaining data for the five species the O_2 rise contributed between 10 and 40%, the pH increase between 26 and 38% and the DIC decline between 29 and 56% of the combined inhibition of net-O_2 evolution, when all three factors acted together. Although all three factors contributed to reduce photosynthetic O_2 output, there was no evidence of a much larger contribution to inhibition by one factor over another among the five macroalgae.

Use of external molar ratio of $O_2 : HCO_3^- + CO_2$ to predict net-O_2 evolution

Because O_2, pH and DIC all affect net-O_2 evolution, it is difficult to predict photosynthesis based on any of these factors alone. Knowing that CO_2 and O_2 interact competitively for RUBISCO (Lorimer 1981), we used the external molar ratio of O_2 to the assimilable components of DIC (i.e. free CO_2 and HCO_3^-) as a single factor which is influenced by the three variables simultaneously. The dependence of net-O_2 evolution of the five microalgae on changing external ratios of O_2 to $HCO_3^- + CO_2$ is shown in Fig. 1. All five macroalgae showed the same general response to variable ratios of $O_2 : HCO_3^- + CO_2$, with net-O_2 evolution decreasing as the ratio increased. Fitting all data points to a linear regression gave coefficients of determination (r^2) between 61 and 79%. To improve the fit we next removed some outlying points (depicted by arrows in Fig. 1) where photosynthetic rates were very low immediately after changes in solution or in water highly supersaturated with O_2. In this way, between 72 and 84% of the observed variation in net-O_2 evolution could be explained by the external molar ratio of $O_2 : HCO_3^- + CO_2$.

The ratio of $CO_2 : HCO_3^-$ in seawater declines from about 1:10 at pH 7 to 1:1000 at pH 9, and CO_2 is recognized as being more readily utilized than HCO_3^- in photosynthesis (e.g. Sand-Jensen and Gordon, 1984). We examined the predictive value of the regressions further by artificially increasing the affinity of free CO_2 relative to HCO_3^- by factors of 2, 5 and 10 and recalculating the regressions. This did not noticeably increase the r^2 values for any of the species. With the molar ratio of $O_2 : HCO_3^- + 2CO_2$, the r^2 values ranged from 77 to 85% among the five species. With a 5-fold increase in CO_2 affinity, the r^2 values were 78 to 85%.

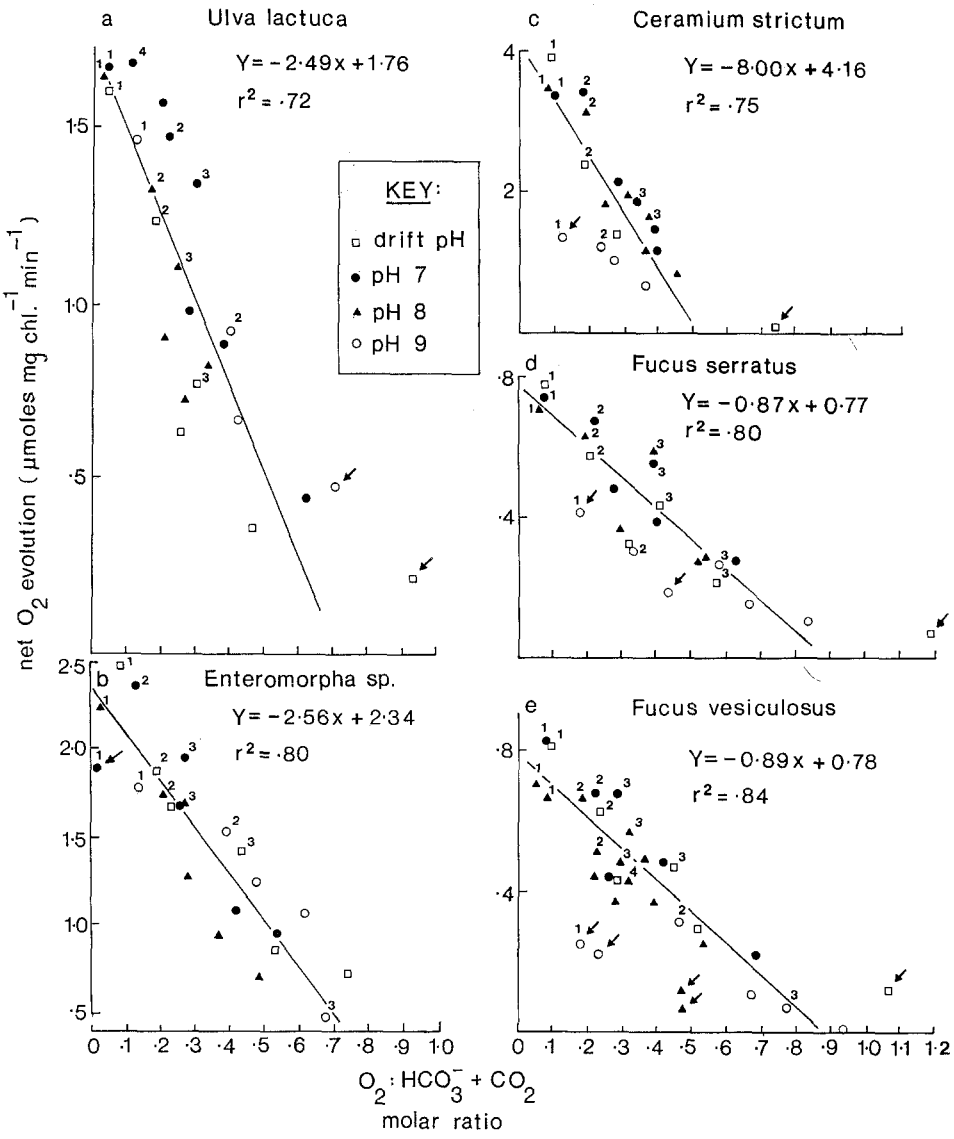

Fig. 1. Net-O_2 evolution by five marine macroalgae (a to e) as a function of increasing molar ratios of $O_2:HCO_3^- + CO_2$ in solution. Photosynthesis is shown with pH drifting upwards from pH values of about 8 (drift) and with pH fixed close to 7, 8 and 9. Numbered points (1, 2, 3 . . .) refer to the start of every experimental run, where DIC (dissolved inorganic carbon) was constant at natural concentrations (2.08 m*M* for algae shown in a, b, c and e; 1.70 m*M* for algae shown in d), but at successively higher O_2 concentrations. Points excluded from the regressions are indicated with arrows

The five algae displayed different absolute rates of photosynthesis under the same conditions of light, temperature and stirring. The greatest O_2 output per unit of chlorophyll was obtained with *Ceramium strictum* (large proportions of accessory red pigments relative to chlorophyll *a*), then *Enteromorpha* sp. and the least with the two fucoid species. To compare the relative effects of the changing molar ratio of $O_2:HCO_3^- + CO_2$ among the five species, the regression lines were standardized by setting the intercept with the ordinate-axis (i.e. P_{max}) at unity. Recalculation of the slopes shown in Fig. 1 gave values ranging from −0.92 to −1.92 for the five algae. Reciprocals of these slopes indicate the extrapolated molar ratios of $O_2:HCO_3^- + CO_2$ where net-O_2 evolution is zero. These values were from 0.52 to 0.91 for the five algae (Fig. 1).

Discussion

Oxygen inhibition

Similar responses by the five macroalgae (*Ulva lactuca*, *Enteromorpha* sp., *Ceramium strictum*, *Fucus serratus* and *F. vesiculosus*) to variable CO_2 and O_2 concentrations suggest that the same principles are operating to inhibit net-O_2 evolution and that the processes have similar magnitudes.

The results showed an inhibitory effect of rising O_2 concentration in solution on photosynthetic net-O_2 evolution under conditions where pH and DIC were kept constant. Oxygen inhibition of similar magnitudes has been observed previously for a wide range of marine macroalgae (Table 3 in Turner et al. 1956, Table 1 in Downton et al. 1976, Table 10 in Wheeler 1982), whereas other investigations have found little or no O_2 inhibition (e.g. Colman and Cook 1985, Beer and Israel 1986, Beer and Shragge 1987). It is indeed possible that the extent of O_2 inhibition observed is dependent on the physiological status and pre-acclimatization of the macroalgae as well as the experimental techniques used to determine O_2 evolution (e.g. the algae totally submerged and unaerated, the medium bubbled with gas mixtures, or the algae in damp air).

In a recent comprehensive comparison of three species of marine macroalgae (*Codium*, *Udotea* and *Sargassum*) Bowes (1985) reported between 9 and 30% inhibi-

tion of net-O_2 evolution with 21% O_2 in the gas phase. If we assume that our value of 285 μM O_2 at air saturation at 15 °C corresponds to 21% air-equivalent in the gas phase (information not provided in the reference), then the O_2 inhibition is equivalent to between 3 and 10.5% per 100 μM O_2 rise. This range of O_2 inhibition resembles that observed here under fixed pH and DIC levels (3 to 16% per 100 μM O_2, Table 1). This O_2 sensitivity is likely to be reduced most when the plant has a high access to free CO_2 in the medium or is able to utilize external HCO_3^- efficiently (Colman and Cook 1985). In support of this notion, O_2 inhibition was small (3 to 6% per 100 μM O_2) at high CO_2 concentration at pH 7 for the O_2 range 40 to 450 μM (Table 1).

The O_2 inhibition of net-O_2 evolution at constant pH and DIC reported here also resembles the O_2 effects on cell photosynthesis and the activity of ribulose 1,5-bisphosphate carboxylase isolated from lower and higher plants (Servaites and Ogren 1978, Badger 1980, Bird et al. 1982). Re-calculation of data in Fig. 2 in Servaites and Ogren (1978) indicates that oxygen inhibition of soybean cell photosynthesis was about 2 to 3% per 100 μM O_2 at 2 mM HCO_3^- and, as expected, lower with higher bicarbonate concentrations (less than 2% per 100 μM O_2 at 5 mM HCO_3^- and less than 1% per 100 μM O_2 at 10 mM HCO_3^-). The O_2-inhibition of RUBISCO carboxylase activity in enzyme isolated from the cyanobacterium *Anabaena variabilis* was about 3 to 4% per 100 μM O_2 rise over the O_2 range 0 to 100% (gas phase) in the presence of about 1.5 and 2.8 mM $HCO_3^- + CO_2$ (re-calculated from Fig. 5 in Badger 1980). Similarly, re-calculation of the effects of O_2 on the carboxylase activity of RUBISCO isolated from a variety of crop plants indicated an inhibition of about 6% for a 100 μM O_2 increase, using the average velocity ratios for carboxylase activity in nitrogen and air under the conditions specified (Bird et al. 1982).

Influence of O_2, pH and DIC

Increasing O_2 concentration in solution influences photosynthesis in different ways, including competition with CO_2 at the reaction sites of RUBISCO as well as effects on respiration and the energy transfer processes of the photosystems of the plants. Despite the similarities apparent between inhibition of photosynthesis of the five marine algae and reductions in carboxylase activity of a range of lower and higher plants under increasing O_2, it is not possible here to infer clear links between the inhibition of net-O_2 evolution and O_2 consumption via RUBISCO because other O_2 consuming reactions, not measured here, may contribute to the effect. These include Mehler-type reactions associated with the electron transport system, which has a high affinity for oxygen (Heber 1985) and O_2-sensitive dark respiration (Bidwell 1983). Mehler-type reactions are not directly coupled to the photosynthetic carbon oxidation (PCOC) cycle, but are important to energy-driven reactions involving the electron transport chain in the photosystems, including the production of ATP and NADPH. Though O_2 consumption in dark respiration is not always saturated at low O_2 concentrations in marine macroalgae (Table 1 in Downton et al. 1976), the O_2 effects on photosynthesis were similar among the five species of algae over wide O_2 ranges, making it unlikely that O_2-sensitive dark respiration was a major factor contributing to O_2-inhibition.

Solution pH influences the relative proportions of carbon species, as well as membrane, and possibly tonoplast pH. Most pH effects here were registered below 9.6, where membrane and tonoplast pH are probably little affected (Wheeler 1982). Accordingly, the adverse effects of high pH more likely reflect the decreasing proportions of free CO_2 and HCO_3^- relative to CO_3^{2-} (assumed to be unusable; Steeman Nielsen 1960) in the external solution and in the diffusive boundary layer.

The effects of reduced DIC concentrations on net-O_2 evolution are dependent on transport resistance across the diffusive boundary layer, the changing proportions of free CO_2 and HCO_3^- accompanying pH changes and finally the ability to maintain high CO_2 concentrations at the fixation site of RUBISCO by possible active transport and conversion processes (carbonic anhydrase activity). The importance of active HCO_3^- utilization and carbonic anhydrase activity for photosynthesis is apparently variable among macroalgae (Bowes 1985, Smith and Bidwell 1989). Addition of an inhibitor of carbonic anhydrase, for example, more than doubled the inhibitory effect of O_2 on net-O_2 evolution by *Codium* sp. but had little effect on O_2 inhibition of *Udotea* sp. (Bowes 1985). Although we did not examine aspects of carbon transport and carbonic anhydrase activity, we did find that the relative proportion of CO_2 and HCO_3^- in solution influenced photosynthesis. Inhibition of net-O_2 evolution clearly increased when the ratio of $CO_2 : HCO_3^-$ declined.

The external molar ratio of $O_2 : HCO_3^- + CO_2$ could serve as a unifying variable to predict the decline of net O_2 evolution when O_2, pH and DIC change simultaneously (Fig. 1). This behaviour is qualitatively in agreement with the competition between carboxylase and oxygenase reactions in RUBISCO, but the internal concentrations of CO_2 and O_2 at the site of RUBISCO may be very different from the external ones, and several other processes also influence the net-O_2 evolution of the plant.

We compared the responses of the macroalgae to that of carboxylase isolated from the cyanobacterium *Anabaena variabilis*. Recalculation of the original data for *A. variabilis* (Fig. 5 in Badger 1980) shows that the activity of the enzyme was reduced at higher ratios of $O_2 : HCO_3^- + CO_2$. If we crudely estimate changes in carboxylase activity of *A. variabilis* based only on lowest and highest ratios of O_2: carbon, the resulting slopes range from −0.77 to −3.61, comparable to those of the five algae, here, whose standardized slopes were −0.92 to −1.92.

The interaction of all three factors (O_2, pH and DIC) reflected in the ratio of $O_2 : HCO_3^- + CO_2$, provided a reasonably sensitive means of predicting net-O_2 evolution of the five macroalgae, given the wide range of O_2, pH and DIC conditions in the experiments, the known differences in activities and kinetics of plant RUBISCO (Badger 1980; Jordan and Ogren 1983), and the spatial separation between external gases and the internal site of fixation.

Ecological considerations

Marine macroalgae in well-stirred waters of exposed habitats are not normally subjected to conditions with strongly fluctuating O_2, pH and DIC unless the are growing as dense stands under sheltered conditions, where there will be reduced physical exchange with open-water masses and reduced gas exchange with the atmosphere. The combined inhibitory effects of increasing O_2 and pH and decreasing DIC on net-O_2 evolution of the macroalgae provide clear evidence that all three factors must be taken into account when net-O_2 evolution is used as a measure of photosynthetic performance in situations which promote bloom conditions and therefore push these factors to their extremes.

In Danish waters green algae such as *Ulva lactuca* and *Enteromorpha* sp. can develop dense mats under highly eutrophic conditions. Under such conditions O_2 may be greatly supersaturated, and pH may approach the possible upper limit for photosynthesis (10.4 to 10.5) (Anonymous 1980). The responses of the five marine macroalgae examined here provided little evidence that some species (e.g. in the genera *Ulva* and *Enteromorpha*) should be able to resist these adverse conditions better than others. Clearly, other aspects such as the efficiency of light and nutrient utilization, grazing resistance and the ability to survive during winter are more important for regulating the composition and density of the communities of macroalgae.

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