

Photosynthesis of marine macroalgae and seagrasses in globally changing CO₂ environments

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ABSTRACT: Photosynthetic rates of many marine macroalgae are saturated by the present day inorganic carbon (Ci) composition of seawater, while those of seagrasses (or marine angiosperms) are CO₂-limited. In this study we attempted to simulate the Ci conditions of near-shore seawater during the time that seagrasses colonised the sea (in the Cretaceous), and compare the photosynthetic performance of representatives of the 2 plant groups under those versus present day conditions. The results show that the seagrasses have an affinity for Ci at least as high as the algae under the low pH and high CO₂/HCO₃⁻ concentration ratios simulating near-shore areas of the Cretaceous seas, indicating that their photosynthetic capacity then matched that of macroalgae. However, in the high pH and high CO₂/HCO₃⁻ ratios of today, their affinity for Ci is lower than that of the macroalgae, and it is suggested that this deficiency renders them a lower ability for Ci utilisation. This situation may possibly be reversed again as global CO₂ levels of the atmosphere and, consequently, of near-shore marine habitats increase in the future.

KEY WORDS: Marine macroalgae · Seagrasses · Photosynthesis · CO₂ · Global change

INTRODUCTION

Two major groups of marine macrophytes exist today, macroalgae (seaweeds) and seagrasses (submerged angiosperms). While the former group has evolved in the oceans from the early times of plant-life existence [from cyanobacteria (ca 3.5 billion years ago), via microalgae (ca 1.3 billion years ago), to the first occurrence of macroalgae (ca 1 billion to 750 million years ago)], fossil evidence dates the earliest seagrasses to the Cretaceous period, only ca 90 million years ago. This plant group originated from terrestrial angiosperms which invaded the seas either directly or via freshwater forms (den Hartog 1970). For these plants, life in the oceans required adaptations to factors such as high salinity (by systems which could

compartmentalise salts away from metabolically active sites; Beer et al. 1980, Jagels 1983), attenuated light (by having the chloroplasts in the outermost, epidermal cell layer; Tomlinson 1980) and anoxic sediments (by a system of aerial canals, called lacunae, allowing photosynthetically derived O₂ to be transported to the roots; Smith et al. 1984). In addition, and concerning the focus of this study, as seagrasses colonised the sea they had to adapt to diffusivity of CO₂ in the liquid orders of magnitude lower than in the aerial medium from which they came. However, at the time when seagrasses started to inhabit the oceans the atmospheric, and hence the near-shore seawater equilibrium, CO₂ concentration was higher than it is today (Arthur et al. 1991, Berner 1991). This, and the potential advantage of seagrasses in having roots [and thus being able to extract nutrients other than inorganic carbon (Ci) from the sediment] apparently allowed them to develop into a prolific group of marine macrophytes. Despite their low diversity (there are only some 50 species), seagrasses are the dominant macrophytes of many near-shore soft-bottom ecosystems, where they comprise an impor-

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tant, highly productive, component of the benthic environment (McRoy & McMillan 1977). However, present seagrass ecosystems seem to be sensitive to human-induced perturbations, and a decline in seagrass dominated areas has been noted in several regions of the world (Dennison et al. 1993).

Presently, seawater in equilibrium with air contains ca 13 μM CO_2 and 2.2 mM anionic carbon, mainly in the form of HCO_3^- . Under these conditions, photosynthesis of marine macroalgae is often saturated by the seawater Ci-composition, largely because they can efficiently use HCO_3^- (Beer 1994). In contrast, seagrasses possess less efficient systems for utilising HCO_3^- , and their photosynthesis is therefore Ci-limited because of the low concentration and slow diffusive supply of CO_2 to the leaves (Beer 1989). During the Cretaceous era, however, the atmospheric CO_2 concentration was 3 to 12 times higher than it is today, and the pH of the seawater has also been estimated to have been lower in the past (Berner et al. 1983, Spivack et al. 1993), causing a proportionally lower Ci fraction to be present as ionic Ci (see below). Assuming equilibrium between the air and near-shore marine habitats where seagrasses and marine macroalgae grew (and disregarding a slight effect of different temperatures on the solubility of CO_2), the corresponding concentration of dissolved CO_2 was thus 40 to 150 μM . Therefore, we hypothesised that seagrasses had a better photosynthetic ability during past eras in shallow marine habitats. Accordingly, in this work we explored the effects of the possible differences in the marine Ci-environment of today and that in which seagrasses evolved during the Cretaceous on the photosynthetic yields of 2 common seagrasses and that of some common macroalgae.

MATERIALS AND METHODS

The seagrasses used in this study were the temperate species *Zostera marina* (from Avery Point, Connecticut, USA) and the sub-tropical *Thalassia testudinum* (shipped overnight from St. Petersburg, Florida, USA, courtesy of Dr M. Durako), while the macroalgae consisted of the ubiquitous green species *Ulva lactuca* and the common temperate red and brown algae *Palmaria palmata* and *Laminaria saccharina*, respectively (all collected at Avery Point).

Cut sections (3 cm long) from young leaves of the seagrasses, or 1.2 cm diameter circular thallus discs of the macroalgae (cut out with a cork borer), were pre-incubated in normal, air-sparged, seawater at 20°C and at an irradiance of 200 $\mu\text{mol photons (400 to 700 nm) m}^{-2} \text{ s}^{-1}$ for at least 1 h prior to experimentation. Photosynthetic rates were then measured for each leaf

section or thallus disc using Hansatech DW1 O_2 electrode chambers (set to 2 ml volume) connected to CB1-D control units and interfaced with a computer via an IF1/2 interface card (Hansatech, King's Lynn, UK). The temporal resolution of the O_2 -tracings was <1 s, and rates were calculated between 227 (air equilibrium) and 300 μM O_2 ; no inhibitory O_2 response was observed within this range. The temperature and irradiance (saturating for photosynthesis of all species) were kept the same as during the pre-incubation, and the media in the measuring vials were agitated by fast spinning magnetic stir-bars (so as not to limit the Ci supply to the plant material).

Photosynthetic rates of the various marine macrophytes were first measured in normal seawater (2.1 to 2.2 mM Ci at pH 8.0 to 8.1; the natural seawater used was of slightly lower pH and total Ci than that calculated for Table 1). After steady-state photosynthetic rates had been reached (ca 5 and 2 min for the seagrasses and macroalgae, respectively), Ci was added to the closed system by injecting μl amounts of a 100 mM NaHCO_3 solution so as to create higher than ambient Ci levels. In other experiments, a synthetic seawater medium was used instead of natural seawater. This medium consisted of 450 mM NaCl, 30 mM MgSO_4 , 10 mM KCl and 10 mM CaCl_2 (Beer & Eshel 1983), and it was found to be adequate for maintaining steady-state photosynthetic rates (after the addition of NaHCO_3) for at least 2 h. The pH was adjusted by injecting 1 M solutions of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) for the pH values 8.2 and 7.0 and 2-N-morpholinoethanesulfonic acid (MES) for pH 6.0, yielding final concentrations of 50 mM. The plants were left to photosynthesise in the buffered synthetic seawater medium till zero gas exchange had been reached (which could take up to 20 min at pH 6.0), and μl aliquots of the NaHCO_3 solution were then injected so as to create increasing Ci concentrations. At pH 6.0, 1 low (but saturating) NaHCO_3 concentration was injected, and the plants were left to photosynthesise till zero gas exchange had again been reached. The hyperbolic curves of O_2 evolution thus created (as Ci from the synthetic seawater was used up) were utilised in order to determine both Ci contents at each instance (assuming a photosynthetic quotient of 1.0 and a CO_2 compensation point close to zero) and photosynthetic rates (calculated as the tangents of the curve at various Ci concentrations). The rationale and details for such determinations of Ci responses has been described previously (Beer & Björk 1994). Concentrations of the various Ci forms were calculated using the program CARBON (Beer & Eshel 1983) for either the solutions in the O_2 -electrode measuring systems ('closed system' calculations) or for the values in Table 1 ('open system' calculations).

Table 1. Concentrations of various Ci forms in seawater of different pH values at various CO₂ concentrations in the air phase (assuming equilibrium with the air, a salinity of 35‰ and a temperature of 20°C; calculated using program CARBON, Beer & Eshel 1983)

Air CO ₂ conc. (ppm)	pH 8.2 (today)			pH 7.0			pH 6.0		
	CO ₂ (μM)	HCO ₃ ⁻ (μM)	CO ₃ ²⁻ (μM)	CO ₂ (μM)	HCO ₃ ⁻ (μM)	CO ₃ ²⁻ (μM)	CO ₂ (μM)	HCO ₃ ⁻ (μM)	CO ₃ ²⁻ (μM)
340 (today)	12.5	1991	363	12.5	126	1.4	12.5	12.6	0.0
1020 (3×)	37.5	5973	1089	37.5	378	4.2	37.5	37.8	0.0
1700 (5×)	62.5	9955	1815	62.5	630	7.0	62.5	63.0	0.0
2720 (8×)	100	15928	2904	100	1008	11.2	100	101	0.0

After experimentation, all plant samples were dissolved in 5 ml N,N-dimethyl formamide overnight, and chlorophyll concentrations were measured spectrophotometrically (Inskeep & Bloom 1985); all photosynthetic rate values were normalised to a chlorophyll *a+b* basis.

RESULTS

Table 1 summarises the expected effects of atmospheric CO₂ concentrations and pH on the dissolved Ci composition of near-shore seawater during the time that seagrasses evolved in comparison to today. Since the dissolution of CO₂ is virtually independent of pH, there is a linear relationship between the increase in aerial and aqueous CO₂ concentrations. However, since high pH is coupled with the presence of ionic Ci, the lower pH of past periods (Spivack et al. 1993), possibly 1 to 2 pH units lower in the Cretaceous than today (Berner et al. 1983) means that a smaller percentage of the Ci was in the form of HCO₃⁻ and carbonate ions (CO₃²⁻) and, thus, that the total Ci concentration was lower.

The results of the photosynthetic measurements in normal seawater to which NaHCO₃ had been added are shown in Fig. 1. Both seagrass species were Ci-limited in ambient seawater (where they photosynthesised only ca 50% of maximal rates), and photosynthesis was saturated at 5 to 7 mM Ci. On the other hand, the 3 macroalgae were at, or close to, Ci saturation in normal seawater.

We then measured net photosynthetic rates in synthetic seawater media, buffered to various pH values, to which NaHCO₃ was added so as to form increasing concentrations of Ci. At pH 8.2, both seagrass species showed a significantly lower affinity for Ci [expressed as higher half-saturation (*K*_{1/2}) values] than did the algae (Fig. 2), and photosynthetic rates of the latter were saturated (or nearly saturated for *Laminaria saccharina*) at the present day Ci level (1× in Fig. 2a). At pH 7.0, the seagrasses had a higher affinity for Ci than

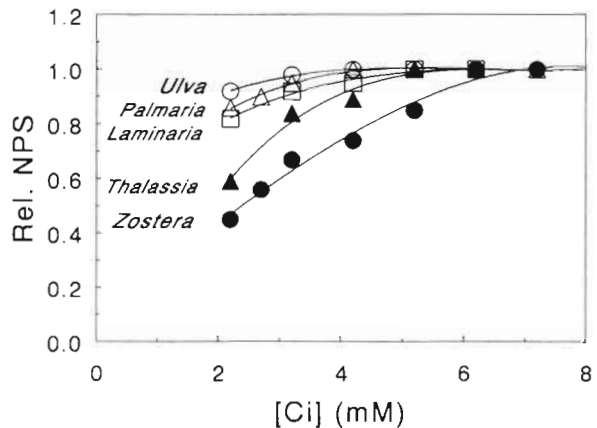


Fig. 1. Net photosynthetic rates (NPS) of 2 seagrasses (solid symbols) and 3 marine macroalgae (open symbols) in natural seawater (2.2 mM Ci) following additions of Ci. Rates are given relative to the maximal mean rate for each species; the coefficient of variance was less than 10%. Maximal mean rates, \pm SD (*n* = 9 to 10), of net photosynthesis (in $\mu\text{mol O}_2 \text{ mg chl}^{-1} \text{ h}^{-1}$) were for *Zostera marina* 38.3 ± 5.4 ; *Thalassia testudinum* 25.1 ± 4.7 ; *Ulva lactuca* 105 ± 8.9 ; *Palmaria palmata* 91.0 ± 3.5 ; *Laminaria saccharina* 30.0 ± 3.1

2 of the macroalgae, and at pH 6.0 there was no significant difference in affinity for any of the marine plants investigated, and saturation of photosynthesis occurred at ca 0.2 mM Ci.

DISCUSSION

Our results show that although both the seagrasses and marine macroalgae have a higher affinity for Ci when a larger part of the carbon pool consists of CO₂ (i.e. at lower pH values), the macroalgae are Ci saturated in the present day Ci composition of seawater (in which HCO₃⁻ is present at a concentration some 160 times that of CO₂), while the seagrasses are not. This corroborates previous conclusions for the 2 plant groups (Beer 1989), and may be related to the fact that marine macroalgae have evolved for a much longer time in the sea than have the seagrasses and/or that

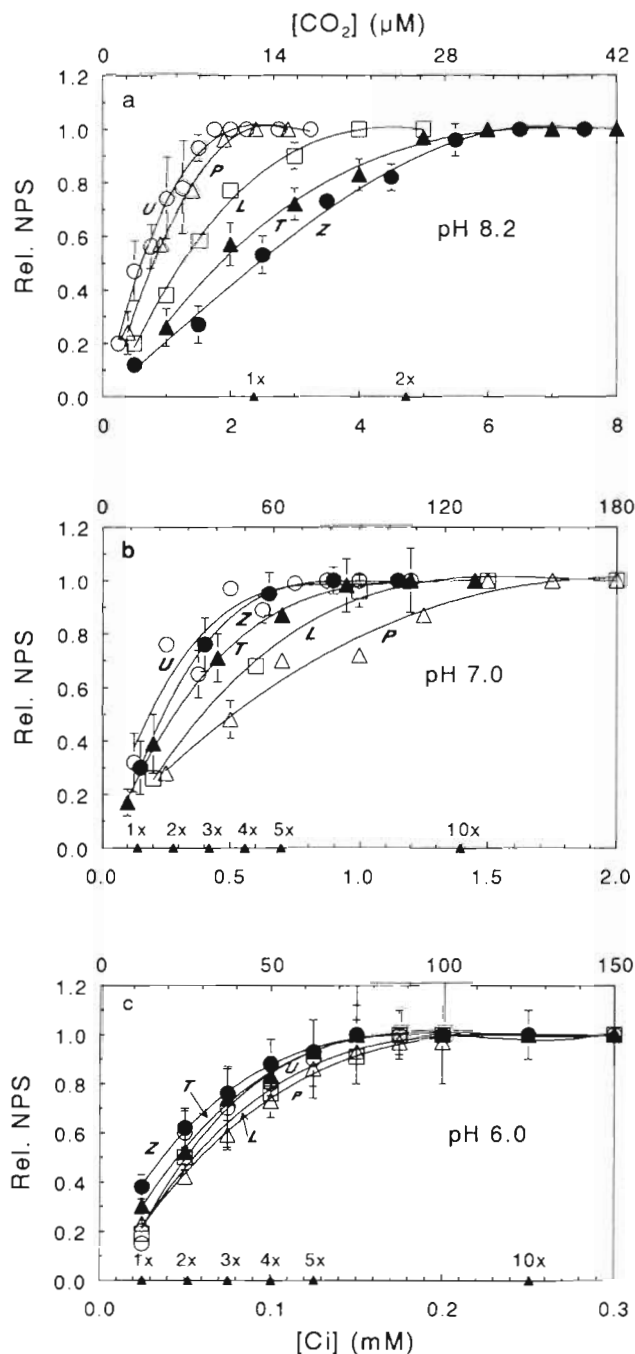


Fig. 2. Net photosynthetic rates (NPS) of 2 seagrasses (solid symbols) and 3 marine macroalgae (open symbols), at 3 different pH values, in synthetic seawater to which various amounts of Ci were added. Rates are given relative to the maximal mean rate for each species \pm SD ($n = 9$ to 10). Maximal average rates of net photosynthesis (in $\mu mol O_2 mg chl^{-1} h^{-1}$) at pH 8.2, 7.0 and 6.0, respectively, were for *Zostera marina* (Z): 21.2 ± 1.7 , 28.4 ± 3.0 , 21.6 ± 6.8 ; *Thalassia testudinum* (T): 18.6 ± 2.6 , 21.7 ± 2.0 and 17.6 ± 3.4 ; *Ulva lactuca* (U): 59.4 ± 10.1 , 62.0 ± 21.9 and 66.9 ± 3.2 ; *Palmaria palmata* (P): 94.7 ± 6.2 , 102.3 ± 13.2 and 86.4 ± 9.2 ; *Laminaria saccharina* (L): 29.1 ± 7.6 , 40.1 ± 3.0 and 31.2 ± 0.4 . Triangles on the x-axes designate multiple increases in Ci of today's value (1x)

several groups of the macroalgae feature a genetic flexibility from which a better HCO_3^- utilisation system could have developed (Beer 1994). The high photosynthetic capacity of *Ulva lactuca* (Drechsler et al. 1993) and *Palmaria palmata* (Kübler & Raven 1994) is likely due to their efficient HCO_3^- -utilisation systems, and a trans-membranal HCO_3^- transporter has been suggested for *Ulva* sp. (Drechsler et al. 1993). Bicarbonate utilisation by the seagrass *Zostera marina* is partly driven by external, carbonic anhydrase mediated, HCO_3^- dehydration (Rehnberg & Beer unpubl.), but such a system has been viewed as less efficient than direct HCO_3^- transport as found in some macroalgae at potentially CO_2 -limiting conditions (Drechsler et al. 1993, 1994, Axelsson et al. 1995). The recently found ambient CO_2 limitation for growth in *Zostera marina* (Thom 1995) may be due to this apparent disadvantage. On the other hand, it is also shown here that seagrasses can have a higher affinity than some (at pH 7.0), or the same affinity as (at pH 6.0), marine macroalgae to Ci at lower pH values where increasingly more of the Ci is present in the form of CO_2 rather than HCO_3^- (the ratio between CO_2 and HCO_3^- is 1:160 at pH 8.2, 1:10 at pH 7.0 and 1:1 at pH 6.0, see also Table 1).

Based on the present results, it can be argued that since near-shore dissolved- CO_2 levels during the Cretaceous were higher (and since the pH was lower and, consequently, the CO_2/HCO_3^- ratio was higher), it follows that the Ci acquisition by seagrasses was likely conducive to a higher productivity per se (as well as relative to that of marine macroalgae) at the time when they colonised the seas than it is now. If we assume that the pH was 6.0, then all tested species would photosynthetically saturate at 0.2 mM Ci (Fig. 2c). Since the CO_2/HCO_3^- ratio is ca 1:1 at that pH, and since a HCO_3^- concentration of 0.1 mM could not significantly contribute to photosynthesis of any of the tested species (Fig. 2a), it follows that photosynthetic saturation would occur at a seawater CO_2 concentration of around 0.1 mM, corresponding to an atmospheric CO_2 concentration of 8 times the present one. If we assume a pH of 7.0, then the higher affinities for Ci of the 2 seagrasses than those of 2 of the macroalgae (Fig. 2b) indicate that they would have had an absolute advantage in Ci acquisition over these 2 macroalgae, and their photosynthetic potential would equal that of the highly photosynthetic, opportunistically growing, *Ulva lactuca*. This higher affinity may be due to a shorter diffusion path for Ci to the chloroplasts of the epidermal seagrass cells than to that of the mesophyll cells of the 2 macroalgae (Koch & Beer unpubl. obs.), which could be beneficial under these intermediate CO_2/HCO_3^- conditions. Assuming that mainly CO_2 was utilised also at pH 7.0, saturation for this substrate would occur at

ca 0.09 mM (at 1 mM total Ci), corresponding to an aerial CO₂ concentration 7 times that of today. Both this and the 8 times higher CO₂ concentration which would saturate seagrass photosynthesis (at pH 6.0) are in agreement with the paleo-oceanographic data of atmospheric CO₂ concentrations during the Cretaceous. In contrast today, given the comparably lower air equilibrium CO₂ concentration of seawater and the higher pH (linked with a higher HCO₃⁻ level), there is a clear disadvantage to the seagrasses; they are only ca half-saturated with regard to Ci while 1 of the macroalgae is 80% saturated and the other 2 are fully (Fig. 2a) or almost fully (Fig. 1) saturated.

While this research has omitted other potentially limiting factors for growth of marine macroalgae and seagrasses (including temperature effects on metabolic rates), it has been our contention that steady-state photosynthetic rates well represent the potential for productivity of submerged macrophytes (Lipkin et al. 1986) (Since the partitioning of photosynthate was not considered for the different morphological forms studied here, we have favoured the use of relative photosynthetic rates for each species at close to ambient Ci levels as a measure of growth potential.) If so, then the above relationships between the 2 marine macrophyte groups may also point out possible trends for the future in that seagrasses may regain higher photosynthetic yields and growth rates as future global atmospheric, and thus the in near-shore seawater dissolved, CO₂ concentrations increase. On the other hand, as most marine macroalgae are already Ci saturated, no such change is expected for this plant group. Since the global CO₂ level is predicted to double within the next ca 50 yr, and assuming that the seawater pH stays stable (but it will probably decrease some; Wetzel & Grace 1983), it may be predicted from Fig. 2a that seagrasses will be able to nearly saturate their photosynthetic systems. However, it is also possible that ongoing eutrophication might counteract this advantage since those algae that are already Ci saturated could then utilise other nutrients (such as nitrogen and phosphorus) directly from the seawater (as they have no roots) and thus increase their growth potential. In places where both types of organisms grow together (e.g. where *Zostera marina* and *Ulva lactuca* or *Thalassia testudinum* and *Caulerpa* spp. coexist, or where the seagrasses are epiphytised) the drifting or epiphytic algae could both shade out the seagrasses and/or contribute to increased organic loads of the sediment to such an extent that the formation of reducing compounds (e.g. sulphide) could poison the seagrass roots (Carlson et al. 1994). Thus, while it is concluded in this paper that the deficient Ci acquisition system of seagrasses may be partly responsible for diminishing seagrass beds today, it may depend on future successful

management of eutrophication whether these plants will continue to inhabit (or re-inhabit) future, more CO₂-rich, waters.

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