

Growth rate and carbon affinity of *Ulva lactuca* under controlled levels of carbon, pH and oxygen

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Abstract

We examined the growth rate (μ) of *Ulva lactuca* L. (collected from Roskilde Fjord, Denmark in 1987) at different levels of dissolved inorganic carbon (DIC), pH and oxygen in two culture facilities. Growth was faster in Facility A (μ_{\max} ca 0.3 d^{-1}) than in B (μ_{\max} ca 0.2 d^{-1}), probably because of more efficient stirring and higher light intensity. The growth-DIC response curve exhibited low half-saturation constant ($K_{1/2}$) values (0.35 mM DIC in A, 0.55 mM in B) and growth rates close to μ_{\max} at natural seawater concentration of 2 mM DIC. Growth rate showed a low sensitivity to oxygen over a wide range of DIC and oxygen concentrations. Collectively, the results demonstrated an efficient mechanism for DIC use, unaffected by acclimatization to DIC concentrations between 0.2 and 3 mM . The growth rate decreased little between pH 7.5 and 9 at 2 mM DIC, but steeply above pH 9 approaching zero just above pH 10 . The decline of growth at high pH may result from direct pH effects on cell pH, reduced HCO_3^- availability and impaired operation of the carbon uptake process. The growth responses of *U. lactuca* to DIC, pH and oxygen resembled those observed in previous short-term photosynthetic experiments. This similarity is probably due to the fast growth of *U. lactuca* which means that photosynthetic products are rapidly converted into cell growth. Based on the culture experiments we argue that field plants of *U. lactuca* not exposed to stagnant water and DIC depletion are likely to be limited in growth by environmental factors other than DIC (e.g. light and nutrients). Dense mats of *U. lactuca*, however, may show reduced growth as a result of DIC depletion, high pH and self-shading.

a high growth potential (doubling time ca 2 d) and may develop dense free-floating mats in shallow, eutrophicated estuaries following nutrient stimulation of growth and reduced biomass losses by zoospore formation and herbivore grazing (Geertz-Hansen and Sand-Jensen unpublished).

Rapid photosynthesis in dense plant stands leads to depletion of dissolved inorganic carbon (DIC) and increases in pH and oxygen levels. These changes may all inhibit carbon fixation and thus reduce growth. Within dense floating mats of *Ulva lactuca* in shallow, protected areas, DIC reduction and pH and oxygen build-up may be very substantial (Anon. 1980), and it would, therefore, be important for the plant to possess an efficient and oxygen-insensitive carbon uptake mechanism to maintain growth.

Previous photosynthetic experiments have shown that species of *Ulva* have a high affinity for DIC, which was only weakly affected by oxygen levels, whereas pH approaching 10 was highly inhibitory (Beer and Eshel 1983, Colman 1984, Sand-Jensen and Gordon 1984, Beer and Israel 1986). These measurements, however, were conducted with unacclimated plants in brief experimental trials. The ecologically more relevant long-term growth response may be different from the short-term photosynthetic response, if inhibitory effects are aggravated over time or, alternatively, if biochemical and physiological adjustments to the chemical environment take place.

Our purpose, therefore, was to examine the steady-state growth response of *Ulva lactuca* at different levels of DIC and pH, and combinations of DIC and oxygen. Further, we examined the carbon affinity of fully acclimated plants. Finally, we discuss the possibility of carbon limitation of *U. lactuca* growth in the field.

Introduction

Ulva lactuca is a common and ubiquitous macroalga in coastal waters of temperate and subtropical regions. It has

Materials and methods

Free-floating *Ulva lactuca* L. was collected in 1987 in the eutrophic Danish estuary, Roskilde Fjord (Borum 1985, study Site IV). Circular discs were punched from the thallus

with a sharpened Perspex tube (12 mm diam.) and grown in freshly collected, filtered estuarine seawater (ca 19‰ salinity, ca 2 mM DIC) enriched with 100 μM NH_4NO_3 and 10 μM K_2HPO_4 to ensure nutrient saturation for growth.

Growth experiments were conducted at 15°C in two growth facilities; two facilities were used in order to substantiate the generality of the findings. In the first, (A), *Ulva lactuca* discs were placed in 500 ml closed glass-bottles circulating at 12 rpm in an incubator and illuminated at 140 $\mu\text{E m}^{-2} \text{s}^{-1}$ by fluorescent light tubes in a 16 h light:8 h dark cycle. In the second growth facility, (B), *U. lactuca* discs were exposed to 70 $\mu\text{E m}^{-2} \text{s}^{-1}$ of continuous light under shaking and otherwise similar growth conditions. A glass-bottle contained 4 to 6 discs, and their surface areas were determined daily by measuring the diameter. The growth rate (μ) was calculated by linear regression of \ln to surface area versus time. New discs were punched from the old ones when they exceeded 25 mm diameter. Experiments continued until a constant growth rate was obtained. Discs for affinity experiments were used when this condition was achieved.

In Facility A the medium was renewed twice daily, before and after the light incubation. The initial and final levels of DIC, pH and oxygen were determined in conjunction with every change of media. The mean values were used as a measure of the chemical conditions that *Ulva lactuca* were exposed to. In Facility B, the medium was renewed daily and initial values of DIC, pH and oxygen were used throughout. The daily changes of medium composition were only small. DIC was measured by an infrared gas-analyser (Vermaat and Sand-Jensen 1987), pH by a Radiometer pH-meter and oxygen by Winkler titration or by a Clark-type oxygen electrode.

Media with different DIC, pH and oxygen levels were prepared from estuarine seawater. Reduced DIC levels were obtained by acidifying with HCl to pH 5, sparging with CO_2 -free air or nitrogen, adjusting to pH 8.0–8.2 with freshly prepared NaOH and adding NaHCO_3 to the desired DIC level. Increased DIC levels were obtained by NaHCO_3 addition without prior removal of CO_2 . Different pH levels were obtained from 2 mM DIC solutions by adding NaOH or HCl. Different oxygen levels were created from seawater of pH 8 by bubbling with different gas mixtures, or by mixing oxygen-free and oxygen-saturated seawater. Care was taken to prevent gas exchange with the atmosphere while filling the growth bottles.

The carbon affinity of *Ulva lactuca* discs acclimated to different DIC and pH levels was measured in a small photosynthetic chamber equipped with a fast-response oxygen electrode and a magnetic stirrer (Kusk 1980). The chamber was illuminated at 150 $\mu\text{E m}^{-2} \text{s}^{-1}$, kept at 15°C, and filled with DIC-free seawater at the same pH as the culture medium. After 1 h, when oxygen production had dropped to a low level, but normally not ceased completely, NaHCO_3 was added in increments of 10 to 100 μM and photosynthesis was measured. The kinetics of the photosynthesis-DIC relationship were calculated by linear regression of Lineweaver-Burk plots. The carbon affinity was expressed as the

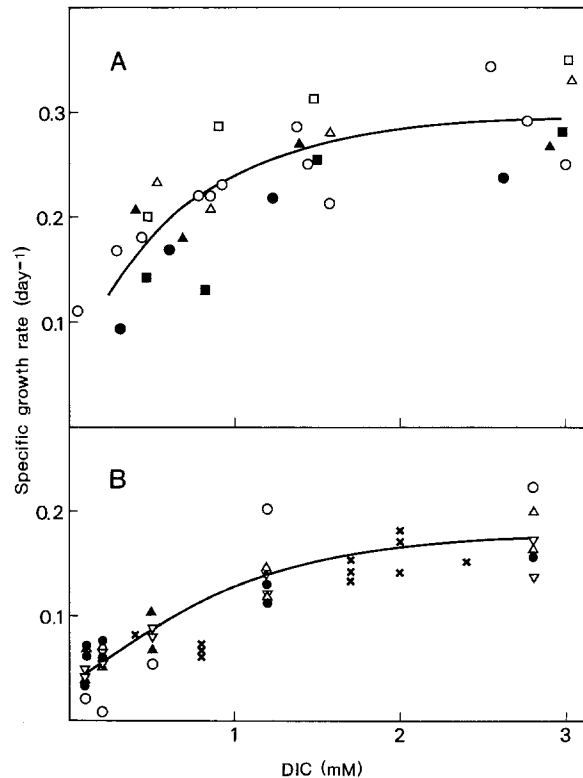


Fig. 1. *Ulva lactuca*. Specific growth rates versus dissolved inorganic carbon (DIC) concentration at constant pH of ca 8, in two culture facilities: (A) continuous circulation of incubation bottles kept at 140 $\mu\text{E m}^{-2} \text{s}^{-1}$ in a 16 h light:8 h dark cycle at 15°C, and (B) reciprocating shaking at 70 $\mu\text{E m}^{-2} \text{s}^{-1}$ with continuous light and at 15°C. Mean growth rates attained over 7 d are shown for different experimental trials (different symbols represent experimental trials with plants collected at different times of the year). Curves fitted according to average growth rates in intervals of ca 0.2 mM DIC conc.

quotient between maximal photosynthesis (V_{max}) and the half-saturation constant ($K_{1/2}$). This quotient gives $K_{1/2}$ representing the slope of the photosynthesis-DIC curve at zero carbon concentration.

Results

Growth rate vs DIC

Growth rate (μ) of *Ulva lactuca* as a function of DIC concentrations showed the same overall pattern in the two growth facilities, but was higher in A than in B (Fig. 1) probably because of a more suitable light and current regime. The DIC concentration of ca 2 mM at atmospheric air equilibrium in natural seawater was close to saturating. In Facility A, μ was ca 0.18 d^{-1} at 0.5 mM, or 60% of μ_{max} , which was ca 0.3 d^{-1} at 3 mM. In Facility B, μ was ca 0.08 d^{-1} at 0.5 mM, or 44% of μ_{max} , which was ca 0.18 d^{-1} at 2.8 mM.

Growth rate vs pH

Growth rate was examined as a function of pH ranging from 7.5–8 to 10 at 2 mM DIC (Fig. 2). The pattern was the same

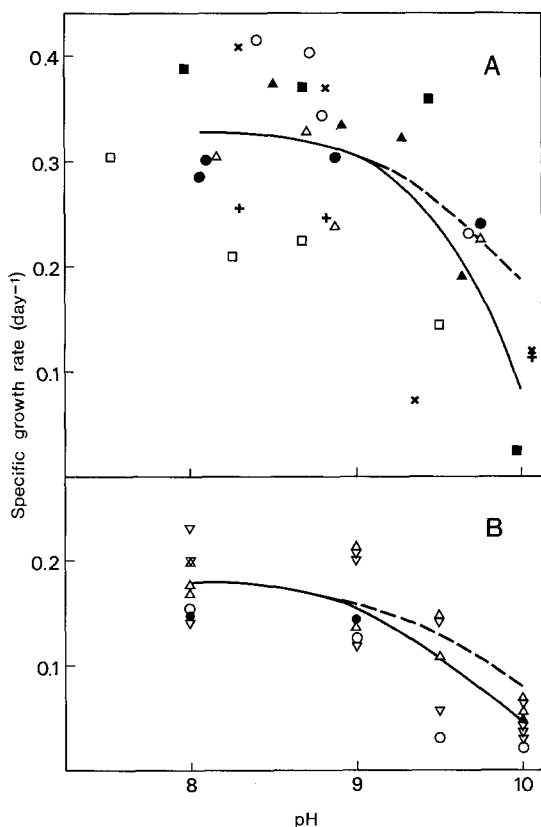


Fig. 2. *Ulva lactuca*. Specific growth rates versus pH at a constant DIC of ca 2 mM in two growth facilities (A and B; see Fig. 1). Continuous line fitted according to average growth rates in pH intervals of ca 0.25. Dashed line shows estimated growth rates at HCO₃⁻-concentrations available at different pH's according to Fig. 1. For explanation of symbols, see Fig. 1

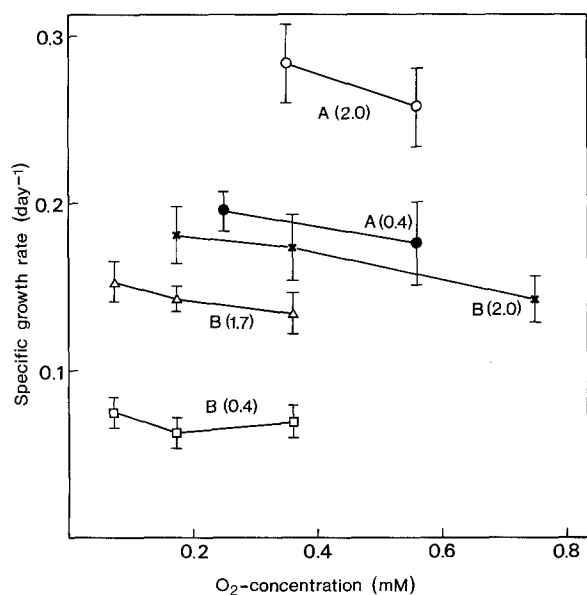


Fig. 3. *Ulva lactuca*. Specific growth rates versus oxygen concentration at different DIC levels and at a constant pH of 8 in two culture facilities (A and B; see Fig. 1). DIC level is shown in parentheses. For explanation of symbols, see Fig. 1

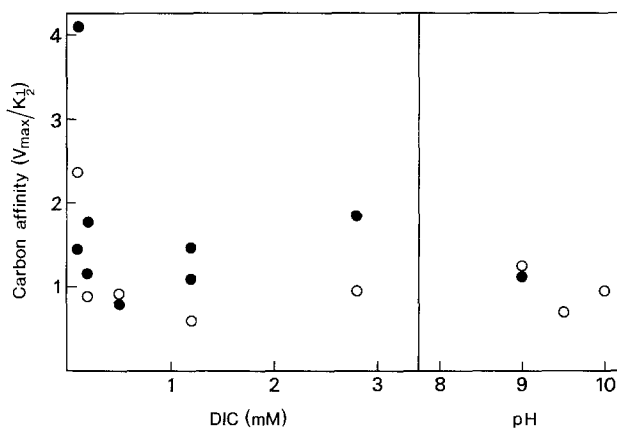


Fig. 4. *Ulva lactuca*. Photosynthetic carbon affinity of plants acclimated to different DIC levels at pH 8 and to pH 9, 9.5 and 10 at 2 mM DIC. Affinity expressed as the quotient between V_{max} (maximal photosynthesis; μmol O₂ cm⁻² h⁻¹) and K_½ (half-saturation constant; mM DIC). Open and closed circles refer to two different thallus samples

in both facilities, again with faster growth in A than in B. The growth rate decreased little from pH 7.5–8 to pH 9. At higher pHs the growth rate declined steeply and approached zero at ca pH 10.5. The HCO₃⁻ concentration declines with increasing pH and is ca 1 mM at pH 9.4 and 0.4 at pH 10 (Riley and Skirrow 1975). The growth rate declined more steeply with decreasing HCO₃⁻ concentration following increase of pH (Fig. 2) than with DIC, and hence HCO₃⁻, decline at a constant pH of 8 (Fig. 1).

Growth rate vs oxygen

Growth rates generally declined with increasing oxygen concentrations at constant DIC and pH ca 8 in both culture facilities (Fig. 3), but only in one case (Facility B, 2 mM DIC) was there a significant decrease in growth rate between the highest and lowest oxygen concentration. Furthermore, there was no indication of oxygen having a greater effect at lower DIC concentrations.

Carbon affinity

Determination of carbon affinity was based on photosynthetic experiments at varying DIC concentrations. Algal material was acclimated to specific DIC levels at pH 8 or to pH 9, 9.5 and 10 at 2 mM DIC (Fig. 4). Carbon affinity was unaffected by previous culture conditions with DIC levels ranging from 0.2 to 3 mM DIC and pHs ranging from 8 to 10. Only at the lowest DIC level of 0.1 mM did we observe an increase in carbon affinity. The apparent half-saturation constants for photosynthesis ranged from 0.4 to 0.7 mM DIC.

Discussion

It is common practice to evaluate growth regulation in the field based on short-term photosynthetic experiments. This

may be problematic because the two processes are different, as are the appropriate time scales and regulations (Harris 1986). In that sense, our laboratory growth experiments are more suitable, as they continued until constant growth rates were established. *Ulva lactuca* also attained maximum growth rates in culture similar to maximum field rates in the estuary (Roskilde Fjord) from where it was collected (0.2 to 0.35 d^{-1} , Geertz-Hansen and Sand-Jensen unpublished). Inorganic carbon requirements should, therefore, resemble each other, making it more legitimate to evaluate in situ growth based on the laboratory experiments. However, flow conditions are highly variable in situ, and this is likely to affect the kinetics of growth vs DIC concentration.

Laboratory growth rates of *Ulva lactuca* were affected by the medium DIC concentrations at pH 8 (Fig. 1). However, the response curve was rather flat and growth was close to saturation at the ambient DIC concentration of 2 mM in air-equilibrated seawater. This suggests that DIC availability would not usually limit *U. lactuca* growth at field sites, provided that water movements resembled those in the cultures, and that DIC availability was not greatly reduced. This view was also strengthened by the fact that seasonal mean growth rates of *U. lactuca* between April and October in Roskilde Fjord were about 0.1 d^{-1} (Christiansen and Hansen 1989, Geertz-Hansen and Sand-Jensen unpublished) and carbon demands were accordingly much lower than when the plant grows at maximum rates. Thus, a growth rate of 0.1 d^{-1} was attained at only ca 0.2 mM DIC in growth Facility A and 0.6 mM in B; such low DIC concentrations are unlikely to occur under most field conditions. Light, temperature and nitrogen availability, on the other hand, are known to be very important for in situ growth rates as demonstrated by field experiments in Roskilde Fjord over the season with simultaneous manipulations of light and nitrogen availabilities (Geertz-Hansen and Sand-Jensen unpublished).

The apparent half-saturation constant, $K_{1/2}$, (DIC) for growth of *Ulva lactuca* was low in both culture facilities, i.e., 0.35 mM in A and 0.55 mM in B, and they resembled those attained in the present as well as in previous photosynthetic experiments (Beer and Eshel 1983, Sand-Jensen and Gordon 1984). Thus, for *U. lactuca* it is permissible to use the results of short-term photosynthetic experiments when describing regulation of growth by DIC availability, belying our initial scepticism. A similar correspondance was observed by Sand-Jensen (1988 a, b) in photosynthetic and growth experiments with *U. lactuca* acclimated to different light levels. The findings are perhaps not surprising considering that the carbon affinity of *U. lactuca* was virtually unaffected by acclimatization to DIC levels between 0.2 and 3.0 mM (Fig. 4). The correspondance between short-term photosynthetic experiments and long-term growth experiments may rely on the fact that *U. lactuca* is a fast growing organism, where carbon fixed during photosynthesis is rapidly used in cell production, thereby minimizing the importance of accumulation or mobilization of organic storage products. On the other hand, great discrepancies between photosynthesis and growth are found in slow-growing perennial macroalgae,

where photosynthetic products stored at one time of the year are mobilized for growth during later periods, when nutrients become available (Hatcher et al. 1977).

The growth rate of *Ulva lactuca* declined steeply from pH 9 to 10 which is similar to results of photosynthetic experiments (Beer and Eshel 1983, Sand-Jensen and Gordon 1984) and, as the dashed lines of Fig. 2 infer, part of this decline must be due to a specific pH-effect in addition to the reduced availability of HCO_3^- -ions. The same general pattern has been observed in many other growth and photosynthetic experiments with macroalgae and aquatic angiosperms (Blinks 1963, Sand-Jensen and Gordon 1984, Lignell and Pedersen 1989). High pH may impair photosynthesis and growth in several ways. It may create greater energetic costs to maintain less alkaline pHs in the cells. The reduced CO_2 and HCO_3^- levels in the water and the associated higher concentrations of OH^- and CO_3^{2-} may impair the ability to take up inorganic carbon from the medium (Sand-Jensen 1983). Finally, the general operation of ion-pumps in the plasmalemma may be depressed and reduce uptake of other essential ions as well.

The low sensitivity of *Ulva lactuca* growth to a 4 to 5-fold increase in oxygen concentration (Fig. 3) also corresponded with photosynthetic experiments (Colman 1984, Bidwell and McLachlan 1985, Beer and Israel 1986). In addition, these authors reported a low and oxygen-insensitive carbon compensation point for *U. lactuca*, and enzymatically characterized (Beer and Israel 1986) the alga as a C_3 plant. Together with the high carbon affinity for growth and photosynthesis, these findings may suggest that *U. lactuca* possesses a carbon concentrating mechanism similar to the one found in microalgae (Raven 1985). This argument, however, has recently been questioned (Smith and Bidwell 1989). The carbon affinity (Fig. 4) and oxygen concentration (Fig. 3) experiments showed, that this carbon acquisition process was virtually unaffected by the tested variations in DIC, pH and oxygen. Another consequence of stable carbon affinity is that the reduced growth rates at decreasing DIC concentration and at increasing pH most plausibly are explained by a reduced diffusive carbon supply to the surface of the thallus perhaps, at high pH, combined with a less efficient operation of the carbon acquisition process. It does not rule out, however, that high CO_2 or HCO_3^- levels, above those that are likely to occur in marine environments, may reduce the carbon affinity of *U. lactuca*.

Among the marine macroalgae tested, many show the same insensitivity of photosynthesis to oxygen (Bidwell and McLachlan 1985, Beer and Shrage 1987, Holbrook et al. 1988, Reiskind et al. 1988), and in most instances this has been explained by the operation of a carbon concentrating mechanism. However, sensitivity of photosynthesis to oxygen has been found in other macroalgal species, and carboxylating enzymes other than Rubisco have also been found at high enough activities to consider them important in the carbon fixation process (Bowes and Reiskind 1987, Holbrook et al. 1988). Thus, the versatility of the carbon fixation processes in macroalgae is far from being known in detail.

Extensive photosynthesis of *Ulva lactuca* in dense mats is accompanied by pH and oxygen increases and a DIC decline. If we assume that *U. lactuca* starts to photosynthesize in a closed system with natural seawater of 2.0 mM DIC at air-equilibrium, then, when pH 10 is reached, the calculated DIC level will be ca 1.15 mM (0.23 mM HCO_3^-) and the corresponding oxygen level will be about 1.1 mM. According to the culture experiments of Fig. 1 the growth rate would then decline by 15% by DIC reduction alone and by 60% when the decline in HCO_3^- concentration is taken into account. It is evident from Fig. 2, however, that at pH 10 the actual growth rate is approximately halved compared to what should be expected from the decline in HCO_3^- concentration alone. So, the combined effects of diminished HCO_3^- concentration and increased pH will result in a decline in growth rate of 80%; the increase in oxygen will only have a small effect.

We conclude that both the high pH and, particularly, the reduced HCO_3^- availability may impair *Ulva lactuca* growth under mass accumulation. The relative importance of reduced HCO_3^- availability for growth at pH 10 may also increase in dense mats because of reduced bulk movements in the water and reduced transport of HCO_3^- to the thallus surface. However, it is important to acknowledge that dense mats are characterized by extensive self-shading as well. At present, we are unable to account for the interactive effects of light, DIC and pH on algal photosynthesis and growth in dense mats.

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