A comparative study of the effect of pH and inorganic carbon resources on the photosynthesis of three floating macroalgal species of a Mediterranean coastal lagoon

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Abstract

This study examines the effect of pH changes on photosynthetic characteristics and the role of dissolved inorganic carbon (DIC) in determining the dominance of three species of macroalgae *Chaetomorpha linum* (O.F. Müller) Kützing, *Gracilaria verrucosa* (Hudson) Papenfuss and *Ulva* sp. in a Mediterranean coastal lagoon. Fluctuations of pH were measured in the lagoon in summer. Water column CO₂ and HCO₃⁻ concentrations inside the algal mat showed significant diurnal fluctuations, from a morning peak to an afternoon low, decreasing 96 and 40%, respectively. The response of photosynthesis to increased pH was examined in laboratory conditions in spring (May) and summer (July). The photosynthetic rate declined rapidly at pH above 8.5 and below 6.5. *G. verrucosa* responded differently in spring and summer showing acclimation to higher pH in summer than in spring. In *Ulva* sp. incubations, we observed optimum photosynthesis between pH 6 and 7.5. The decrease in photosynthetic rate below pH 6 was lower (12.30%) than above pH 8 (81.03%). This difference may be related to the origin of the macroalgae, suggesting acclimation to the original pH of the environment. Results from instantaneous photosynthesis measurements indicate that low DIC-availability limits the photosynthetic capacity of *G. verrucosa*, *C. linum* and *Ulva* sp. in spring and of *C. linum* at high irradiances in summer. Our results also show that *G. verrucosa* has a higher efficiency at low CO₂ concentrations than *C. linum* and *Ulva* sp. It is suggested that *G. verrucosa* may be better adapted to maintain higher photosynthetic rates than other macroalgae in conditions of tissue N sufficiency and low water DIC concentrations which are typical of shallow coastal environments in summer.

Keywords: Coastal lagoon; Inorganic carbon; Macroalgae; pH; Photosynthesis

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1. Introduction

The total amount of dissolved inorganic carbon in water (DIC) includes CO$_2$ (free CO$_2$ + H$_2$CO$_3$), HCO$_3^-$ and CO$_3^{2-}$. All aquatic plants use CO$_2$ for photosynthesis, and some species also use HCO$_3^-$ (Raven, 1991). The availability of HCO$_3^-$ and CO$_2$ is variable in freshwater habitats and the CO$_2$ concentration can be subjected to large variations when biological activity is high. The concentrations of the two carbon forms vary greatly among sites, and can also fluctuate temporally and spatially within a site as a result of photosynthesis or respiration, tempered by atmospheric, sedimentary and hydrological exchange (Maberly, 1996). At atmospheric equilibrium, most freshwaters have pH between 7 and 8.5 and HCO$_3^-$/CO$_2$ ratios from about 4 to 140, whereas the pH of seawater is close to 8.2 and the HCO$_3^-$/CO$_2$ ratio is about 150 (Stumm and Morgan, 1970).

The pH of water in coastal areas and enclosed seas normally ranges from 7.9 to 8.2 (Dromgoole, 1978). It has been suggested that pH changes within this range have little ecological significance for marine algae (Chapman, 1962), however the concentration of dissolved CO$_2$ alters by approximately 10% for every pH unit near pH 8. Moreover, the solubility coefficients are temperature and salinity dependent and are higher for CO$_2$ (1019.0 ml CO$_2$ l$^{-1}$ water at 15°C and for 1 atm partial pressure) than for O$_2$ (34.1 ml O$_2$ l$^{-1}$ water at 15°C and for 1 atm partial pressure) and CO$_2$ solubility declines faster with increasing temperature than that of O$_2$ (Madsen and Sand-Jensen, 1991). In summer, higher temperatures and high conductivity owing to the water evaporation observed in shallow environments such coastal lagoons, decreased CO$_2$ solubility. Thus, for algae which can assimilate only free CO$_2$ and with a low affinity for HCO$_3^-$, such pH changes may affect photosynthesis by limiting the rate of inorganic carbon (C$_i$) uptake (Dromgoole, 1978). Recent studies have shown that DIC may also limit the growth of submerged macrophytes in lakes with an alkalinity higher than 1 mEq l$^{-1}$ (Madsen and Maberly, 1991; Jones et al., 1996), even in species that can use HCO$_3^-$ as a carbon source for photosynthesis (Van Wijk, 1989).

Tancada is a shallow coastal lagoon with a mean conductivity of 33 mS cm$^{-1}$ (ranging between 10 and 65 mS cm$^{-1}$). The DIC concentration in the water column varies between 3.0 and 3.9 mmol l$^{-1}$, and the pH remains fairly constant at about 8.2 except at the end of summer when it rises to 9. In the last few years, proliferation of floating macroalgae, mainly Chaetomorpha linum and Gracilaria verrucosa, has been observed in sheltered zones of the lagoon in spring and summer (Comin et al., 1995). Ulva was observed only in winter and early spring and normally accumulated near the sediment and close to the border of the lagoon; in summer it died and rapid decomposition occurred. In late spring and summer an increase in pH, reaching 10, was observed within the macroalgae mats at the end of the day. A reduction of alkalinity from 3.22 mEq l$^{-1}$ in February to 2 mEq l$^{-1}$ in August 1997 was observed and was presumably related to photosynthetic DIC macroalgae uptake and CaCO$_3$ precipitation. Therefore, the buffering influence of bicarbonate and carbonate decreased and pH increased resulting in CO$_2$ depletion and a diminution of HCO$_3^-$ concentration.

The aim of this study was to examine the seasonal (spring and summer) photosynthetic responses of Chaetomorpha linum, Gracilaria verrucosa and Ulva sp., the
most abundant macroalgae in Tancada lagoon, to changes in the availability of dissolved inorganic carbon, as a consequence of pH changes, and the potential consequences of such changes for species competition and dominance. Laboratory incubations were carried out at two DIC levels (ambient and enriched) and the photosynthetic characteristics of the three species were measured under several pH conditions.

2. Materials and methods

2.1. Study area

Tancada is a brackish coastal lagoon located in the Ebro River Delta (NE Spain, 40°40’N, 0°40’E) with an area of 1.8 km² and average depth of 37 cm. In the past, this lagoon received freshwater inputs from rice fields annually from May–October, but in the last 10 years, the freshwater inflows have decreased progressively due to agricultural management practices. This long-term hydrological change has caused disturbances in the submerged vegetation and has led to a decrease in diversity and coverage of rooted macrophytes and an increase in floating macroalgae growth during spring and summer (Comin et al., 1995) with Chaetomorpha linum and Gracilaria verrucosa accounting for the highest biomass.

2.2. Diurnal variations of water-column pH above and within the macroalgae mat.

Field measurements of pH were performed in July when photosynthesis of algae is maximum and the conditions of temperature and calm waters are more likely to induce a maximum increase in water pH. Temperature, pH (WTW P-4 multiparametric sensor) and alkalinity of the water (potentiometric titration) inside (3 cm within an algal mat) and outside the macroalgal mat were measured between 09:00 and 17:00 h GMT. A 25-ml syringe with an L-shaped plastic tube with a 2-mm diameter was used to sample water inside the algal mat. The tube was inserted gently inside the mat and 25 ml of water was sampled.

2.3. Laboratory experimental setup

During spring (May) and summer (July) 1998, macroalgae were collected from the lagoon and maintained them aerated under ambient temperature and nearly saturating photosynthetically active radiation (PAR) photon flux densities (400 μmol m⁻² s⁻¹) under a natural light/dark cycle as found in the field, for no longer than 4 days. We selected healthy fronds for laboratory experiments. Winkler 100 cm³ bottles were filled with filtered (0.45 μm) water from the coastal lagoon. Water was kept in darkness overnight in order to maintain oxygen concentration in equilibrium with air after oxygen increase due to water mixing with air related to filtration (initial O₂ content about 6–7 mg l⁻¹). Macroalgal samples (approximately 0.05 g dry weight) were incubated in each Winkler bottle. Dry weight was determined after each experiment by drying at 65°C until constant weight (48 h).
Mixing was provided by stirring bars (ca. 200 rpm) to help \( \text{O}_2 \) and \( \text{CO}_2 \) diffusion throughout the stagnant boundary layers surrounding the thalli, and to avoid oxygen stratification in the bottle. The bottles were placed in an incubator at 20 and 25°C in May and July, respectively, which corresponded to the water temperatures observed in the lagoon. Light was provided by daylight fluorescent tubes (400–700 nm waveband). Photosynthetic active radiation was measured with a quantum radiometer (LiCor). After 1.5 h of incubation, dissolved oxygen in the water within the bottles was measured using an oxygen electrode (WTW P-4 multiparametric sensor) (precision 0.01 mg l \(^{-1}\)), and then compared to the concentration in control bottles without plants \((n = 3)\). All the results are expressed in mg \( \text{O}_2 \) g \(^{-1}\) dry weight (DW) h \(^{-1}\).

Photosynthetic measurements were made under ambient DIC (pH 8.4, 0.023–0.0027 mM \( \text{CO}_2 \) and 3.55 and 3.07 mM DIC in May and July, respectively) concentrations and enriched (10 mM NaHCO\(_3\)) (pH 7.9, 0.38–0.41 mM \( \text{CO}_2 \), final concentration 13 mM DIC). The irradiances needed to assess photosynthesis–irradiance relationships \((0, 40, 70, 160, 220, 350, 450, 550, 650, 750 \mu \text{mol photon m}^{-2} \text{s}^{-1}\) were obtained by covering the bottles with appropriate neutral filters. Three replicate experiments were performed on three consecutive days. At the end of incubation, a photosynthesis vs. irradiance curve was generated using the Enzfit program (Menendez and Sanchez, 1998). We determined the maximum rates of net primary production \((P_{\text{max}})\) by averaging points on the light-saturated portion of each \(P\) vs. \(I\) fitted curve (i.e. as the slope approached zero).

Respiration rate \((R)\) was determined by the oxygen consumption in dark bottles. A linear regression of photosynthetic rates at 0, 40 and 70 \(\mu\text{mol m}^{-2} \text{s}^{-1}\), was used to calculate the initial slope \((\alpha)\). Photosynthetic compensation photon flux densities \((I_c)\) (where the net production is balanced by respiration) were determined from the interception between initial slope and the \(x\)-axis.

The preference of macroalgae for different forms of DIC was determined from the photosynthetic response to ambient DIC at several pH values ranging from 4 (99.4% \(\text{CO}_2\) and 0.0056% \(\text{HCO}_3^-\)) to 9.5 (0.0004% \(\text{CO}_2\) and 79% \(\text{HCO}_3^-\)). The pH of the incubation water was adjusted using 1 N NaOH and 1 N HCl at saturating photon flux densities of 400 \(\mu\text{mol m}^{-2} \text{s}^{-1}\). We repeated the experiment twice, on day 1 and 4 after macroalgae collection from the lagoon. Alkalinity was measured by potentiometric titration and DIC, \(\text{CO}_2\), \(\text{HCO}_3^-\) and \(\text{CO}_3^{2-}\) concentrations were computed from pH values (initial and final incubation pHs were recorded, and the average pH was used for calculations), alkalinity, conductivity and temperature were measured following Mackereth et al. (1978) using a computer program (WATEQ).

### 2.4. Statistical analysis

To test the effect of seasonality (spring vs. summer) and DIC availability (ambient and enriched 10 mM NaHCO\(_3\)) on \(P_{\text{max}}, I_c\) and \(\alpha\) on each of the macroalgae species analysis of variance (ANOVA) was used. The fitted characteristics of the curves were log transformed to obtain homogeneity of variance and tested by two-way ANOVA followed by a comparison of means (Tukey test, \(P < 0.05\)). In addition, differences between the type of algae were tested with data obtained in May and July. Student’s \(t\)-tests were used to compare diurnal fluctuations of pH and \(\text{CO}_2\) and \(\text{HCO}_3^-\) concentrations in water.
column, inside and outside algal mat. The CSS-Statistica computer program was used for statistical analysis.

3. Results

3.1. Diurnal variation of pH in the water column

The pH of the water in the lagoon outside the macroalgal mats varied from 8.3 (09:00 h) to 9 (17:00 h) during the daily period studied, while inside the algae mat it increased from 8.4 (09:00 h) to 9.6 (17:00 h) ($t = 3.3$, $P < 0.05$) (Fig. 1). While total water column DIC daily variation was generally 2.5–3 mM both outside and inside the algal mat ($t = 2.2$, $P > 0.05$), dissolved CO$_2$ concentrations varied throughout the day during the

![Graph](image)

Fig. 1. Time course of daily pH changes, dissolved HCO$_3^-$ and CO$_2$ concentrations (mmol l$^{-1}$) measured in the water outside and inside an algal mat in Tancada lagoon during summer (July) ($n = 3$). Irradiance at water surface is also given.
summer (July), ranging from a morning peak of approximately 20 μM, to an afternoon low of 4 and 0.7 μM outside and inside the algal mat, respectively (t = 4.33, P < 0.05). This diurnal variation was presumably related to CO₂ uptake by the active algal mat. The time course of HCO₃⁻ was similar to that of CO₂, but the concentration was 2.98±0.02 mM (about 100-fold that of CO₂) and the decrease from the morning to the afternoon was less pronounced than that the observed for CO₂. Significant differences between the concentrations of HCO₃⁻ outside and inside the algal mat (t = 4.53, P < 0.05) were observed beginning from pH 9 (Fig. 1).

3.2. pH study

The photosynthetic rate of Gracilaria showed an optimum at pH 6–7.5 in May but this value increased in July when a maximum photosynthetic rate was observed between pH 6 and 8.5 (Fig. 2). At pH above 8 and 9 and below 6 and 7 in spring and summer, respectively, its photosynthetic rate declined rapidly. In Ulva sp. incubations, we observed optimum photosynthesis between pH 6 and 7.5. At pH above 7.5, the photosynthetic rate declined rapidly while below 6 this decline was less pronounced (Fig. 2). Chaetomorpha showed the same behaviour in spring and summer, with maximum photosynthetic rates between pH 6 and 7.5, but with higher photosynthetic rates in spring.

3.3. Photosynthesis measurements

We studied the effect of DIC enrichment on photosynthetic capacity during spring (May) and summer (July) in Chaetomorpha, Gracilaria and Ulva (Fig. 3, Table 1). In May, all the species exhibited a DIC enhancement effect (F = 18.19, P < 0.005), however no differences were observed between species. In contrast, during summer only Chaetomorpha linum showed enhanced photosynthetic capacity under DIC enrichment (F = 10.54, P < 0.05). In Gracilaria incubations, we observed inhibition of photosynthesis at high irradiance both in enriched and in ambient treatment, whereas in Chaetomorpha photoinhibition was observed only under ambient DIC treatment (Fig. 3). The enrichment effect was also positive in spring, lowering photosynthetic compensation photon flux densities (Iₖ), and also showing significant differences between species (F > 60.57, P < 10⁻⁶). The effect of enrichment on Iₖ was more marked in Chaetomorpha (82.43%) than in Gracilaria (54.43%) and in Ulva (46.84%). In July, the opposite effect of DIC enrichment was observed on Chaetomorpha. Iₖ increased, probably due to the high photosynthetic rate observed. No significant differences were observed in the initial slope (α) between either DIC concentrations or different algal species (F < 2.20, P > 0.05).

4. Discussion

The water in Tancada coastal lagoon contains a high concentration of dissolved inorganic carbon, but in general only a small proportion of this occurs as CO₂, and the
Fig. 2. Photosynthetic rates of *Gracilaria verrucosa*, *Ulva* sp. and *Chaetomorpha linum* with increasing pH in spring (May) and summer (July). Curves were fitted by third order regression ($r^2 > 0.83$, $n > 11$, $P < 0.001$, $r^2 = 0.83$ was the lowest value calculated of regression coefficient).

The remainder is found in ionic forms such as HCO$_3^-$ and as CO$_3^{2-}$. If CO$_2$ is removed, for example due to photosynthetic uptake, new CO$_2$ is formed spontaneously from the ionic forms, but this process is slow when uncatalysed (Miller, 1985). In addition, the diffusion rates of all DIC forms are slow in water (Axelsson, 1988). In response to this situation, macroalgae have developed several adaptive mechanisms which allow them to use the HCO$_3^-$ pool in shallow more productive environments such as coastal lagoons.
Fig. 3. Photosynthetic rates (mg O$_2$ g$^{-1}$ DW h$^{-1}$) of macroalgae at varying photon flux rate densities (µmol m$^{-2}$ s$^{-1}$) under ambient and enriched (10 mM) dissolved inorganic carbon (DIC) in spring (May) and summer (July) ($r^2 > 0.90$, $P < 0.001$, $r^2 = 0.90$ was the lowest value calculated of the regression coefficient). Vertical bars are standard errors ($n = 3$).
Table 1
Photosynthetic parameters estimated from *G. verrucosa*, *C. linum* and *Ulva* incubations under ambient and enriched (10 mM) dissolved inorganic carbon (DIC) in spring (May) and summer (July)

<table>
<thead>
<tr>
<th>Species / DIC (mM)</th>
<th>$P_{\text{max}}$ (mgO$_2$ gDW$^{-1}$ h$^{-1}$)</th>
<th>$I_c$ (µmol m$^{-2}$ s$^{-1}$)</th>
<th>$\alpha$ (mgO$_2$ gDW$^{-1}$ h$^{-1}$/µmol m$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. linum</em>/3.5</td>
<td>6.00±0.25</td>
<td>34.96±1.26</td>
<td>0.035±0.004</td>
</tr>
<tr>
<td><em>C. linum</em>/10</td>
<td>8.14±1.00</td>
<td>5.87±0.54</td>
<td>0.028±0.005</td>
</tr>
<tr>
<td><em>G. verrucosa</em>/3.5</td>
<td>7.92±0.57</td>
<td>79.79±9.15</td>
<td>0.018±0.005</td>
</tr>
<tr>
<td><em>G. verrucosa</em>/10</td>
<td>13.58±1.55</td>
<td>36.36±2.71</td>
<td>0.026±0.009</td>
</tr>
<tr>
<td><em>Ulva</em>/3.5</td>
<td>12.90±1.16</td>
<td>43.00±12.7</td>
<td>0.022±0.004</td>
</tr>
<tr>
<td><em>Ulva</em>/10</td>
<td>16.54±3.22</td>
<td>22.86±4.89</td>
<td>0.021±0.002</td>
</tr>
<tr>
<td>July</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. linum</em>/3.1</td>
<td>Photoinhibition</td>
<td>16.69±5.90</td>
<td>0.035±0.003</td>
</tr>
<tr>
<td><em>C. linum</em>/10</td>
<td>17.39±1.75</td>
<td>39.06±1.65</td>
<td>0.033±0.007</td>
</tr>
<tr>
<td><em>G. verrucosa</em>/3.1</td>
<td>Photoinhibition</td>
<td>13.08±4.59</td>
<td>0.027±0.002</td>
</tr>
<tr>
<td><em>G. verrucosa</em>/10</td>
<td>Photoinhibition</td>
<td>3.11±1.30</td>
<td>0.032±0.004</td>
</tr>
</tbody>
</table>

Extracellular carbonic anhydrase activity seems to be very common among macroalgae (Larsson and Axelsson, 1999), but the mechanism has essentially no capacity to utilize DIC in seawater at high pH (Axelsson et al., 1999). This, together with a very high affinity for CO$_2$, supports the hypothesis that this mechanism operates via external dehydration of HCO$_3^-$ followed by (passive or active) CO$_2$ entry through the plasma membrane (Axelsson et al., 1995). The second mechanism for utilizing the bicarbonate pool is the direct uptake of HCO$_3^-$ through the plasma membrane and is, in addition, capable of supporting photosynthesis at high pH values (Larsson et al., 1997). It has been suggested that the two HCO$_3^-$ utilization mechanisms described in *Ulva lactuca* (Axelsson et al., 1995) may function in distinct environments and, therefore, confer an adaptive advantage on algae growing in those conditions.

The data presented here illustrate the plasticity response of floating macroalgae in Tancada lagoon to inorganic carbon. Although photosynthesis was not measured in situ the decrease in the CO$_2$ concentration inside the algal mat observed at relatively low pH was probably associated with CO$_2$ uptake during the morning. Differences observed between decrease on HCO$_3^-$ concentrations inside and outside the algae mat were presumably related to bicarbonate uptake when pH rose to 9. Diurnal decrease in CO$_2$ levels in the water column outside the algal mat was probably related to the low volume of this shallow lagoon and to the huge development of phytoplankton observed in summer (maximum productivity in summer of 49–108 mg C m$^{-3}$ h$^{-1}$; Comin, 1984). This ability to use various forms of DIC would enhance the photosynthetic capacity of these macroalgae under conditions of saturating irradiance and high algal biomass, as occurs in shallow areas of eutrophic systems (Valiela et al., 1992; Peckol et al., 1994; Rivers and Peckol, 1995). We estimated a daily CO$_2$ concentration in the water column ranging from 0.004 to 0.026 mmol l$^{-1}$ at pH 8.3–9.2. Under this low availability, most macrophytes use bicarbonate as a source of DIC, but this use is inefficient — in terms of energy cost — compared with that of CO$_2$ (Beer, 1994). Our results suggest that all
three species of macroalgae studied can use various forms of DIC, however the photosynthesis response to distinct pH and DIC concentrations seems different.

In *Gracilaria* we detected an increase in the pH range at which this species reached a high photosynthetic rate in summer in relation with the optimum range of pH in spring. This observation indicates that *Gracilaria* acclimates to increases in the pH of water, as reported for some freshwater and marine macrophytes (Maberly and Spence, 1983; Beer et al., 1990; Madsen and Sand-Jensen, 1991; Madsen et al., 1996; Invers et al., 1997; Van den Berg et al., 1999). In contrast, in *Chaetomorpha*, we did not observe this seasonal different response in photosynthesis at high pH. The pH values at which this species reached a maximum photosynthetic rate were the same in summer and in spring. The observation that *Gracilaria* showed enhanced photosynthesis at higher pH in summer than spring, whereas *Chaetomorpha* did not show this response, suggests an adaptive advantage of *Gracilaria* over *Chaetomorpha* at high pH observed in Tancada lagoon in summer.

At initial pH above 9, a decrease in the final pH was observed. These results are consistent with reduced alkalinity occurring at high pH (Fig. 4). The alkalinity at the end of experiments at pH 9 and 9.2 (Fig. 4) was reduced from initial alkalinity of 4.98 mEq l$^{-1}$ to 3.12 and 3.59 mEq l$^{-1}$ in *Gracilaria* and to 2.89 and 3.74 mEq l$^{-1}$ in *Chaetomorpha* suggesting HCO$_3^-$ uptake or precipitation of CaCO$_3$. The efflux of hydroxide ions, which is associated with HCO$_3^-$ uptake, causes the precipitation of CO$_2$. In coastal lagoons Ca$^{2+}$ is generally present in excess of the concentrations in equilibrium with CaCO$_3$, meaning that the solution is supersaturated. Calcification leads to a loss of one Ca$^{2+}$ for each atom of carbon precipitated. This loss is compensated by the formation of H$^{+}$ which shifts the equilibrium of the inorganic carbon system to the formation of CO$_2$, thus reducing the concentration of total dissolved inorganic carbon.

When initial pH was below 8.5, these two species increased pH and hence extracted inorganic carbon (Fig. 4). For every HCO$_3^-$ ion taken up by the macroalgae one hydroxide ion is excreted to maintain electrical neutrality (Lucas and Smith, 1973). The uptake of HCO$_3^-$ results in higher water pH values. In general pH increase (when initial water pH was low) was more important in July than in May in both species of macroalgae, *Gracilaria* and *Chaetomorpha*, suggesting higher HCO$_3^-$ uptake in summer than in spring (Fig. 4). This increment in final pH was also more evident in *Gracilaria* than in *Chaetomorpha*. The graph is also helpful to show HCO$_3^-$ uptake and/or precipitation of CaCO$_3$ at high pH (above initial pH 9), related to decrease of initial alkalinity observed after the incubation.

In *Ulva*, a decrease in photosynthesis was observed at pH 8--9 while it reached maximum values of production between pH 6 and 8, these observations contrast with values reported by Invers et al. (1997). These results are probably related to the origin of *Ulva* in Tancada lagoon. In winter, spring and early summer, *Ulva* develops mainly in the canals of communication with the sea, which are often closed, and characterized by high accumulations of organic matter and an intense decomposition process, thus providing high CO$_2$ availability in the water column. The high production observed at pH 5, probably reflects an adaptation to this environment. *Ulva* only was observed in the lagoon, and had a low density, when the sea level rose, water flow from the sea to the lagoon increases and transports the macroalgae that grow in the canal into the lagoon. If
Fig. 4. Relation between initial and final water pH in spring (May) and summer (July) observed during laboratory pH experiments with *Gracilaria verrucosa* and *Chaetomorpha linum* (a). Relation between initial and final values in water alkalinity observed during the same experiments in summer. Comparison with constant value of alkalinity during experiment (1:1) is shown (b).
the dissolved nitrogen concentration in the lagoon is high, water temperature relatively low and primary production moderate, thus allowing pH values around 8 and sufficient dissolved CO$_2$ in water, as occurs in winter, Ulva could survive. However, if dissolved nitrogen and CO$_2$ are depleted by active uptake of primary producers, which are better adapted to low nitrogen concentrations and have a high affinity for bicarbonate, such as Chaetomorpha or Gracilaria (Lavery and McComb, 1991; Rivers and Peckol, 1995; Anderson et al., 1996), Ulva dies, decomposes and disappears as observed in Tancada lagoon in summer.

As Lavery et al. (1991) reported from experimental data obtained in Peel Inlet estuary (Western Australia), Chaetomorpha and Gracilaria were seldom nutrient-limited except after prolonged periods of low nutrient input to the system and, Gracilaria is able to maintain a high growth rate even when ambient N levels are low. It can store N for long periods, and can persist if the nitrogen concentration in the water is low or only sporadically high (Anderson et al., 1996). Chaetomorpha can perform luxury consumption in winter (Pedersen, 1995; McGlathery et al., 1996), and its ability to promote and utilize sediment remineralization of nutrients under the thickest banks of macroalgae (Josselyn and West, 1985; Lavery and McComb, 1991) may support growth in summer.

Our enrichment experiments have shown photosynthesis enhancement under enriched DIC conditions in these species of macroalgae in spring, and also decreased $I_c$, which indicates that under high carbon availability these macroalgae could maintain a positive carbon balance under low light conditions. The latter effect is more important in Chaetomorpha than in Gracilaria or Ulva. This could be related to the fact that under enriched DIC conditions, the relative CO$_2$ concentration increased more than the other forms of DIC (from 0.78 to 2.25% DIC whereas % of HCO$_3$ remained constant at 97% and CO$_3^{2-}$ decreased from 1.95 to 0.67%).

In summer, the effect of DIC enrichment was observed only in Chaetomorpha incubations when irradiance reached values over 500 $\mu$mol m$^{-2}$ s$^{-1}$, showing limitation of photosynthesis by carbon at high irradiances and high pH. In Gracilaria, a positive effect of DIC enrichment on photosynthesis was not observed, suggesting that inhibition of production at higher irradiances was not related to carbon availability and high pH, but to high irradiance itself, as reflected by the whitish colour observed in algae growing on the surface of the mat in the lagoon, suggesting photodestruction of pigments.

In Tancada lagoon, inorganic carbon limitation is probably strong enough in summer, to contribute to a shift dominance of Gracilaria over Chaetomorpha in the banks of the lagoon where high pH values were observed due to both higher irradiance, which leads to high photosynthetic rates, and to small water volumes. In these shallow environments Chaetomorpha grows in thick mats near the sediment–water interphase where low oxygen concentrations (4.5 mg l$^{-1}$) and relative low pH values (around 7.2) were observed, which probably favours the release of nutrients and CO$_2$ from the sediment to the water, thus contributing to algal growth. High temperature and irradiance in the banks of the lagoon could be over-come by shelf shading. For example, the temperature and irradiance were 35$^\circ$C and 201 $\mu$mol m$^{-2}$ s$^{-1}$ above a dense Chaetomorpha mat in a shallow (15 cm) zone of the lagoon on 20th July 1996 at 17:00 h, whereas they were 29$^\circ$C and 350 $\mu$mol m$^{-2}$ s$^{-1}$ inside algal mat. Moreover, a brown algae in the upper part of the mat and an intense green algae in the inner part were observed, suggesting degradation of chlorophyll and acclimation to low irradiance, respectively.
Gracilaria has high nitrogen storage capacity (Fujita, 1985; Anderson et al., 1996) and high affinity for dissolved CO$_2$. Therefore it may have a competitive advantage compared to Ulva and Chaetomorpha in Tancada lagoon in summer, when dissolved nitrogen and CO$_2$ are greatly reduced compared to winter and spring values (Comin, 1984; Comin et al., 1995), due to increase water temperatures and metabolic demands of the highly productive opportunistic species in this system.

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References