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Photosynthetic utilization of $CO_{2(aq)}$ and HCO_3^- in *Thalassia testudinum* (Hydrocharitaceae)

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Abstract. The effects of total dissolved inorganic carbon (DIC), free carbon dioxide [$CO_{2(aq)}$], and bicarbonate (HCO_3^-) concentrations on net photosynthetic oxygen evolution of the marine angiosperm *Thalassia testudinum* Banks ex König collected from Biscayne Bay (1988) and from Tampa Bay (1990), Florida, USA, were examined. Rates of photosynthesis declined by 85% from pH 7.25 to 8.75 in buffered seawater media with constant DIC concentration (2.20 m*M*), suggesting a strong influence of $CO_{2(aq)}$ concentration. A plateau in the pH-response curve between pH 7.75 and 8.50 indicated possible utilization of HCO_3^-. Responses of photosynthesis measured in buffered seawater media of varying DIC concentrations (0.75 to 13.17 m*M*) and pH (7.8 to 8.61) demonstrated that photosynthesis is rate-limited at ambient DIC levels. Photosynthesis increased in media with increasing HCO_3^- concentrations but near-constant $CO_{2(aq)}$ levels, confirming HCO_3^- assimilation. Calculated half-saturation constants (K_s) for $CO_{2(aq)}$ and HCO_3^- indicated a high affinity for the former [$K_s(CO_2)$ = 3 to 18 μM] and a much lower affinity for the latter [$K_s(HCO_3^-)$ = 1.22 to 8.88 m*M*]. Calculated V_{max} values for HCO_3^- were generally higher than those for $CO_{2(aq)}$, suggesting relatively efficient HCO_3^- utilization, despite the apparent low affinity for this carbon form.

Introduction

Unlike the terrestrial plants from which they presumably evolved, submersed aquatic macrophytes are surrounded by a medium in which dissolved inorganic carbon (DIC) is present in three available forms: free carbon dioxide ($CO_{2(aq)}$), bicarbonate ions (HCO_3^-), and carbonate ions (CO_3^{-2}). All aquatic plants use $CO_{2(aq)}$ for photosynthesis. Carbon dioxide solubility in freshwater is high, with a partition coefficient of ~1 between 10 and 20 °C (Stumm and Morgan 1981). In the marine environment, CO_2 solubility is reduced by 10 to 15% compared with air-equilibrated freshwater due to the effect of salinity on the partition coefficient (Helder 1988); seawater at 25 °C typically contains about 10 to 15 μM free $CO_{2(aq)}$. In contrast, HCO_3^- concentrations are relatively high (2.0 to 2.5 m*M*) in the marine environment because of high and stable pH. Because 90% of the total DIC in seawater is present as HCO_3^-, this ionic carbon species is the most likely source of photosynthetic carbon for marine macrophytes (Steeman-Nielsen 1975, Benedict and Scott 1976, Beer and Waisel 1979, Sand-Jensen and Gordon 1984, Millhouse and Strother 1986, Beer 1989).

Various species of seagrasses use HCO_3^- in addition to $CO_{2(aq)}$ as carbon sources for photosynthesis (Beer et al. 1977, Beer and Waisel 1979, Sand-Jensen and Gordon 1984, Millhouse and Strother 1986). These seagrasses have a high potential for photosynthesis that is limited under natural conditions of low $CO_{2(aq)}$ by their relatively inefficient HCO_3^- utilization systems (Beer 1989). Although the efficiency of HCO_3^- utilization at high pH is low, there is no evidence for the use of CO_3^{-2} in aquatic photosynthesis (Steeman-Nielsen 1960, Raven 1970, Prins and Elzenga 1989).

As most seagrasses are Calvin cycle (C_3) plants (Beer et al. 1977, 1980, Andrews and Abel 1979, Benedict et al. 1980), carbon must be in the form of $CO_{2(aq)}$ at the site of carboxylation by ribulose bisphosphate carboxylase/oxygenase. Consequently, seagrasses should be unable to directly assimilate seawater HCO_3^-. Only two reports suggest that seagrasses utilize only dissolved $CO_{2(aq)}$ in photosynthesis, and these are based on experiments with the two species of the genus *Thalassia*. Benedict et al. (1980) and Abel (1984) performed photosynthesis vs pH measurements on *T. testudinum* Banks ex König and *T. hemprichii* (Ehrenb.) Aschers., respectively. Photosynthesis decreased with increasing pH [and associated decreasing $CO_{2(aq)}$ concentration], which led these authors to conclude that both species of *Thalassia* were unable to use exogenous HCO_3^-. Photosynthetic carbon fixation in *T. hemprichii* was reportedly limited by the concentration of $CO_{2(aq)}$ in seawater (Abel 1984).

Rates of photosynthesis in terrestrial C_3 plants (Strain and Bazzaz 1983) and freshwater-submersed macrophytes

(Bowes 1985) are also limited by ambient levels of inorganic carbon. Carbon limitations are especially severe for submersed plants because the rate of diffusion of $CO_{2(aq)}$ in water is four orders of magnitude slower than in air. The characteristically heavy $\delta^{13}C$ values of seagrasses primarily reflect diffusional limitation of carbon supply, resulting in what can be viewed as a closed carbon-fixation system (Andrews and Abel 1977, Beer 1989). If rates of photosynthesis in seagrasses are limited by DIC under natural conditions, then the basis for the reportedly high productivity of seagrasses is not readily understandable (Beer 1989).

Whether HCO_3^- (as well as free CO_2) is taken up during photosynthesis at high pH cannot be resolved by the simple pH-response-curve technique utilized in most studies. This inability is evidenced by the conflicting conclusions regarding HCO_3^- use reached by Beer et al. (1977, 1980) and Benedict et al. (1980) on the basis of similar experimental results. The purposes of the present study were to determine whether HCO_3^- is a carbon source for photosynthesis in *Thalassia testudinum* and to assess the degree of carbon limitation in this species. The approach employed here, using various total DIC and pH combinations in well-buffered media, allowed separate observations on the effects of total DIC, $CO_{2(aq)}$ and HCO_3^- on the kinetics of exogenous inorganic carbon use in photosynthesis. Calculated K_s (the substrate concentration at which photosynthetic rate is half maximal) values for total DIC, $CO_{2(aq)}$, and HCO_3^- were then used to assess the relative affinity and potential for limitation for each carbon species.

Materials and methods

Plant material

Laboratory-cultured seedlings of *Thalassia testudinum* Banks ex König and field-collected *T. testudinum* short-shoots were used in this investigation. To ensure that experimental units represented separate plants (genets) rather than subsamples of the same plant (ramets), seedlings were used for most experimental measurements. Seedlings were collected from shoreline drift material at Matheson Hammock Park (25°40′N; 80°15′W) in Biscayne Bay, Florida, USA, on 10 August 1988. Seedlings were placed in plastic bags containing seawater-saturated paper towels, transported to the laboratory at 27 to 30 °C, and placed in floating racks within 24 h in an outdoor vault-culture system (Short 1985) filled with seawater adjusted to environmental salinity (30‰). After the seedlings began to form roots (7 to 14 d), they were planted in peat pellets in liner trays (Jiffy-7 pellet paks) and placed on the surface of the gravel-bed filtration system of the culture vaults (water depth ~50 cm). Vaults were located in a greenhouse covered with neutral-density nursery cloth that reduced sunlight by ~50%. Experiments were conducted after the seedlings had been in culture for 9 to 16 mo.

Field-collected short-shoots of *Thalassia testudinum* were utilized in an experimental series designed to increase the potential for carbon limitation. Short-shoots were collected from a seagrass bed adjacent to Lassing Park (27° 45′N; 82°38′W) in Tampa Bay, Florida. They were collected with a small spade to ensure that the shoots remained intact and undamaged. They were transported back to the laboratory within 2 h of collection and were stored in the dark in aerated seawater until required. All experiments on field-collected short-shoots were conducted within 4 d of collection.

Table 1. Calculated free carbon dioxide [$CO_{2(aq)}$] and bicarbonate (HCO_3^-) concentrations (mM) for media (Treatment Series I) with constant total dissolved inorganic carbon concentrations (2.20 mM) but varying pH (Park 1969, Mehrbach et al. 1973). $CO_{2(aq)} = [CO_2 + H_2CO_3] = (h^2c)/(h^2 + hk_1 + k_1k_2)$; $HCO_3^- = [HCO_3^-] = (hk_1c)/(h^2 + hk_1 + k_1k_2)$, where h = hydrogen ion activity measured by a pH meter, 10^{-pH}; c = total carbon dioxide concentration (mM); k_1 = first apparent dissociation constant of carbonic acid at 30‰ S and 25 °C, $10^{-6.03}$; k_2 = second apparent dissociation constant of carbonic acid at 30‰ S and 25 °C, $10^{-9.18}$

pH	$CO_{2(aq)}$	HCO_3^-
7.25	0.124	2.052
7.50	0.071	2.086
7.75	0.040	2.083
8.00	0.022	2.043
8.25	0.012	1.958
8.50	0.006	1.815
8.75	0.003	1.602

Table 2. Calculated $CO_{2(aq)}$ and HCO_3^- concentrations (mM) in media (Treatment Series II and III) with varying total dissolved inorganic carbon (DIC) concentrations but relatively constant pH (Park 1969, Mehrbach et al. 1973)

Total DIC	pH 7.80		pH 8.21		pH 8.61	
	$CO_{2(aq)}$	HCO_3^-	$CO_{2(aq)}$	HCO_3^-	$CO_{2(aq)}$	HCO_3^-
0.75	0.012	0.708	0.004	0.673	0.002	0.590
2.35	0.038	2.220	0.014	2.110	0.005	1.848
6.64	0.106	6.272	0.039	5.962	0.014	5.221
13.17	0.211	12.44	0.078	11.82	0.027	10.35

Incubation media

Concentrations of individual inorganic carbon species in treatment media were manipulated by altering pH and/or total dissolved inorganic carbon (DIC) concentrations according to the principles described by Sand-Jensen (1983) and Abel (1984). Experiments were conducted using 30‰ S synthetic seawater (Instant Ocean, Eastlake, Ohio) of measured pH and DIC concentrations, buffered with 10 mM Bicine [N,N-bis(2-hydroxyethyl) glycine, $pK_a = 8.35$ at 20 °C]. The addition of 10 mM Bicine did not significantly affect photosynthetic rates (Durako unpublished data).

Buffered synthetic seawater was acidified to below pH 4 with concentrated HCl and was purged overnight with N_2 to remove $CO_{2(aq)}$. The pH of the CO_2-free medium was adjusted to the required level with carbonate-free NaOH (Vogel 1961). Medium pH was measured using a combination glass pH electrode calibrated using buffers referenced to the NBS (National Bureau of Standards) pH scale. Inorganic carbon in the form of $NaHCO_3$ was added to the medium to a known final DIC concentration. Free carbon dioxide ($CO_{2(aq)}$) and HCO_3^- concentrations were calculated from the pH and DIC concentration using the first and second dissociation constants for carbonic acid in seawater (Park 1969, Mehrbach et al. 1973). Since Bicine is a chelator of Ca^{2+} and hence prevents precipitation of $CaCO_3$ (Good and Izawa 1972), no $CaCO_3$ solubility term was required in the calculations, even for media supersaturated with respect to calcite and aragonite.

Two types of media treatments were tested. Treatment Series I consisted of media with variable pH but with a constant, near-seawater DIC concentration (Table 1). Treatment Series II and III utilized media at various DIC concentrations, with pH values maintained close to that of natural seawater (Table 2). Combinations of pH and DIC concentration were chosen such that an increment in

both pH and total DIC maintained an approximately constant $CO_{2(aq)}$ concentration while changing the HCO_3^- concentrations (Abel 1984). Change in total carbon at constant pH has the same proportional effect on both carbon species. Change in pH at constant total DIC concentration has a proportionally greater effect on the $CO_{2(aq)}$ concentration and a relatively small effect on HCO_3^-.

Photosynthesis measurements

Measurements of photosynthesis during Treatment Series I and II were conducted using leaf tissue from laboratory-cultured seedlings. In these two series, rates of photosynthesis of leaf tissues were measured by continuously monitoring the dissolved oxygen concentration of the Bicine-buffered assay medium using a Clark-type polarographic oxygen micro-electrode (Microelectrodes, Inc., Londonderry, New Hampshire). The electrode was calibrated using N_2-purged and air-saturated media. Rates of photosynthesis were determined under well-stirred conditions in a closed, temperature-controlled, glass reaction cell (cell volume = 34 ml). Irradiance was provided by two 500 W, quartz-halogen bulbs at a photon flux density of ~ 500 μE m^{-2} s^{-1} PAR (photosynthetically active radiation), which is above saturation for this species (≈ 300 μE m^{-2} s^{-1} PAR, Dawes and Tomasko 1988). During the measurements of photosynthesis, medium temperature was maintained at 25 °C by a refrigerated recirculating water bath.

Leaf tissue used in the determination of photosynthesis consisted of a group of twenty 2 mm-long sections of blades cut under water from the mid-section of the youngest mature leaf of a short-shoot. Each group of leaf segments was placed in N_2-sparged buffered synthetic seawater (pH 8.21), aspirated at 20 cm Hg vacuum for 30 min, and then allowed to equilibrate at atmospheric pressure overnight to avoid the effects of wound respiration (Dawes and Tomasko 1988). Aspiration floods the lacunae, minimizing problems associated with photosynthetic transients of oxygen exchange and lacunar gas buildup (Sorrell and Dromgoole 1986).

Treatment media were prepared the day before the first determinations of photosynthesis and were stored with no head space in 300 ml acid-washed glass biological oxygen-demand bottles in a temperature-controlled water bath at 25 °C. The following morning, the aspirated leaf segments were placed in the reaction chamber that was filled with the first treatment medium. The chamber was sealed with a stopper so that no gas bubbles were present, and the tissue was allowed to acclimate in the dark for 10 min. The chamber was then illuminated, and the change in dissolved oxygen was continuously recorded for a 20 min interval using an X-Y mV recorder connected to a digital pH/mV meter. The lights were extinguished, the chamber opened, and the medium removed using a 50 ml syringe. The next treatment medium was added to the chamber slowly to minimize turbulence and gas exchange. The chamber was resealed and the cycle repeated. Each group of leaf segments was subjected to the 7 (first treatment series) or 12 (second treatment series) treatment media in random order. After a treatment series was completed, the leaf segments were rinsed in deionized water, dried at 60 °C, and weighed. Four replicate leaf-segment groups, each representing leaf material from an individual plant (seedlings), were used for each media series.

Treatment Series III utilized the same media combinations as Series II, but measurements of photosynthesis were conducted under unstirred conditions using single 4 cm leaf segments from field-collected short-shoots. Leaf segments were excised from the short-shoots the day before each experiment and were held overnight in N_2-purged buffered synthetic seawater (pH 8.21). Treatment media were made up as described above and then distributed into 52, 50 ml Erlenmeyer flasks (12 treatment media × 4 replicates + 4 blanks) that were stoppered with no head space. The flasks were placed randomly in a constant-temperature water bath (25 °C). The following morning, the initial dissolved oxygen concentration in each flask was measured immediately after an individual leaf segment had been placed in the flask. The flask was resealed and returned to its original position in the now-illuminated water bath. For each flask, initial oxygen concentration measurements were taken at 3 min intervals. After 3.5 h in the light, the dissolved oxygen concentration was again determined for each flask, again at 3 min intervals. Leaf tissue was removed from each flask, rinsed in deionized water, and dried at 60 °C for dry-weight determinations.

Rates of photosynthesis were calculated using changes in dissolved oxygen concentration in the well-stirred reaction cells during steady-state photosynthesis (i.e., the linear portions of the oxygen concentration versus time curves) or from the difference in dissolved oxygen concentrations between time, $t=0$ and $t=3.5$ h in the unstirred flasks. In the reaction cells, steady-state rates were usually attained within 2 to 5 min after illumination. Rates of photosynthesis are expressed as mg O_2 g^{-1} dry wt h^{-1}. The kinetic parameters K_s and V_{max} (maximum photosynthetic rate at "infinite" substrate concentration) were estimated using three linear transformations of the Michaelis-Menten equation (see Dowd and Riggs 1965): (1) the Lineweaver-Burk (LB) equation $[1/v=(1/V_{max})+(K_s/V_{max})\ (1/S)]$; (2) the Eadie-Hofstee (EH) equation $[v=V_{max}-K_s(v/S)]$; (3) the Hanes (HA) equation $[S/v=K_s/V_{max})+(1/V_{max})S]$, where v=the photosynthetic rate and S=the substrate concentration of DIC, CO_2, or HCO_3^-.

Results

Photosynthesis at variable pH and constant total dissolved inorganic carbon concentration (Treatment Series I)

The effect of pH on the rates of photosynthesis of *Thalassia testudinum* at the normal seawater concentration of dissolved inorganic carbon (DIC) is shown in Fig. 1. From pH 7.25 to 8.75, the $CO_{2(aq)}$ concentration decreased by $\sim$40-fold while HCO_3^- concentration declined by only $\sim$30% (Table 1). Over the same pH range, rates of photosynthesis declined only 7-fold (85%), suggesting some contribution by HCO_3^-. The use of HCO_3^- is also suggested by the plateau in photosynthesis between pH 7.75 to 8.50. The variation in photosynthesis with pH was curvilinear ($r=0.92$ for a fourth-order regression compared to $r=0.86$ for a first-order regression, see Fig. 1).

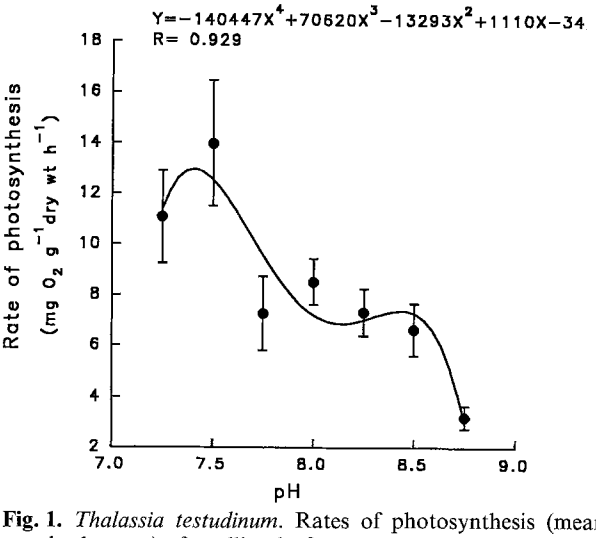

Fig. 1. *Thalassia testudinum.* Rates of photosynthesis (means ± standard errors) of seedling leaf segments as a function of pH at a constant dissolved inorganic carbon concentration (DIC) of 2.20 m*M*

Kinetic parameters were not calculated for Treatment Series I because photosynthesis versus HCO_3^- did not follow saturation kinetics. A wide variation in photosynthetic rates occurred at the three lowest pH levels (Fig. 1). Over this pH range (7.25 to 7.75), the HCO_3^- concentration was virtually constant, while $CO_{2(aq)}$ concentration decreased 3-fold (see Table 1). Although the results of Treatment Series I suggest that $CO_{2(aq)}$ concentration has a greater effect on rates of photosynthesis than does HCO_3^--concentration, at least at lower pH levels, they do not allow an accurate assessment of the contribution of HCO_3^- to photosynthesis at the higher pH levels.

Photosynthesis at various DIC concentrations with pH maintained close to that of seawater (Treatment Series II and III)

Rates of photosynthesis of the seedling leaf segments increased with increasing DIC up to the highest levels tested (Fig. 2). The rate of increase was greatest between the two lowest DIC levels. For a particular DIC concentration, photosynthetic rates were highest at the lowest pH [i.e., where $CO_{2(aq)}$ concentration was highest]. Kinetic data (Table 3) calculated using LB and HA transformations indicated that photosynthetic rates at environmental seawater DIC levels were about half that at saturating DIC concentrations. The K_s (DIC) and V_{max} (DIC) values calculated using the EH equation were the lowest of the three transformations; this equation also provided the poorest fit to the data.

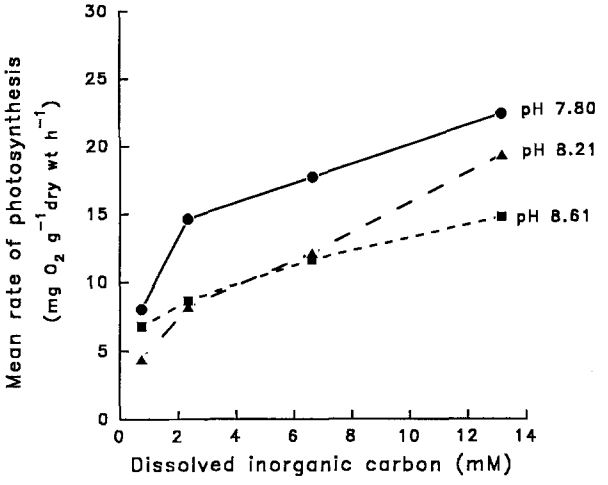

Fig. 2. *Thalassia testudinum*. Rates of photosynthesis of leaf segments at pH 7.80, 8.21, and 8.61 as a function of DIC concentration. Each data point represents mean of four laboratory-cultured seedlings

For similar $CO_{2(aq)}$ concentrations, rates of photosynthesis as a function of $CO_{2(aq)}$ were highest only at the highest pH (i.e., highest HCO_3^- concentrations; Fig. 3). In contrast, rates as a function of HCO_3^- were highest only at the lowest pH [i.e., highest $CO_{2(aq)}$ concentrations]. The kinetic data (Table 3) indicate a very high affinity for $CO_{2(aq)}$ and a much lower affinity for HCO_3^-, similar to those values calculated for the first treatment series. The K_s values calculated using the LB and EH equations were similar, and indicated that at seawater concentrations $CO_{2(aq)}$ would be available at saturation levels and HCO_3^- would be only slightly limiting (ambient concentration approximately twice the K_s); K_s values calculated using the HA equation indicated that both substrates would be limiting. All three transformations resulted in higher calculated V_{max} values for HCO_3^- compared with CO_2.

To increase the potential for carbon limitation through an increase in the thickness of the diffusive sublayer, the pH/DIC media series was repeated using unstirred media containing individual, non-aspirated, 4 cm leaf segments. Photosynthetic characteristics during this third series showed several distinctions from the previous series. First, the photosynthetic rates were about half those of the previous two series (Fig. 4). Second, photosynthetic rates versus DIC generally leveled off at the higher DIC concentrations, indicating possible substrate saturation or diffusional limitation. Finally, for a particular DIC concentration, the photosynthetic rate was always higher as pH decreased.

Kinetic data calculated using the LB and HA equations (Table 3) resulted in K_s (DIC) values similar to those obtained using the aspirated leaf segments from labora-

Table 3. Comparison of calculated photosynthetic kinetic parameters from linear transformations of the Michaelis-Menten equation (see "Materials and methods", Paragraphs 5 and 10, for explanation of treatment series and linear transformations). r=correlation coefficient; K_s=carbon concentration (mM) at which photosynthetic rate is half maximal; and V_{max}=maximum photosynthetic rate (mg O_2 g^{-1} dry wt h^{-1})

Treatment	Lineweaver-Burk (LB)			Eadie-Hofstee (EH)			Hanes (HA)		
	r	K_s	V_{max}	r	K_s	V_{max}	r	K_s	V_{max}
Treatment Series II									
DIC	0.98	1.94	14.04	0.77	1.21	10.75	0.93	2.61	21.09
CO_2	0.70	0.003	13.78	0.62	0.004	15.47	0.99	0.018	23.40
HCO_3^-	0.83	1.22	17.08	0.64	0.97	16.95	0.51	8.88	34.04
Treatment Series III									
DIC	0.99	2.76	7.18	0.81	4.70	11.88	0.92	2.55	7.30
CO_2	0.95	0.007	5.83	0.84	0.009	6.54	0.99	0.18	8.02
HCO_3^-	0.93	2.22	7.34	0.75	1.68	6.79	0.93	2.55	7.74

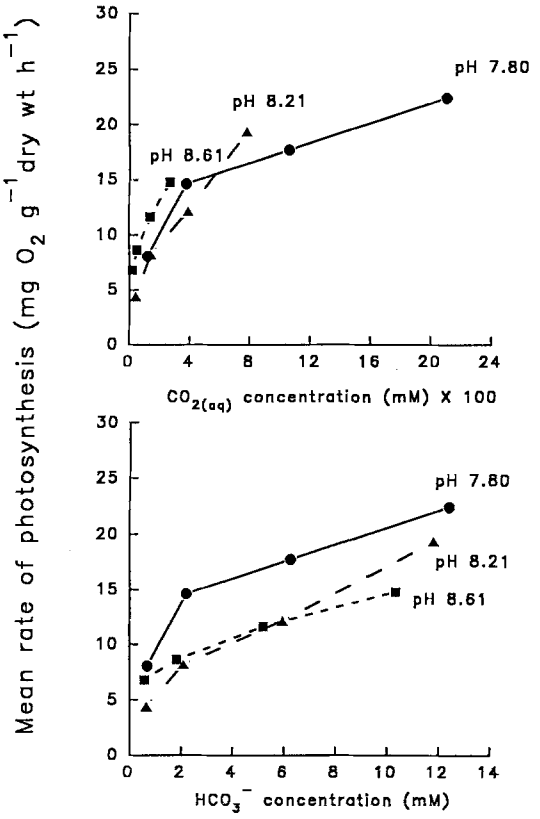

Fig. 3. *Thalassia testudinum.* Rates of photosynthesis of leaf segments at pH 7.80, 8.21, and 8.61 as a function of $CO_{2(aq)}$ (top graph) and HCO_3^- (bottom graph) concentrations. Each data point represents mean of four laboratory-cultured seedlings

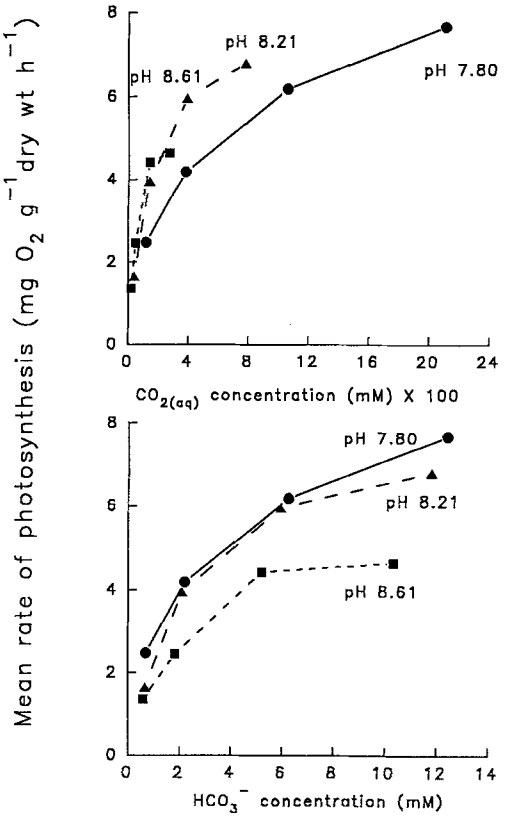

Fig. 5. *Thalassia testudinum.* Rates of photosynthesis of short-shoot leaf segments at pH 7.80, 8.21, and 8.61 as a function of $CO_{2(aq)}$ (top graph) and HCO_3^- (bottom graph) concentrations. Each data point represents mean of four field-collected short-shoots

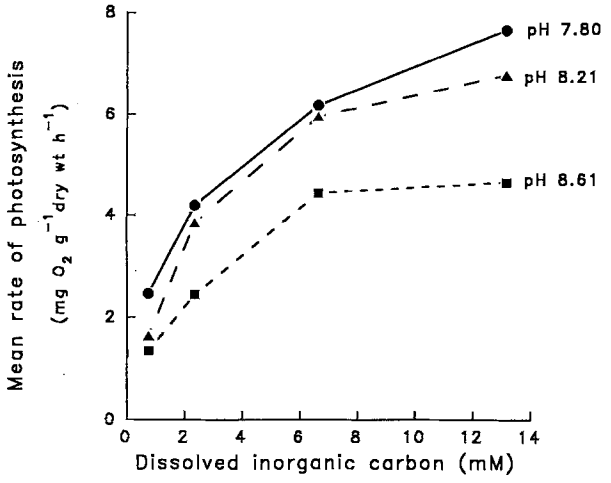

Fig. 4. *Thalassia testudinum.* Rates of photosynthesis of short-shoot leaf segments at pH 7.80, 8.21, and 8.61 as a function of DIC concentration. Each data point represents mean of four field-collected short-shoots

tory-cultured seedlings; K_s (DIC) calculated using the EH equation was about twice those of the other two estimates. All three K_s (DIC) values indicated carbon limitation. Lower photosynthetic rates in the third series were reflected by relatively low apparent V_{max} values.

The patterns of photosynthetic rates plotted as a function of calculated $CO_{2(aq)}$ and HCO_3^- concentrations (Fig. 5), were similar to those for the aspirated leaf segments: highest rates at the highest pH for $CO_{2(aq)}$ and highest rates at the lowest pH for HCO_3^-. The K_s constants calculated for $CO_{2(aq)}$ and HCO_3^- using the LB and EH equations were higher for non-aspirated leaf segments compared to those of the aspirated segments (Series II, Table 3), while the HA equation yielded an equal K_s ($CO_{2(aq)}$), but a lower K_s(HCO_3^-) for the non-aspirated segments. The V_{max} values were all lower for the field-collected material.

Discussion

The results indicate that *Thalassia testudinum* utilizes HCO_3^- in addition to $CO_{2(aq)}$ as a photosynthetic substrate and exhibits a high degree of plasticity in its photosynthetic carbon metabolism. Benedict and Scott (1976) suggested that HCO_3^- was the photosynthetic substrate for *T. testudinum* and that this species utilized a C_4-type of carbon metabolism. However, these conclusions were largely based on indirect evidence of heavy $\delta^{13}C$ values. O'Leary (1988) suggested that the characteristically heavy $\delta^{13}C$ values of seagrasses primarily reflect diffusional limitations on carbon supply resulting in a relatively "closed" carbon-fixation system rather than reflecting their mode of carbon fixation (i.e., C_3 versus C_4). Benedict et al. (1980) concluded that *T. testudinum* was indeed a C_3 plant because phosphoglyceric acid was the first

stable product of photosynthetic carbon fixation. They also suggested that $CO_{2(aq)}$ was the only species of inorganic carbon utilized for photosynthesis, based on photosynthetic responses of leaf sections at pH levels from 4 to 9.

These conclusions contrast with those of Beer et al. (1977, 1980), who interpreted plateaus in the pH-response curves from pH 7.5 to 9.2 for four seagrass species as indicating HCO_3^- uptake. In their studies, pH was changed during the photosynthesis measurements, whereas Benedict et al. (1980) used three different buffer systems to measure photosynthesis. Since buffers can reduce the rate of HCO_3^- utilization while stimulating the use of $CO_{2(aq)}$ (Lucas 1977, 1983, Prins et al. 1982, Prins and Zanstra 1985), the responses observed by Benedict et al. (1980) may have been an artifact.

The conflicting conclusions reached by Beer et al. (1977, 1980) and Benedict et al. (1980) indicate that examination of the pH response curve does not reveal whether HCO_3^- in addition to $CO_{2(aq)}$ is taken up during photosynthesis. In agreement with Abel's (1984) conclusion regarding the limitations of this technique, the photosynthesis versus pH responses observed for *Thalassia testudinum* in the present study were not highly correlated with the calculated concentrations for either substrate ($r_{CO_2}=0.74$, $r_{HCO_3}=0.81$). The pH-response data suggest a strong $CO_{2(aq)}$ influence at lower pH levels, but do not preclude HCO_3^- uptake, especially at the higher pH levels. The decrease in photosynthetic rate over the pH range tested was much lower than the exponential decline in $CO_{2(aq)}$ concentration, suggesting some potential for HCO_3^- uptake.

The media pH/DIC combinations utilized in Series II and III experiments followed those of Abel (1984) and were so chosen that an increment increase in both pH and DIC resulted in relatively constant $CO_{2(aq)}$ concentrations with an increase in HCO_3^- concentrations, all within a small pH range that would not be expected to affect photosynthesis directly. This approach can reveal uptake of either $CO_{2(aq)}$ or HCO_3^-, or of both (Abel 1984, Sand-Jensen and Gordon 1984) and avoids the problems of conflicting interpretations regarding $CO_{2(aq)}$ and HCO_3^- attendant upon the examination of pH-response curves. However, this approach cannot distinguish the mechanism of HCO_3^- use, i.e., direct uptake of HCO_3^- via an electrogenic pump versus conversion of HCO_3^- to $CO_{2(aq)}$ by an active carbonic anhydrase system. In assessing the relative contributions of $CO_{2(aq)}$ and HCO_3^- to the photosynthetic performance of *Thalassia testudinum*, the data obtained in this study suggest characteristics quite distinct from those observed by Abel (1984) for *T. hemprichii*, illustrating plasticity in photosynthetic characteristics within this genus.

Abel (1984) concluded that field-collected *Thalassia hemprichii* did not exhibit appreciable use of HCO_3^- as a carbon source, based on her interpretation of pH independence in photosynthetic responses when plotted against $CO_{2(aq)}$ and on pH-dependent responses when plotted against HCO_3^- concentrations. In contrast, photosynthetic rates for laboratory-cultured and field-collected *T. testudinum* exhibited pH dependence when plotted against both CO_2 and HCO_3^-, indicating a contribution by both carbon species. However, there were large differences in maximum photosynthetic rates between leaf material from laboratory-cultured *T. testudinum* and field-collected material, suggesting that major physiological modifications accompany changes in growth conditions for seagrasses, as has been observed for freshwater angiosperms (Bowes and Salvucci 1989).

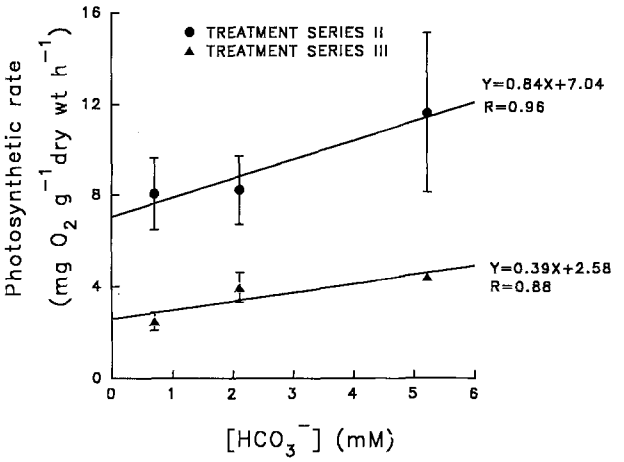

Fig. 6. *Thalassia testudinum.* Rates of photosynthesis (means ± standard errors) of leaf segments at relatively constant $CO_{2(aq)}$ (12 to 14 μ*M*) and increasing HCO_3^- concentration

Because of plasticity in photosynthetic carbon metabolism, the distinction of plants as users and nonusers of HCO_3^- may really be one of degree (Spence and Maberly 1985, Bowes and Salvucci 1989). Both *Thalassia* species have high rates of primary production (McRoy and McMillan 1977, Brouns 1985). Larkum et al. (1989) pointed out that with this similarity in productivity coupled with the difficulties in positively delineating $CO_{2(aq)}$ vs HCO_3^- utilization, it seems likely that HCO_3^- is utilized by both species.

To illustrate the effect of HCO_3^- concentration on photosynthesis of *Thalassia testudinum*, only the photosynthetic rates from the three media with a free $CO_{2(aq)}$ concentration of 12 to 14 μ*M* were plotted (Fig. 6). Although there were physiological differences between Series II and Series III plants, the overall effect is clear: for both experimental series there were positive responses to increasing or high HCO_3^- concentrations at relatively constant $CO_{2(aq)}$ concentrations, reflecting HCO_3^- utilization. Therefore, the results of this study agree with the conclusions of Benedict and Scott (1976), although for different reasons. The results are more similar to those of Beer et al. (1977, 1980), Sand-Jensen and Gordon (1984), and Millhouse and Strother (1986) in that these authors suggested photosynthetic HCO_3^- use in all the marine macrophytes they examined, based on photosynthetic-response data.

Beer (1989) concluded that all evidence based on controlled photosynthetic experiments suggests DIC limitation for seagrasses under natural conditions. Likewise, the kinetic data for photosynthesis versus DIC concentration for *Thalassia testudinum* indicated that photosynthetic rates would generally be below saturation under

natural conditions. The use of the Eadie-Hofstee and Hanes linear transformations of the Michaelis-Menten equation, in addition to the most commonly used but generally inferior Lineweaver-Burk transformation (see Dowd and Riggs 1965 for a discussion of the merits of the various linear transformations), provide a reasonable range of estimates of K_s and V_{max}. These estimates demonstrate a major distinction between the photosynthetic characteristics of *T. testudinum* and those of the previously examined submersed aquatic macrophytes – the apparent great affinity of the former for $CO_{2(aq)}$. Most submersed aquatic macrophytes exhibit high $CO_{2(aq)}$ saturation requirements, with K_s (CO_2) values ranging from 40 to >700 μM (Steeman-Nielsen 1947, Van et al. 1976, Browse et al. 1979, Allen and Spence 1981, Salvucci and Bowes 1982, 1983, Sand-Jensen and Gordon 1984, Millhouse and Strother 1986). In contrast, terrestrial species exhibit K_s (CO_2) values of ~10 μM (Goldsworthy 1968). The calculated K_s (CO_2) values for *T. testudinum* ranged from 3 to 18 μM, comparable to terrestrial plant values but almost two orders of magnitude lower than the K_s(CO_2) values reported by Abel (1984) for *T. hemprichii*. However, my calculations, using values estimated from Fig. 2 of Abel (1984), yielded K_s (CO_2) estimates of 34, 30, and 40 μM for the LB, EH, and HA equations, respectively. Thus, these two closely related species may both have relatively high affinities for $CO_{2(aq)}$.

The high apparent K_s (CO_2) for most submersed aquatic macrophytes has been attributed to the combined effects of slow rates of $CO_{2(aq)}$ diffusion in the aqueous environment (Steeman-Nielsen 1947, Abel 1984, Raven 1984) and the presence of external and internal resistances to $CO_{2(aq)}$ transfer (Sorrell and Dromgoole 1986, Larkum et al. 1989). As the diffusion coefficients for CO_2 are ~10 000 times greater in air than in water, the major resistance to $CO_{2(aq)}$ uptake, which accounts for much of the high apparent K_s (CO_2), is the slow rate of diffusion through the diffusive sublayer surrounding the leaves (Browse et al. 1979, Smith and Walker 1980, Wheeler 1980, Black et al. 1981). The similarity in kinetic values calculated from the Series III data compared with those calculated from the Series II experiments with well-stirred media implies little effect of the diffusive sublayer on K_s (CO_2) using the photosynthetic assay system employed here. Lower absolute rates of photosynthesis may have been due to lacunar oxygen storage in these larger, non-aspirated tissues. Sorrell and Dromgoole (1986) observed a 17% decrease in apparent photosynthetic rates in non-aspirated compared to aspirated tissues.

In contrast to the uniqueness of very high affinity for $CO_{2(aq)}$, the apparent K_s (HCO_3^-) values for *Thalassia testudinum* fall within the range of values reported for other submersed aquatic macrophytes (0.6 to 23 mM; Allen and Spence 1981, Titus and Stone 1982, Sand-Jensen and Gordon 1984, Millhouse and Strother 1986). However, the apparent V_{max} values for HCO_3^- for *T. testudinum* are generally higher than those for $CO_{2(aq)}$. Rates of photosynthesis are usually higher at CO_2 saturation than at HCO_3^- saturation, due to restrictions associated with HCO_3^- transport across the plasmalemma (Prins et al. 1982, Lucas 1983). The comparatively higher V_{max}(CO_2) vs V_{max}(HCO_3^-) values reported for several seagrasses (Beer et al. 1977, Sand-Jensen and Gordon 1984, Millhouse and Strother 1986) may indicate active transport of HCO_3^-. Active H^+-HCO_3^- cotransport (and/or direct uptake of the ionic HCO_3^-) has not been demonstrated in *T. testudinum*. An alternative explanation for the pattern observed in this study may be rapid extracellular conversion of HCO_3^- to $CO_{2(aq)}$, which subsequently enters the leaf by diffusion rather than by an energy-requiring transport system (Larkum et al. 1989). The resulting increase in $CO_{2(aq)}$ concentration would increase the concentration gradient across the diffusive sublayer, leading to greater availability at the site of fixation. Using similar types of carbon-concentrating systems, cyanobacteria exhibit whole-cell K_s (CO_2) values as low as 3 nM, in spite of a K_s (CO_2) for cyanobacterial ribulose bisphosphate carboxylase/oxygenase of about 200 μM (Miller et al. 1990).

It is not possible to examine the effects of one inorganic carbon species in the absence of the other. The approach employed here allowed an assessment of the effects of varying the concentration of one carbon species while maintaining a constant concentration of the other. Abel (1984) and Sand-Jensen and Gordon (1984) applied this approach to marine macrophytes and arrived at conflicting conclusions. The results of the present study suggest differences between the physiological aspects of $CO_{2(aq)}$ and HCO_3^- use by laboratory-cultured and field-collected *Thalassia testudinum* and those reported for other seagrasses. As with freshwater macrophytes (Bowes and Salvucci 1989), plasticity seems to be a major feature of the photosynthetic carbon metabolism of seagrasses. Physiological modifications which accompany changes in growth conditions may form the basis of the reported differences in the utilization of HCO_3^- by seagrasses. This plasticity may allow *T. testudinum* to adjust its carbon assimilation capacities so that the K_s values are close to the inorganic carbon concentrations of its medium.

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