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Impacts of CO_2 Enrichment on Productivity and Light Requirements of Eelgrass

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Seagrasses, although well adapted for submerged existence, are CO_2-limited and photosynthetically inefficient in seawater. This leads to high light requirements for growth and survival and makes seagrasses vulnerable to light limitation. We explored the long-term impact of increased CO_2 availability on light requirements, productivity, and C allocation in eelgrass (*Zostera marina* L.). Enrichment of seawater CO_2 increased photosynthesis 3-fold, but had no long-term impact on respiration. By tripling the rate of light-saturated photosynthesis, CO_2 enrichment reduced the daily period of irradiance-saturated photosynthesis (H_{sat}) that is required for the maintenance of positive whole-plant C balance from 7 to 2.7 h, allowing plants maintained under 4 h of H_{sat} to perform like plants growing in unenriched seawater with 12 h of H_{sat}. Eelgrass grown under 4 h of H_{sat} without added CO_2 consumed internal C reserves as photosynthesis rates and chlorophyll levels dropped. Growth ceased after 30 d. Leaf photosynthesis, respiration, chlorophyll, and sucrose-phosphate synthase activity of CO_2-enriched plants showed no acclimation to prolonged enrichment. Thus, the CO_2-stimulated improvement in photosynthesis reduced light requirements in the long term, suggesting that globally increasing CO_2 may enhance seagrass survival in eutrophic coastal waters, where populations have been devastated by algal proliferation and reduced water-column light transparency.

Photosynthesis of many terrestrial autotrophs is CO_2 limited in the current atmosphere, and future increases in atmospheric CO_2 may have profound consequences for terrestrial plant productivity (Houghton et al., 1990; Koch and Mooney, 1995). In contrast, marine productivity is expected to exhibit little response to increased atmospheric CO_2 because most algae possess CO_2-concentrating mechanisms that effectively utilize bicarbonate, which is 140 times more abundant in seawater than free CO_2 (Raven et al., 1995). Seagrasses (marine angiosperms), however, represent a unique contrast to most marine algae in that light-saturated photosynthesis is limited to less than 50% of their physiological capacity by the availability of dissolved inorganic C (Beer, 1989; Durako, 1993; Zimmerman et al., 1995a; Beer and Koch, 1996). Perhaps not coincidentally, seagrass colonization is limited to depths at which light levels are greater than 10% of surface irradiance (Duarte, 1991a; Onuf, 1991; Zimmerman et al., 1991). In contrast, rates of light-saturated photosynthesis in algae are 2- to 3-fold higher than in seagrasses, algal photosynthesis is not stimulated by increased seawater [CO_2], and algae are commonly found growing at the 1% light-penetration depth or isolume (Kirk, 1994; Raven et al., 1995).

Given the unique anatomical and physiological adaptations to a fully submerged life history, which include the lack of stomata, greatly reduced cuticle and xylem, the presence of arenchyma (lacunae) to support root aerobiosis in permanently flooded sediments, and anoxia tolerance by roots (Tomlinson, 1980; Smith et al., 1984; Smith, 1989), it is surprising that these ecologically successful marine monocots are not similarly well adapted for photosynthesis in a bicarbonate-rich environment.

Acute exposure to seawater enriched in dissolved inorganic C instantaneously increases seagrass photosynthesis 3-fold and increases leaf sugar content by 50% in as little as 2 h, but the long-term effects of increased CO_2 availability on seagrass performance have not been investigated (Beer, 1989; Durako, 1993; Zimmerman et al., 1995a; Beer and Koch, 1996). Terrestrial plant productivity often responds positively to CO_2 enrichment in the short term (Koch and Mooney, 1995, and refs. cited therein), but prolonged exposure to elevated CO_2 can down-regulate photosynthesis through strong feedback from sinks to sources, particularly when other resources (e.g. water, nutrients) become limiting (Stitt, 1991; Tuba et al., 1994; Van Oosten et al., 1994, 1995). Thus, short-term photosynthetic responses do not always predict the long-term impacts of altered environmental conditions.

This study addressed the environmental impact of CO_2 availability on eelgrass (*Zostera marina* L.) productivity and light requirements over time frames longer than the minutes to hours examined previously. Specific objectives were to determine the extent to which high photosynthesis rates were maintained under chronic exposure to elevated CO_2, and whether increased photosynthesis could significantly reduce light requirements for long-term survival. Maintenance of 3-fold higher photosynthesis under CO_2 enrichment should allow plants receiving only 4 h of H_{sat} to perform nearly as well as plants grown without CO_2 en-

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Abbreviations: ANOVA, analysis of variance; E_k, photosynthesis-saturating irradiance; E_{PAR}, photosynthetically active irradiance; H_{sat}, daily period of irradiance-saturated photosynthesis; P, photosynthesis; P_g, gross photosynthesis; P_m, maximum net photosynthesis; R, respiration; SPS, Suc-phosphate synthetase; SS, Suc synthase.

richment under 12 h of H_{sat}. Any down-regulation of photosynthesis or disruption of C allocation to roots exposed to prolonged daily anoxia, however, should measurably degrade plant performance over time (Zimmerman and Alberte, 1996). Thus, an additional objective was to determine whether short, daily photoperiods altered C allocation patterns when plants remained C-replete due to CO_2 enrichment.

MATERIALS AND METHODS

Source Material

Eelgrass (*Zostera marina* L.) plants were harvested by hand in April 1995 from a subtidal population located near Del Monte Beach in southern Monterey Bay, California (36°36'10" N, 121°53'10" W). Breaking of roots and rhizomes was avoided as individual plants were gently removed from the sediment, placed in coolers containing fresh seawater, and transported immediately to the laboratory. Eelgrass plants possessing at least 10 rhizome internodes were transplanted separately into 4-L plastic pots filled with natural sediment collected from the eelgrass meadow. Transplants were allowed to recover for 10 d in running-seawater aquaria illuminated by natural sunlight screened to 25% of ambient above-water irradiance, approximating the natural environment of the eelgrass meadow.

Photosynthetic Response to Elevated CO_2

Photosynthesis versus irradiance relationships were determined polarographically, according to Zimmerman et al. (1989), in temperature-controlled, water-jacketed chambers (5 mL, Rank Bros., Cambridge, UK) using CO_2-enriched and normal seawater. The chambers were stirred magnetically to prevent boundary-layer depletion of CO_2 and flow limitation of photosynthesis. Seawater $[O_2]$ was reduced to 50% of air saturation by bubbling with a mixture of 99.99% N_2 plus 350 nmol L^{-1} CO_2 to prevent CO_2 stripping and alteration of seawater pH (8.2). Enrichment was achieved by bubbling with CO_2 derived from subliming dry ice until the pH was 6.2. The partitioning of dissolved inorganic C into free CO_2, HCO_3^-, and CO_3^{-2} in normal and enriched seawater was calculated from measured temperature, pH, and salinity using the CO_2-solubility equations of Weiss and Price (1980) and the dissociation constants of Hansson (1973). Alkalinity was assumed to be 2340 microequivalents kg^{-1}. Electrode chambers were sealed immediately to prevent outgassing from the CO_2-supersaturated water. Irradiance generated by slide projectors was adjusted with neutral-density filters to obtain a range of E_{PAR}. Respiration was determined in the dark. Temperature was maintained at 15°C using a circulating water bath. Leaf sections were homogenized on ice in 90% (v/v) acetone to extract pigments. The homogenates were centrifuged and chlorophyll (*a/b*) concentrations of the supernatants were quantified spectrophotometrically using the extinction coefficients of Jeffrey and Humphrey (1975). *P* versus E_{PAR} data were normalized to total chlorophyll (*a/b*) to minimize variability among leaves resulting from differences in pigment content. The parameters P_m and E_k were determined using the exponential function of Webb et al. (1974) and a direct, nonlinear curve-fitting and error-estimation routine (Zimmerman et al., 1987).

Prolonged Whole-Plant Response to Elevated CO_2

Plants exhibited no visual evidence of transplant shock (e.g. soft shoot bundles, discolored or negatively buoyant leaves) after a recovery period of 10 d and were transferred to separate running-seawater aquaria (120 L) illuminated by timer-controlled quartz-halogen lamps that delivered E_{par} well in excess of photosynthetic saturation (200 μmol photons m^{-2} s^{-1}) to the bottom of each tank. Flow in all aquaria was maintained by vigorous delivery of seawater simultaneously to the bottom and top of each tank at a rate of 4 turnovers h^{-1}; aquaria drained from the top.

Thirty plants were grown under 12 h of H_{sat} in unenriched seawater (Table I). Eelgrass normally requires 5 to 8 h of H_{sat} for the maintenance of positive C balance (Zimmerman et al., 1996, and refs. cited therein), and this condition was designed as a C-replete treatment to provide about twice the photosynthesis required to maintain daily C balance. A second set of 30 plants, designed as a C-deplete treatment, was grown under 4 h of H_{sat}, which provided about one-half of the photosynthesis required to meet the daily C demand in unenriched seawater.

A third set of 30 plants was grown in CO_2-enriched seawater (Table I), but received only 4 h of H_{sat} to provide a second C-replete treatment that was roughly equivalent to that of plants grown under 12 h of H_{sat} in unenriched seawater. The enriched aquaria were bubbled with compressed CO_2 metered by a pH-controlled solenoid valve that maintained seawater pH at 6.2 (±0.1). No attempt was made to prevent or control CO_2 release from the enriched aquaria, and temperature varied with the source of seawater (12–16°C). Free $[CO_2]$ probably varied about 10% over this temperature range, but bicarbonate and carbonate levels should have varied by less than 1% (Hansson, 1973; Weiss and Price, 1980). There were no stagnant or unstirred layers within the aquaria, as demonstrated by the fact that vertical profiles of pH and temperature were invariant in both un-

Table I. *Equilibrium distribution of dissolved inorganic C in seawater under normal and enriched treatments, based on the dissociation constants of Hansson (1973) and the CO_2-solubility equations of Weiss and Price (1980)*

Calculation assumptions: salinity = 35 ppt; alkalinity = 2340 microequivalents kg^{-1}; and temperature = 15°C.

Parameter	Unenriched (Normal) Seawater	CO_2-Enriched Seawater
pH	8.2	6.2
	μM	
Free $[CO_2]$	14	1340
$[HCO_3^{3}]$	1890	2330
$[CO_3^{-2}]$	170	3
Total $[CO_2]$	2074	3673

enriched (pH 8.2) and CO_2-enriched (pH 6.2) treatments. Plants were maintained under these conditions for 45 d.

Shoot growth rates were measured weekly on six plants harvested from each treatment 1 week after marking leaves with a hypodermic needle (Zieman and Wetzel, 1980; Zimmerman et al., 1995b). Rates of leaf P_m (light-saturated) were measured polarographically under growth CO_2 (normal or enriched) using sections cut from the middle of leaf 3 (the youngest being leaf 1) from four of the harvested plants. Dark respiration of each leaf section was measured in unenriched (pH 8.2) seawater for all treatments. Pigments were measured as above. Sugar content of leaves, roots, and rhizomes and in vitro activities of leaf SPS and root SS were measured according to Zimmerman et al. (1995a) from four plants harvested from each treatment at each time point.

Statistical Analyses

Statistical significance of all treatment effects was evaluated by two-way ANOVA (treatment × time). Time-series observations were measured on separate, individual plants (replicates) destructively harvested at each sampling time. Consequently, time was treated as an independent factor (not nested) in the ANOVA. Significant two-way effects ($P \leq 0.05$) were then evaluated for significant one-way treatment effects within each sampling period and for significant one-way temporal effects within each treatment. Significant one-way effects ($P \leq 0.05$) were then subjected to LSD multiple-comparison analysis for specific determination of differences among treatments at each sampling period and through time within treatments using the CSS (MS-DOS) statistical software package.

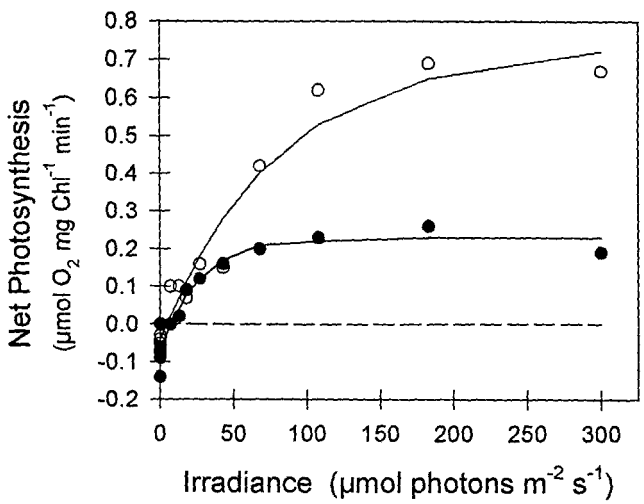

Figure 1. Photosynthesis versus E_{PAR}-response curves of eelgrass leaves incubated in normal (●) and CO_2-enriched (○) seawater. Curves were fit to both data sets using a least-squares direct fitting algorithm (Zimmerman et al., 1987). Chl, Chlorophyll.

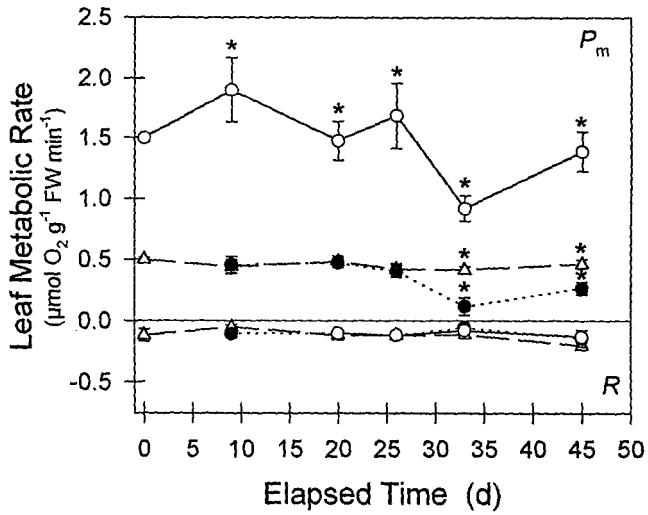

Figure 2. Rates of P_m and dark respiration of eelgrass leaf sections for plants grown in normal seawater under 12 h of H_{sat} (△), 4 h of H_{sat} (●), and 4 h of H_{sat} plus CO_2 enrichment (○). Error bars represent ±1 SE. Asterisks (*) indicate significant differences among treatments at each sampling period ($P \leq 0.05$) identified by post-ANOVA multiple-comparison analysis (Newman LSD). FW, Fresh weight.

RESULTS

Instantaneous Photosynthetic Response to Elevated CO_2

Freshly collected eelgrass leaves placed in O_2 electrode chambers filled with normal (unenriched) seawater exhibited a typical P versus E_{PAR} response curve (Fig. 1). When fit to the exponential function of Webb et al. (1974), net P_m = 0.20 ± 0.03 μmol O_2 mg^{-1} chlorophyll min^{-1} (P_m = 0.5 μmol O_2 g^{-1} fresh weight min^{-1}) and R = 0.07 ± 0.01 μmol O_2 mg^{-1} chlorophyll min^{-1} (R = 0.18 μmol O_2 g^{-1} fresh weight min^{-1}). The resulting instantaneous light-saturated P_g:R of 5.29 was low, but typical of eelgrass (Zimmerman et al., 1995b). The E_k in unenriched seawater was 27 ± 5 μmol photons m^{-2} s^{-1}, again typical of the low light levels required to maximize the C-limited photosynthetic potential of seagrasses in natural seawater. Under CO_2 enrichment, however, photosynthesis of freshly collected leaves increased 260% from 0.30 to 0.78 μmol O_2 mg^{-1} chlorophyll min^{-1} and E_k increased 296% from 27 to 82 μmol photons m^{-2} s^{-1}. There was no difference in the light-limited slopes of the curves under CO_2 enrichment versus normal seawater (α = 0.011 versus 0.010, respectively). Dark respiration, however, was significantly lower under CO_2 enrichment (0.04 ± 0.03 versus 0.07 ± 0.02 μmol O_2 mg^{-1} chlorophyll min^{-1}, t = 3.67, df = 10, $P < 0.01$). Consequently, the CO_2 supplement raised the instantaneous, light-saturated P_g:R ratio to 20.5, a 400% increase over the P_g:R (5.29) ratio in unenriched seawater.

Prolonged Response to CO_2 Enrichment

After 45 d rates of eelgrass P_m grown under CO_2 enrichment remained 3-fold higher than normal (Fig. 2). Treatment effects were the overwhelming source of variation (Table II). Photosynthetic rates of CO_2-enriched plants

Table II. *Two-way ANOVA for the effects of time and treatment (12 h of H_{sat}, 4 h of H_{sat}, and 4 h of H_{sat} + CO_2 enrichment) on total shoot length, absolute growth, and specific growth rates*

Effect	Sum of Squares	*df*	Mean Square	*F*	*P*
Leaf P_m					
Time	0.629	4	0.158	2.35	0.068
Treatment	18.46	2	9.23	138	<0.001
Time × treatment	0.539	8	0.067	1.01	0.44
Within	3.01	45	0.067		
Leaf *R*					
Time	0.043	4	0.011	3.74	0.01
Treatment	0.0038	2	0.0019	0.67	0.52
Time × treatment	0.024	8	0.003	1.07	0.40
Within	0.128	45	0.0028		
Leaf chlorophyll *a* + *b*					
Time	15.52	4	3.88	16.36	<0.001
Treatment	1.52	2	0.76	3.21	0.048
Time × treatment	11.54	8	1.44	6.08	<0.001
Within	10.67	45	0.24		
Total length					
Time	85,122	5	17,024	2.34	0.045
Treatment	258,303	2	129,151	17.73	<0.001
Time × treatment	43,337	10	4,334	0.590	0.820
Within	750,053	103	7,282		
Rhizome 1 Suc					
Time	12,544	4	3,136	1.69	0.162
Treatment	50,213	2	25,106	13.49	<0.001
Time × treatment	32,681	8	4,085	2.20	0.037
Within	133,986	72	1,861		
Rhizome 3 Suc					
Time	26,917	4	6,729	0.99	0.420
Treatment	73,346	2	36,673	5.39	0.007
Time × treatment	99,592	8	12,449	1.83	0.085
Within	482,857	71	6,801		
Leaf SPS					
Time	0.015	4	0.0037	14.97	<0.001
Treatment	0.0038	2	0.0019	7.71	0.002
Time × treatment	0.0047	8	0.00059	2.39	0.03
Within	0.011	45	0.00025		
Root SS					
Time	0.89	4	0.222	0.77	0.55
Treatment	0.79	2	1.37	1.37	0.26
Time × treatment	2.86	8	0.36	1.23	0.30
Within	13.05	45	0.29		
Absolute growth					
Time	51	5	10.20	6.81	<0.001
Treatment	260	2	129.70	86.50	<0.001
Time × treatment	25	10	2.46	1.64	0.106
Within	153	102	1.50		
Specific growth					
Time	11.03	5	2.21	11.71	<0.001
Treatment	29.39	2	14.70	77.98	<0.001
Time × treatment	5.73	10	0.57	3.04	0.024
Within	19.22	102	0.19		
Leaf Suc					
Time	14,816	4	3,704	4.23	0.004
Treatment	50,004	2	25,002	28.53	<0.001
Time × treatment	15,727	8	1,966	2.24	0.033
Within	62,225	71	876		
Root Suc					
Time	618	4	154	3.49	0.011
Treatment	2,215	2	1,107	25.04	<0.001
Time × treatment	666	8	83	1.88	0.076
Within	3,140	71	44		

were about 1.5 μmol O_2 g^{-1} fresh weight min^{-1} throughout the experiment except for d 33, when P_m dropped to 1.0 μmol O_2 g^{-1} fresh weight min^{-1}. P_m remained at about 0.5 μmol O_2 g^{-1} fresh weight min^{-1} in plants grown in unenriched seawater receiving 12 h of H_{sat}, but declined to about one-half of that value after 30 d in plants grown under 4 h of H_{sat} without CO_2 enrichment, probably as a result of depleted internal C reserves. Long-term exposure to elevated [CO_2] had no significant effect on leaf respiration among treatments (Table II; Fig. 2). Leaf *R* was significantly lower only in plants growing under 4 h of H_{sat} in unenriched seawater on d 33, again a possible effect of internal C depletion. There was, however, no significant difference in leaf *R* among treatments on d 45.

Leaf pigment content varied significantly over time and among treatments during the course of this experiment, but there was no consistently identifiable treatment effect. Temporal effects were responsible for most of the variance and treatment effects were marginally significant (Table II). Leaf chlorophyll (*a* + *b*) content of CO_2-enriched plants was significantly higher than that in both unenriched treatments on d 9 (Fig. 3). There was no significant difference in leaf chlorophyll among treatments on d 20 and 26. Leaf chlorophyll was significantly higher in the 12-h unenriched treatment on d 33. On d 45 leaf chlorophyll was significantly lower in the 4-h unenriched treatment than in the two C-replete treatments, which did not differ significantly from each other.

Daily C budgets and H_{sat} requirements of whole plants (shoot plus rhizome plus root) were calculated and averaged across time for each treatment, according to Zimmerman et al. (1996). CO_2 enrichment reduced H_{sat} requirements to only 2.2 ± 0.2 h, approximately one-half of the daily light dose received by these plants growing under 4 h of H_{sat} (Table III). Eelgrass required 6.9 ± 0.6 and 7.4 ± 0.6 h of H_{sat} to maintain a positive C balance in unenriched seawater under 12 and 4 h of H_{sat}, respectively. Like the eelgrass grown under CO_2 enrichment, plants grown under 12 h of H_{sat} received approximately twice their daily light requirement (Table III). Plants grown in unenriched seawater with only 4 h of H_{sat}, however, received an average of only 60% of their required daily light dose (Table III). Daily C gain (5.6 mmol C $plant^{-1}$ d^{-1}) and demand (2.9–3.3 mmol C $plant^{-1}$ d^{-1}) for whole plants were similar for the two C-replete treatments throughout the experiment. Daily C gain was only 1 mmol C $plant^{-1}$ d^{-1} for plants receiving 4 h of H_{sat} in unenriched seawater. C demand was also considerably lower (1.8 mmol C $plant^{-1}$ d^{-1}), primarily as a result of the decline in plant size over time (Fig. 4).

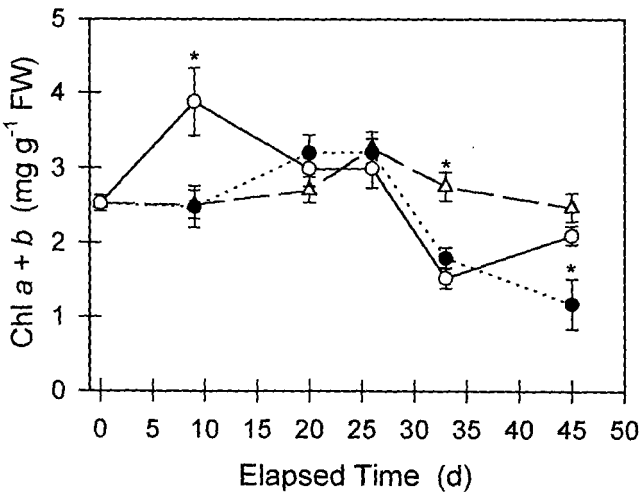

Figure 3. Changes in chlorophyll (Chl) *a* + *b* content of eelgrass leaves grown in normal seawater under 12 h of H_{sat} (△), 4 h of H_{sat} (●), and 4 h of H_{sat} plus CO_2 enrichment (○). Error bars represent ±1 SE. Asterisks (*) indicate significant differences among treatments at each sampling period ($P \leq 0.05$) identified by post-ANOVA multiple-comparison analysis (Newman LSD). FW, Fresh weight.

Sizes and growth rates of plants were similar in the two C-replete treatments (Fig. 4, A–C). Both maintained constant plant size and vigorous, specific growth rates of 1.5 to 2.0% d^{-1}, which were statistically indistinguishable from each other. In contrast, shoot lengths and growth rates of the C-deplete treatment declined steadily (Fig. 4, A–C). Growth of these severely light- and C-limited plants virtually ceased by d 45, and plants were reduced to one-half of their original size. Treatment effects were responsible for most of variance in plant size and growth rate (Table II).

Sugar content of leaves, roots, and the youngest rhizome internode (internode 1) followed temporal trajectories similar to those observed for growth rates (Figs. 5 and 6). Sugar content in all tissues of C-replete plants remained statistically constant throughout the 45-d experiment (Table II). In contrast, sugar content of leaves, roots, and rhizome internode 1 of the C-deplete treatment declined steadily over 45 d. Rates of net sugar loss under C depletion were −1, −0.3, and −1.33 μmol Suc g^{-1} fresh weight d^{-1} for leaves, roots, and rhizomes, respectively, representing a consumption of more than 70% of the C reserves during the course of the experiment. Sugar levels in rhizome internode 3, however, dropped significantly only on d 33 of the experiment (Fig. 6B; Table II). The weak treatment effect on internode 3 was caused partly by high variability in internode 3 sugar concentration among plants. C stores in the older rhizome internode 3 may have been difficult to mobilize under the short (4-h) daily period of below-ground aerobiosis. Starch, which represents less than 10% of the labile carbohydrate in leaves, roots, and rhizomes of eelgrass (Zimmerman et al., 1989), was not measured in this experiment.

Activity of leaf SPS (V_{max}) declined about 45%, from 4.5 to 2.5 μmol Suc mg^{-1} protein h^{-1}, during the 45-d experiment in eelgrass maintained under both C-replete treatments (Fig. 7). Although most of the variance resulted from temporal effects, there was a statistically significant treatment effect as well (Table II). Leaf SPS activity declined even more dramatically in the C-deplete treatment and was undetectable after 30 d (Fig. 7A). Activity of root SS (V_{max}) was variable (Fig. 7B), but showed no statistically significant treatment or temporal effect (Table II).

DISCUSSION

The CO_2-stimulated increase in photosynthesis found here is consistent with previous short-term observations of

Table III. *C budget calculations and H_{sat} requirements, as calculated according to Zimmerman et al. (1996)*
SEs are listed in parentheses (n = 20 for all treatments).

CO_2 Treatment	H_{sat}	Daily C Gain	Daily Shoot C Demand	Daily Root C Demand	Daily Rhizome C Demand	Daily C Balance	H_{sat} Requirement
	h			*mmol plant^{-1} d^{-1}*			*h*
Normal	4	1.0 (0.2)	1.6 (0.3)	0.1 (0.01)	0.1 (0.01)	0.6 (0.04)	7.4 (0.6)
Normal	12	5.6 (0.5)	2.9 (0.4)	0.2 (0.03)	0.2 (0.03)	1.7 (0.20)	6.9 (0.6)
Enriched	4	5.6 (0.4)	2.7 (0.3)	0.1 (0.01)	0.1 (0.01)	1.9 (0.20)	2.2 (0.2)

responses to dissolved inorganic C enrichment by many seagrasses, including eelgrass and the turtlegrass *Thalassia testudinum* (Beer, 1989; Durako, 1993; Zimmerman et al., 1995a; Beer and Koch, 1996). Collectively, these results indicate that photosynthesis of seagrasses is light-limited below E_k and is CO_2-limited above E_k in normal seawater. The 3-fold increase in E_k under CO_2 enrichment further indicates that the light-harvesting, electron-transport, and C-fixation capacities of eelgrass are 3-fold greater than can be exploited in the CO_2 environment of the modern ocean. This excess photosynthetic capacity is curiously similar to the 3-fold excess Suc-formation capacity found in eelgrass leaves (Zimmerman et al., 1995a).

No decline or down-regulation of eelgrass photosynthesis was observed here after 45 d of exposure to a 100-fold increase in free CO_2 (and a 2-fold increase in total dissolved inorganic C), a level of enrichment that is considerably higher than expected from the projected increase in atmospheric CO_2. In contrast to our observations under short H_{sat}, CO_2 enrichment did not increase eelgrass productivity or growth consistently when plants were maintained under long photoperiods and high irradiance (Thom, 1995). Sustained CO_2 enrichment in high-light environments may

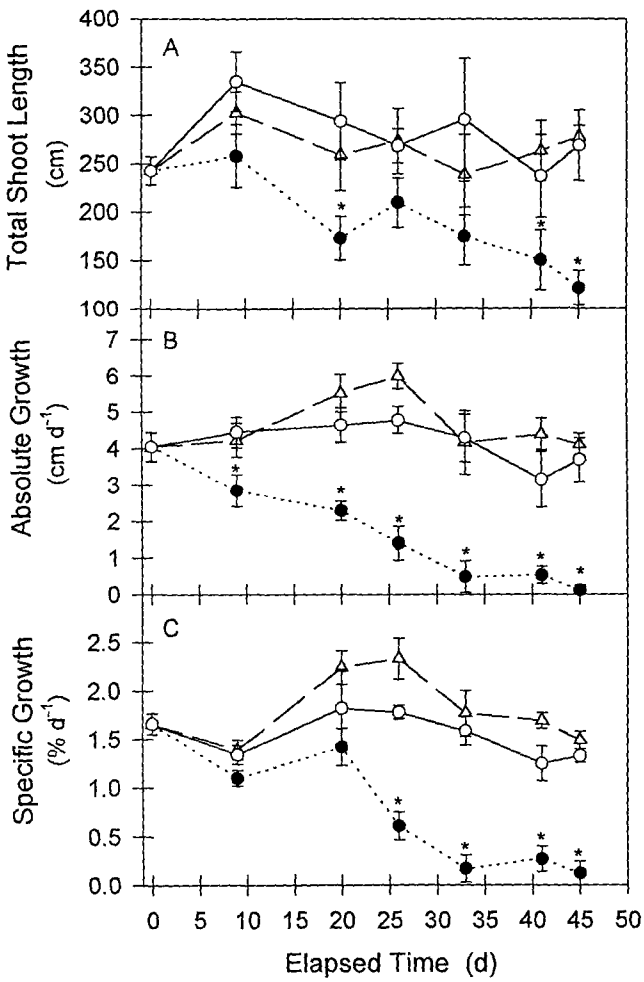

Figure 4. Changes in plant size (A), absolute growth (B), and length-specific growth rates (C) for eelgrass grown in normal seawater under 12 h of H_{sat} (△), 4 h of H_{sat} (●), and 4 h of H_{sat} plus CO_2 enrichment (○). Error bars represent ± 1 SE. Asterisks (*) indicate significant differences among treatments at each sampling period ($P \leq 0.05$) identified by post-ANOVA multiple-comparison analysis (Newman LSD).

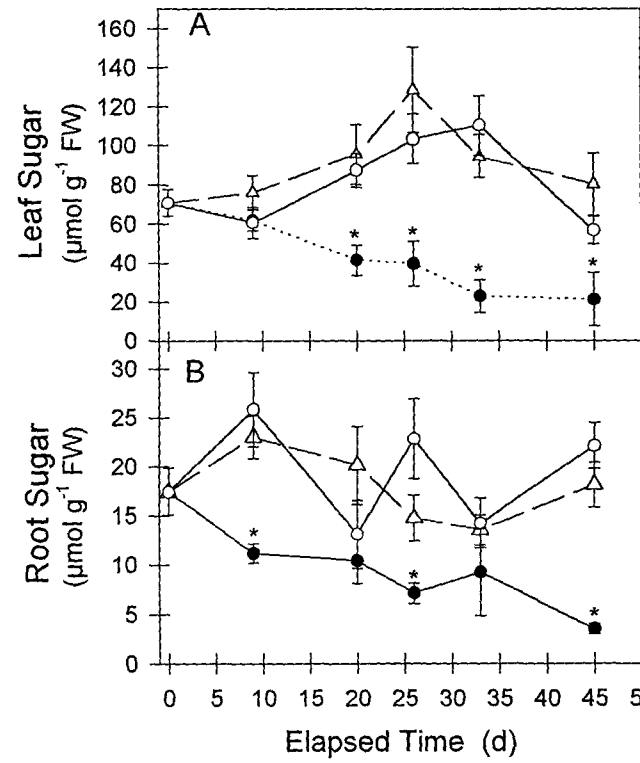

Figure 5. Sugar content of leaves (A) and roots (B) for eelgrass grown in normal seawater under 12 h of H_{sat} (△), 4 h of H_{sat} (●), and 4 h of H_{sat} plus CO_2 enrichment (○). Error bars represent ± 1 SE. Asterisks (*) indicate significant differences among treatments at each sampling period ($P \leq 0.05$) identified by post-ANOVA multiple-comparison analysis (Newman LSD). FW, Fresh weight.

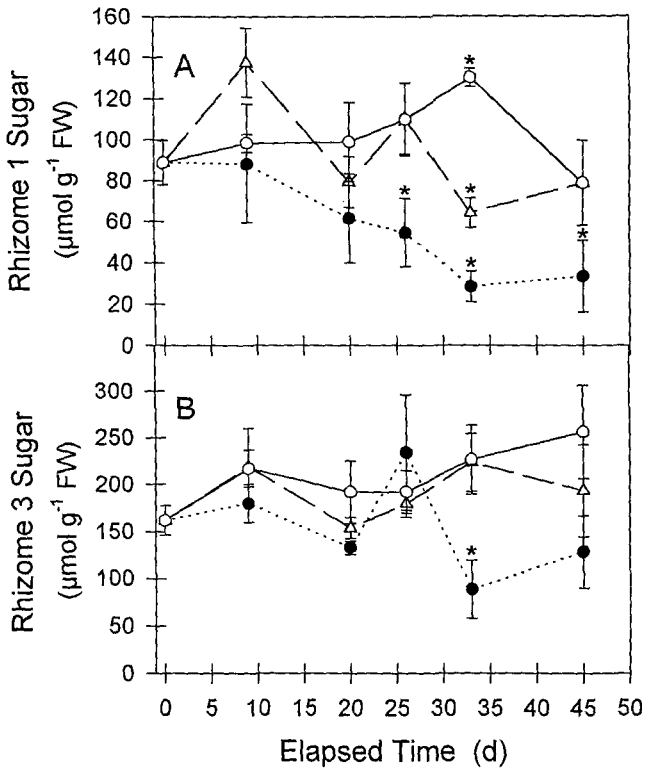

Figure 6. Sugar content of rhizome internodes 1 (A) and 3 (B) for eelgrass grown in normal seawater under 12 h of H_{sat} (△), 4 h of H_{sat} (●), and 4 h H_{sat} plus CO_2 enrichment (○). Error bars represent ± 1 SE. Asterisks (*) indicate significant differences among treatments at each sampling period ($P \leq 0.05$) identified by post-ANOVA multiple-comparison analysis (Newman LSD). FW, Fresh weight.

lead to down-regulation of photosynthesis if Suc production rates exceed sink strength and storage capacity (Arp, 1991; Stitt, 1991), and the decrease in photosynthetic performance is often accompanied by a decline in Rubisco activity (Yelle et al., 1989a, 1989b) and/or chlorophyll abundance (Peet et al., 1986). Those variables, however, were not measured by Thom (1995) and reasons for the apparently inconsistent responses by eelgrass grown in high light remain unclear. Photosynthate accumulation did not exceed utilization capacity under CO_2 enrichment in our study because plants exposed to elevated CO_2 were grown under only 4 h of H_{sat}, which equalized the daily C gain to the 12-h treatment in unenriched seawater. Although we demonstrated that CO_2 enrichment may ease the impact of light limitation on seagrass distributions and production dynamics, the effect of CO_2 enrichment on plants growing in light-replete environments requires more extensive investigation.

As with P_m, acclimation to elevated CO_2 can also decrease respiration (Azcón-Bieto et al., 1994; Bunce and Caulfield, 1991). We did not, however, observe significant effects on leaf respiration that resulted from prolonged exposure to CO_2 enrichment when measured in unenriched seawater. Thus, the low respiration rates we observed at high CO_2 were reversible, as is often seen in plants exposed briefly to elevated CO_2. This effect may result from direct CO_2 inhibition of succinate oxidation and mitochondrial electron transport (Amthor et al., 1992; Wullschleger et al., 1994; Gonzàlez-Meler et al., 1996).

The maintenance of elevated P_m under CO_2 enrichment allowed eelgrass to thrive on a daily light dose that otherwise would not permit long-term survival, as shown by the performance of eelgrass maintained in unenriched seawater with only 4 h of H_{sat} and confirmed by the C budget calculations. Thus, CO_2 limitation of photosynthesis was the prime driver of high light requirements in eelgrass, since below-ground tissues represented only 10% of the daily C demand in this experiment. Roots and rhizomes may be critical for nutrient acquisition and plant stabilization in unconsolidated sediments, but C demand by these tissues was not the first-order determinant of naturally low whole-plant P_g:R or the high light requirements of eelgrass grown in unenriched seawater. C demand by below-ground tissues, however, may play a more significant role in the overall C budget of other species, such as *Thalassia* spp. and *Posidonia* spp., which maintain a larger fraction of nonphotosynthetic biomass (Duarte 1991b; Kraemer et al., 1997).

In addition to controlling daily C gain, the H_{sat} period also determines the daily cycle of root aerobiosis (Smith et al., 1984). Eelgrass roots can tolerate extended periods of anoxia provided that internal C reserves remain sufficient

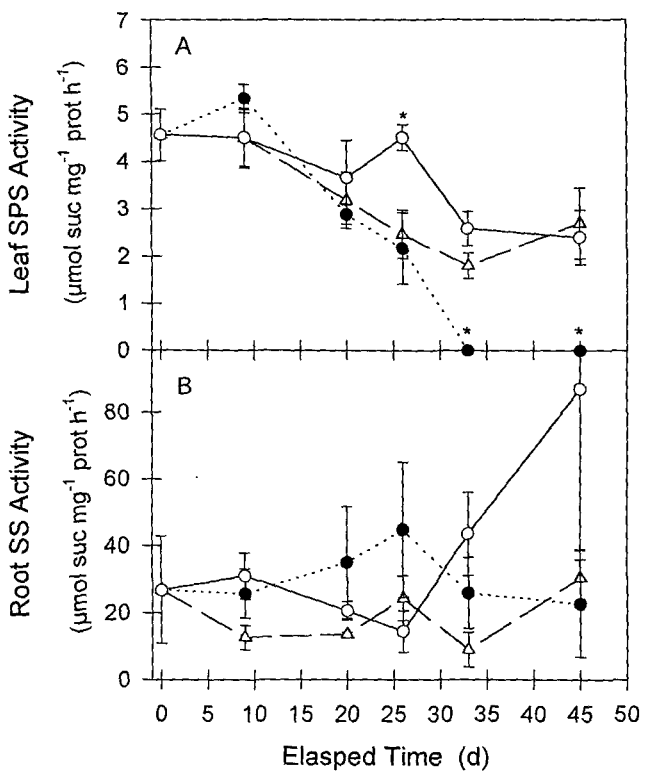

Figure 7. Leaf SPS activity (A) and root SS activity (B) of eelgrass grown in normal seawater under 12 h of H_{sat} (△), 4 h of H_{sat} (●), and 4 h of H_{sat} plus CO_2 enrichment (○). Error bars represent ± 1 SE. Asterisks (*) indicate significant differences among treatments at each sampling period ($P \leq 0.05$) identified by post-ANOVA multiple-comparison analysis (Newman LSD). prot, Protein.

for essential metabolic function (Smith et al., 1988; Smith, 1989). Anoxia, however, blocks translocation and makes roots vulnerable to C limitation even when ample C reserves remain in the shoot (Zimmerman and Alberte, 1996). Although CO_2 enrichment may improve the overall C balance of plants in low-light environments, expansion of seagrass colonization depths will require acclimation of source-sink relationships to accommodate the short windows of root aerobiosis (e.g. short H_{sat} periods). Eelgrass leaves possess excess Suc formation and export capacity (Zimmerman et al., 1995a), and translocation appears to readily accommodate increased photosynthesis rates, which is demonstrated by the fact that we did not observe significant negative impacts on root biomass, sugar content, or SS activity in the CO_2-enhanced plants even though they were aerobic for only 4 h each day. Root sink strength may also be enhanced by up-regulation of SS activity in response to extended daily anoxia produced by short H_{sat} (Freeling and Bennett, 1985; McCarty et al., 1986; Xue et al., 1991). Although temporal patterns observed here suggest that SS activity of eelgrass roots may increase in response to short H_{sat}, plant-to-plant variability in root SS activity was too great for these differences to be statistically significant.

It is curious that eelgrass leaves retain so much capacity for photosynthesis and Suc formation when natural availability of dissolved inorganic C limits the realized activity to about 33% of the maximum. Some of this excess capacity may reflect the terrestrial origins of seagrasses and/or their evolution in a seawater environment that was considerably richer in free CO_2 than it is today. Seagrasses evolved within the last 90 million years (den Hartog, 1970) and the pH of ocean surface waters may have been as low as 7.4 for a considerable portion of that evolutionary history (Spivack et al., 1993), suggesting that seawater free CO_2 may have been more than double the current level. High concentrations of free CO_2 would allow seagrasses to compete more effectively with algae without the need for an efficient mechanism for bicarbonate utilization. Thus, the low photosynthetic performance of modern seagrasses in natural seawater, their excess C-processing capacity, and their present vulnerability to eutrophication and light competition from algae may be combined consequences of evolutionary origins in CO_2-rich environments and relatively recent (<100 years) anthropogenic impacts on coastal biogeochemical processes that have severely reduced water-column transparency and light availability. Further research on CO_2-concentrating mechanisms (e.g. carbonic anhydrase) may shed significant light on the ecological physiology and evolutionary history of these important marine angiosperms.

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