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FEATURE ARTICLE

Response of eelgrass *Zostera marina* to CO_2 enrichment: possible impacts of climate change and potential for remediation of coastal habitats

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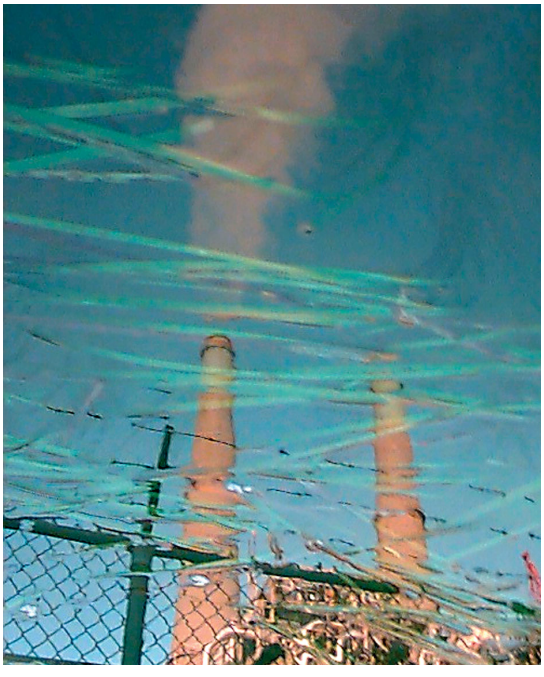
ABSTRACT: Projected increases in dissolved aqueous concentrations of carbon dioxide [CO_2(aq)] may have significant impacts on photosynthesis of CO_2-limited organisms such as seagrasses. Short-term CO_2(aq) enrichment increases photosynthetic rates and reduces light requirements for growth and survival of individual eelgrass *Zostera marina* L. shoots growing in the laboratory under artificial light regimes for at least 45 d. This study examined the effects of long-term CO_2(aq) enrichment on the performance of eelgrass growing under natural light-replete (33% surface irradiance) and light-limited (5% surface irradiance) conditions for a period of 1 yr. Eelgrass shoots were grown at 4 CO_2(aq) concentrations in outdoor flow-through seawater aquaria bubbled with industrial flue gas containing approximately 11% CO_2. Enrichment with CO_2(aq) did not alter biomass-specific growth rates, leaf size, or leaf sugar content of above-ground shoots in either light treatment. CO_2(aq) enrichment, however, led to significantly higher reproductive output, below-ground biomass and vegetative proliferation of new shoots in light-replete treatments. This suggests that increasing the CO_2 content of the atmosphere and ocean surface will increase the area-specific productivity of seagrass meadows. CO_2(aq) enrichment did not affect the performance of shoots grown under light limitation, suggesting that the transition from carbon- to light-limited growth followed Liebig's Law. This study also demonstrated that direct injection of industrial flue gas could significantly increase eelgrass productivity; this might prove useful for restoration efforts in degraded environments. The broader effects of CO_2(aq) enrichment on the function of natural seagrass meadows, however, require further study before deliberate CO_2 injection could be considered as an engineering solution to the problem of seagrass habitat degradation.

KEY WORDS: Eelgrass · *Zostera marina* · Carbon dioxide · Climate change · Productivity



Rising CO_2 concentrations derived from combustion of fossil fuel can increase the productivity and flowering rates of seagrass *Zostera marina*.
Photo: S. L. Palacios

INTRODUCTION

Anthropogenic activity has increased the carbon dioxide concentration of the atmosphere by 30% from pre-industrial concentrations averaging 270 ppm (Trenberth 1996, Keeling 1997). CO_2 concentrations are expected to rise to 450 ppm by 2065 and to 650 ppm by 2100 (Tren-

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berth 1996, O'Neill & Oppenheimer 2002), levels not reached since the Cretaceous (Retallack 2001). These CO_2 increases may have dramatic impacts on global climate (Keeling 1997), global carbon cycles (Trenberth 1996), ocean circulation (Manabe & Stouffer 1994, Sarmiento et al. 1998), biotic diversity (e.g. Kleypas et al. 1999, Ehleringer et al. 2001), and marine ecosystem function (Denman 1996).

Climate change and rising atmospheric CO_2 are predicted to increase the fecundity (Koch & Mooney 1996, DeLucia et al. 1999) and water use efficiency of terrestrial plants (Retallack 2001), alter biomass partitioning between their source and sink tissues (Chu et al. 1992), and decrease the nutritive value of plant material by diluting essential elements (N, Fe, etc.) with carbon (O'Neill & Norby 1996). Additionally, rising atmospheric CO_2 concentration is predicted to favor the survival of C_3 over C_4 species, thereby altering plant community assemblages and their associated herbivore populations (Ehleringer et al. 2001). In contrast, down-regulation of productivity after prolonged exposure to elevated [CO_2] in some terrestrial species indicates that some changes due to CO_2 enrichment may be short-lived (Arp 1991, Woodward 2002).

The ocean environment is also expected to undergo significant changes in response to rising CO_2 concentrations. The greenhouse effect is predicted to increase ocean temperatures by 1 to 3°C, melt polar ice, freshen surface waters at high latitudes and raise sea level by 0.5 m in the next 50 to 100 yr (Trenberth 1996). These temperature changes will affect heat sensitive organisms directly and alter ocean currents (Manabe & Stouffer 1994, Sarmiento et al. 1998). Elevated atmospheric CO_2 will also increase the dissolved aqueous CO_2 concentration [CO_2(aq)] in seawater (Zeebe & Wolf-Gladrow 2001).

The direct response of marine ecosystems to long term CO_2 enrichment is less clear. The resulting drop in seawater pH may cause widespread decline of carbonate accreting systems such as coral reefs (Kleypas et al. 1999). Marine photosynthesis is generally not CO_2 limited, because most marine algae derive 80 to 90% of their dissolved inorganic carbon (DIC) requirements from dehydration of the abundant HCO_3^- (Beer 1996), which represents about 88% of the total DIC content of seawater (Zeebe & Wolf-Gladrow 2001). This efficient utilization of HCO_3^- for photosynthesis contributes to the low minimum light requirement for algal growth, which is on the order of 1% of surface irradiance (Luning & Dring 1975). In contrast, seagrass light requirements are in excess of 11% of surface irradiance (Dennison & Alberte 1985, Duarte 1991), due primarily to carbon limitation of photosynthesis (Zimmerman et al. 1995, 1996, Beer & Koch 1996, Beer & Rehnberg 1997, Zimmerman et al. 1997, Invers et al. 2001). Although seagrasses are capable of dehydrating HCO_3^-, many appear to rely on CO_2(aq) for at least 50% of the carbon used for photosynthesis in nature (Durako 1993, Beer & Koch 1996, Beer & Rehnberg 1997). Short-term enrichment of *Zostera marina* L. (eelgrass) with CO_2(aq) in the laboratory under artificial illumination increased leaf photosynthesis and shoot productivity 3-fold, while simultaneously decreasing daily light requirements (Zimmerman et al. 1997).

Terrestrial studies have demonstrated that long-term effects of changes in important variables, such as CO_2 availability, can be difficult to predict from short-term exposure (Arp 1991, Woodward 2002). Consequently, objectives of this study were to determine (1) if prolonged CO_2(aq) enrichment permanently enhances the productivity of eelgrass shoots growing under natural irradiance regimes, (2) how CO_2 enrichment might affect population dynamics of shoots that ultimately determine the density and spatial extent of eelgrass meadows, (3) if industrial flue gas containing CO_2 derived from fossil fuel combustion promotes eelgrass productivity if deliberately injected into the water. Understanding the impacts of CO_2(aq) availability on seagrasses will provide insight into both responses of these ecologically important macrophytes to global climate change, and techniques for seagrass restoration in turbid coastal waters.

MATERIALS AND METHODS

Experimental site. Four outdoor flowing seawater aquaria were constructed at the Duke Energy-North America Power Plant (DENAPP) at Moss Landing, California, USA. Seawater was pumped from Moss Landing Harbor into a 20 m^3 storage silo and gravity-fed into 4 fiberglass open top aquaria (4 m^3 each). Outflow from the aquaria was fed into the power plant's seawater outfall and transported offshore, more than 1 km away from the source water in Moss Landing Harbor. Seawater volume within the aquaria turned over approximately 10 times per day.

Source population. Eelgrass (512 shoots) was collected by hand in September 2000 from a subtidal population located at Seal Bend in Elkhorn Slough, CA, USA (36.8153° N, 121.7658° W). Care was taken to separate whole shoots from the mud, keeping as many intact root bundles and rhizome internodes as possible. Shoots were placed in coolers containing seawater and transported immediately to the experimental site. Approximately 500 kg of mud, also collected from Seal Bend, was distributed into 128 plastic nursery pots (4 l capacity) lined with plastic bags, and 4 eelgrass shoots were transplanted to each pot. The pots were divided equally among the 4 outdoor flowing seawater aquaria

(Fig. 1). The pot-grown shoots were maintained for 5 mo without CO_2(aq) enrichment to permit recovery from transplant effects (if any) and to evaluate the existence of any aquarium-specific effects that might confound the CO_2(aq) and light treatments. Light availability in all aquaria was reduced to 33% of incident surface irradiance using neutral density screens to simulate the natural submarine light intensity in Elkhorn Slough, and to prevent photoinhibition of the leaves. New shoots created by vegetative proliferation were carefully removed and transferred to a new pot when shoot density exceeded 4 per pot. Shoots growing out of the pots (a result of rhizome elongation) were replanted as necessary to keep roots and rhizomes buried in the sediment.

The 32 pots in each aquarium were randomly segregated into light-replete (33% of surface irradiance) and light-limited (5% of surface irradiance) treatments of 16 pots each, 5 mo after the initial collection. Light was reduced to 5% of surface irradiance by adding more neutral density screening to the south half of each aquarium. The light-limited treatment was designed to provide less than 11% of surface irradiance, which is generally considered necessary for long-term survival (Duarte 1991).

Manipulation of CO_2(aq) and light availability. Manipulation of CO_2(aq) concentration and light availability was initiated in February 2000. Combustion of natural gas for electric power generation by DENAPP produced industrial flue gas containing 10% CO_2, 158 ppm CO and 58 ppm NO_x, the composition of which was monitored continuously by DENAPP. NO_x consisted of a mixture of NO, NO_2, and NO_3, with NO comprising roughly 90%, and NO_2 comprising 1 to 7% of the total NO_x pool (S. Abbott, DENAPP, pers. comm.). Inert components included N_2 (80%) and H_2O (10%). Flue gas generated by the power plant furnace was piped approximately 1 km to the experimental site, at a line pressure of 1.76 kg cm^{-2}. Water was removed through condensate traps placed at regular intervals along the pipeline as the flue gas cooled during its transit from the furnace to the aquaria, raising the final [CO_2] of the nearly dry flue gas to approximately 11%. [CO_2(aq)] treatments were chosen to represent (1) the present day atmosphere, with approximately 16 µM CO_2(aq) (pH 8.1), (2) CO_2 projected for 2100 that increases the CO_2(aq) concentration of seawater to approximately 36 µM CO_2(aq) (pH 7.75), (3) CO_2 projected for 2200 that increases the CO_2(aq) concentration of seawater to 85 µM CO_2(aq) (pH 7.5), and (4) a dissolved aqueous CO_2 concentration of 1123 µM CO_2(aq) (pH 6.2), which triples the light-saturated photosynthesis rate of eelgrass (Zimmerman et al. 1997). These model concentrations were calculated by CO2SYS (ver 1.05) (Lewis & Wallace 1998) using the dissociation constants of Hansson (1973) and the CO_2 solubility equations of Weiss & Price (1980) (Lewis & Wallace 1998) assuming full strength seawater and constant alkalinity (salinity = 35, alkalinity = 2500 µ equiv. kg^{-1}, temperature = 15°C).

Three aquaria were enriched with flue gas delivered by pH-controlled solenoid valves and LED pH/ORP controllers (Cole-Parmer, Model 05656-00) that maintained seawater pH within ± 0.1 unit. The pH electrodes were submerged in each growth aquarium 30 cm below the surface, near the seawater outlet at the end of the aquarium opposite the water input. The electrodes were calibrated weekly using Fisher™ standardized pH buffers. When a solenoid valve was open, flue gas was delivered via two 6 m loops of weighted plastic tubing running through the bottom of the aquarium. The tubing was punctured approximately every 50 cm using a 20-gauge hypodermic needle. Because no other acidifying agents or buffers were added to the seawater, pH served as proxy for the concentration of CO_2(aq) in each aquarium. Salinity was measured every 2 wk using a refractometer calibrated with deionized water. The time series of CO_2(aq) concentration and the total DIC distribution in each aquarium (Table 1) were calculated from pH, temperature, salinity, and alkalinity (assumed to be 2500 µ equiv. kg^{-1}) as described above.

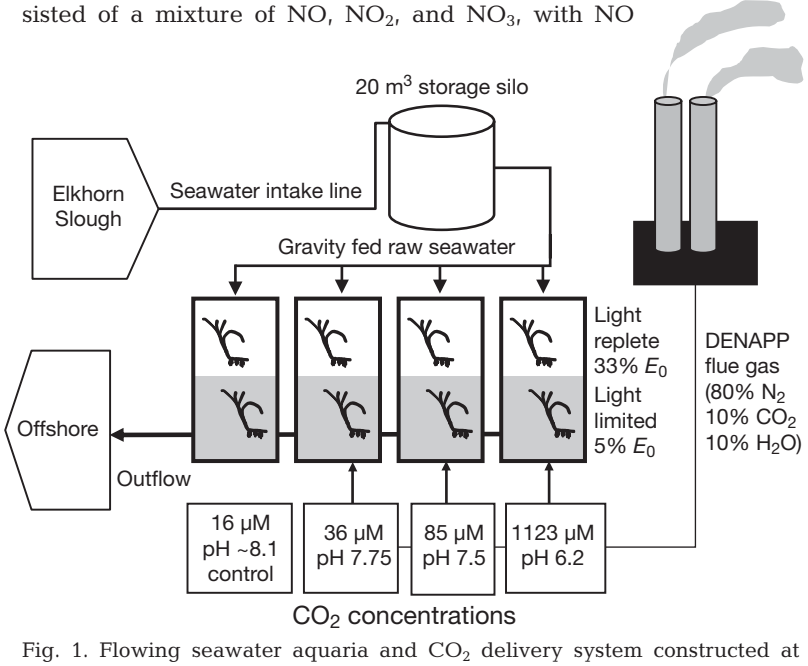

Fig. 1. Flowing seawater aquaria and CO_2 delivery system constructed at the Duke Energy North America Power Plant (DENAPP) at Moss Landing, CA, USA

Environmental conditions. Aquarium water temperature, pH, and irradiance were recorded every 15 min using a BASIC programmable microprocessor-controlled data logger (Tattletale Model 4A). Temperature was monitored using YSI 44033 thermistors calibrated to a precision of 0.1°C over a temperature range of 5 to 25°C using a temperature-controlled water bath. Downwelling (in air) photosynthetically available radiation (PAR = 400 to 700 nm) was measured using a factory calibrated plane irradiance quantum sensor (LI-190SA, LI-COR Biosciences). Periodic gaps in the irradiance observations caused by occasional equipment failure were replaced by data from the plane irradiance quantum sensor incorporated into the Moss Landing Marine Laboratories Weather Station (MLML) (~1 km away). Regression analysis of concurrent data recorded by the 2 sensors produced a slope of 1.06, which was not significantly different from 1 (r^2 = 0.97, N = 230, F = 7076, MSE = 7.52, MSPE = 7.45), and a *y*-intercept of –3.23 µmol quanta m^{-2} s^{-1}. Gaps in the DENAPP data were therefore filled with MLML values that had been converted using the equation of the line fitted to the MLML vs. DENAPP relationship [missing DENAPP PAR = 1.06 × (MLML PAR) – 3.23]. The H_{sat} period, equivalent to the number of h d^{-1} during which irradiance reached photosynthetically saturating levels, was calculated from the irradiance time series according to Zimmerman et al. (2001).

Shoot abundance, growth rates and biomass allocation. All shoots were counted and their flowering status noted in September 2000, and each month from February 2001 to February 2002. All abscised leaves and floating dead shoots were removed from the aquaria every 3 days. In each treatment, 9 shoots were randomly selected each month, beginning in September 2000, and analyzed for growth rate, leaf area, and leaf sugar content. Shoot growth rates, leaf area, and leaf sugar content were never sampled on the same shoots in consecutive months. Shoots were marked for growth estimates 2 wk prior to measurement using the hole-punch method (Zimmerman et al. 1996). Young unmarked leaves were assumed to be new growth. The length of new leaf material below the punch mark and the total length of all leaves were measured to the nearest millimeter using a meter tape. Leaf width (nearest 0.1 mm) was measured with a digital caliper. Photosynthetic shoot size, or leaf area (cm^2 $shoot^{-1}$), was calculated by summing the one-sided area (leaf length × leaf width) of all leaves of the shoot.

Absolute growth (cm^2 $shoot^{-1}$ d^{-1}) was calculated as:

$$\frac{\text{New leaf area per shoot}}{\text{Number of days from hole punch to measure}}$$

Specific growth (% d^{-1}) was calculated as:

$$\frac{\text{Absolute growth}}{\text{Total leaf area}} \times 100$$

Biomass allocation among shoots, rhizomes, and roots was measured only 3 times during the experiment, because it required destructive sampling. Destructive measurements of roots, rhizome, and leaf biomass were made at the following times: in December 2000 prior to the onset of the CO_2(aq) and light manipulations, midway through the experiment in April 2001, and at the end of the experiment in February 2002. Lengths of individual internodes along each rhizome (4 to 18 internodes each) were measured at the end of the experiment to the nearest 0.1 mm using a digital caliper. The date of each internode creation was calculated assuming an average plastochrone interval of 15 d (Hemminga & Duarte 2000). Rhizome extension rate was calculated by dividing total rhizome length by plastochrone age. Internode diameter was measured to the nearest 0.1 mm for the first and third internodes after the meristem at the final destructive sampling in February 2002.

Leaf sugar content. Each month, a segment of leaf #3 (#1 is the youngest leaf) was collected from each of the 9 shoots marked for growth. The leaf samples were dried at 60°C and ground in liquid nitrogen. Sugar was extracted from the ground tissue 3 times using hot (80°C) ethanol (Zimmerman et al. 1989). The 3 extractions were combined, an aliquot was evaporated to dryness under a stream of compressed air, redissolved in distilled water and analyzed spectrophotometrically using a resorcinol assay standardized to sucrose (Zimmerman et al. 1995).

Statistical analyses. Aquarium-specific effects on eelgrass leaf area, absolute growth, specific growth, and leaf sugar content were tested during the pre-enrichment period from September through December 2000 using 1-way ANOVA. The impact of CO_2 enrichment on eelgrass performance was evaluated using linear regression for the light-replete and light-limited treatments separately. The CO_2 treatments were applied to individual aquaria without replication such that replicated performance measures within each CO_2(aq) × Light treatment were used to calculate mean values without error estimates to avoid pseudo-

Table 1. Equilibrium distribution of dissolved inorganic carbon in seawater (February 2001 to February 2002). PR: photosynthesis rate at light saturation

CO_2 level	pH	[CO_2(aq)]	[HCO_3^{-1}]	[CO_3^{2-}]	Total [CO_2]
Present	8.1	16	2005	204	2225
Year 2100	7.75	36	2367	108	2510
Year 2200	7.5	85	2237	55	2377
Triple PR	6.4	1123	2477	10	3610

replication (Hurlburt 1984). Thus, n = 4 for regression analysis of CO_2(aq) effects. In those cases where no CO_2 effects were identified (i.e. slope = 0), performance data within light treatments were pooled across CO_2(aq) treatments and evaluated for irradiance effects over time using 2-way ANOVA (Time × Light) and LSD multiple comparison (Zar 1996). Effects of CO_2 enrichment and light availability were evaluated using Student's *t*-test.

RESULTS

Environmental conditions

Daily-integrated irradiance followed a noisy sinusoidal pattern through time (Fig. 2a). The seasonal amplitude in daily irradiance varied about 3-fold from winter to summer, and cloud effects were randomly scattered throughout the year. The daily H_{sat} period for the light-replete treatment (33% of E_0) was consistently above the 5 h duration required to sustain plant growth (Zimmerman et al. 1996) for 92% of the study period regardless of season (Fig. 2b). Daily H_{sat} in the light-limited treatment (5% of E_0) was consistently lower than the 5 h threshold from October to February. Even in summer (March through September), the minimum H_{sat} period of 5 h was exceeded on only 47% of the days in the light-limited treatment and only 31% of the days over the total study.

Salinity (not shown) ranged from 34 to 37 throughout the experiment and an average of 35 was used for the CO_2 solubility equations. Assuming conservation of alkalinity with salinity, variation in salinity from 34 to 37 produced less than a 3% variation in the calculated DIC distribution and TCO_2 concentration. Annual variation in ambient seawater temperature ranged from 9°C in winter to 17°C in summer (Fig. 3a). On any given day, aquarium temperatures were within 1°C of each other across all treatments. The [CO_2(aq)] of the unenriched aquarium averaged 16 µM CO_2(aq), with transient excursions ranging from 4 to 47 µM CO_2(aq).

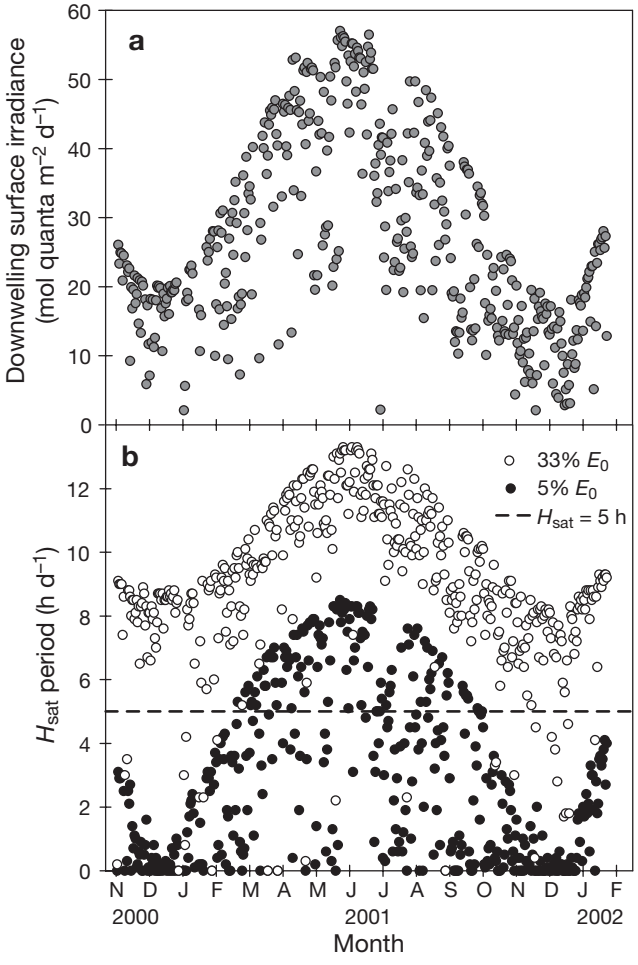

Fig. 2. (a) In-air downwelling plane irradiance (PAR). (b) Daily number of hours during which irradiance attained photosynthetically saturating levels (H_{sat}) for light-replete (○) and light-limited (●) treatments

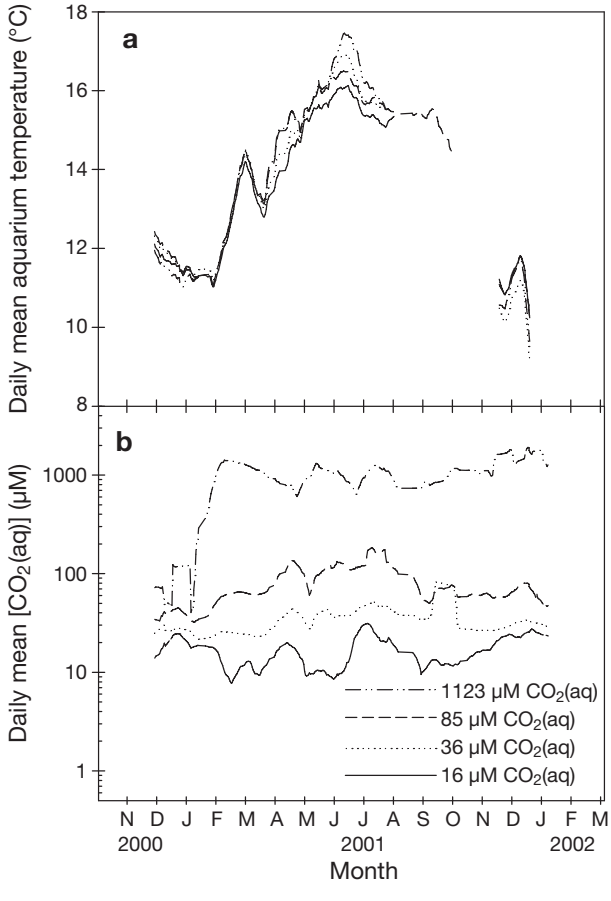

Fig. 3. Environmental conditions during the study period: (a) water temperature in each aquarium. The gap from October 2001 to November 2001 was caused by equipment failure. (b) Calculated CO_2(aq) concentration in each aquarium

[CO_2(aq)] in the manipulated aquaria averaged 36 µM, 85 µM, and 1123 µM CO_2(aq) beginning in February 2001 (Fig. 3b). The data presented here were smoothed to 20 d running averages.

Evaluation of aquarium-specific effects

No significant aquarium-specific effects on eelgrass productivity occurred in the 4 mo (October 2000 to January 2001) prior to initiating CO_2 enrichment (Table 2). The allocation of biomass between above- and below-ground tissues was constant across all aquaria. The 2 statistically significant aquarium effects—leaf sugar content in December 2000 and absolute growth in January 2001—occurred only once for each parameter during this pre-enrichment period.

Shoot size and biomass allocation

Total shoot biomass of light-replete treatments was positively related to CO_2(aq) enrichment at the end of the experiment (Table 3). Shoots growing at 36 µM CO_2(aq) were 25% larger than those in the unenriched treatment [16 µM CO_2(aq)], at 85 µM CO_2(aq) shoots were 50% larger than those in the unenriched treatment and at 1123 µM CO_2(aq) shoots were almost twice as large as those in the unenriched treatment (Fig. 4a). This increase resulted exclusively from an increase in biomass allocated to the rhizome, because leaf and root biomass were unaffected by CO_2(aq) enrichment (Fig. 4a). In contrast, CO_2(aq) enrichment did not affect biomass allocation of plants growing under light limitation (Table 3, Fig. 4b). Leaf biomass was, however, strongly influenced by light availability at 16, 36, and 1123 µM CO_2(aq) concentrations (Table 4). Root and rhizome biomass were greater in the light-replete treatments grown at 1123 µM CO_2(aq) concentration (Table 4).

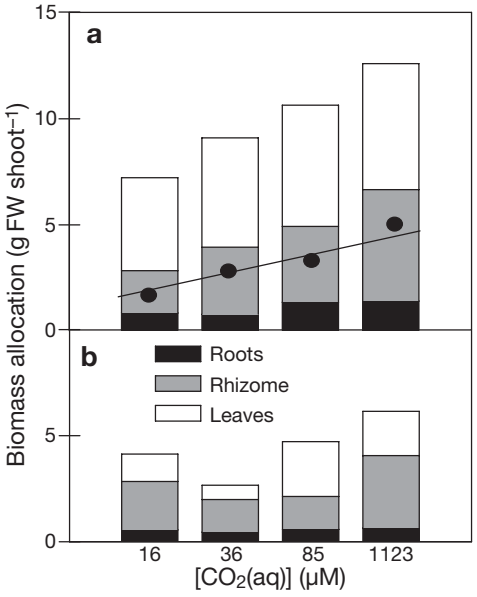

Fig. 4. *Zostera marina*. Biomass allocation (g FW shoot^{-1}) among roots, rhizomes and leaves after 1 yr growth under CO_2 enrichment, plotted as a function of CO_2(aq) concentration for (a) light-replete and (b) light-limited treatments. Mean rhizome biomass (●) with fitted line shown for light-replete treatments ($r^2 = 0.99$, $p < 0.01$)

Table 2. *Zostera marina*. Biomass allocation, leaf area, growth rates and sugar content (mean; SE in parentheses) of plants grown in the 4 aquaria during December 2000, prior to the onset of CO_2 enrichment. *$p \leq 0.05$

Effect	Aquarium				ANOVA			
	1	2	3	4	df	MS	*F*	p
Biomass allocation (g FW)								
Leaves	13 (1)	16 (2)	18 (5)	16 (2)	3	37.90	0.28	0.84
Roots	2 (0)	2 (0)	2 (0)	2 (0)	3	1.77	2.14	0.11
Rhizome	6 (1)	8 (1)	6 (1)	6 (1)	3	4.53	0.50	0.68
Total	21 (2)	26 (3)	25 (6)	23 (3)	3	37.18	0.19	0.90
Leaf area (cm^2)								
Dec 2000	221 (22)	276 (21)	244 (26)	249 (24)	3	9071	0.92	0.43
Jan 2000	272 (20)	236 (26)	282 (28)	290 (34)	3	10170	0.78	0.51
Absolute growth (cm^2 d^{-1})								
Dec 2000	32 (3)	34 (3)	31 (3)	28 (3)	3	138.9	0.90	0.45
Jan 2000	29 (2)	19 (2)	20 (2)	17 (2)	3	473.3	6.46	<0.01*
Specific growth (% d^{-1})								
Oct 2000	2.6 (0.1)	2.5 (0.2)	2.3 (0.1)	2.7 (0.1)	3	0.22	1.40	0.26
Nov 2000	1.4 (0.04)	1.4 (0.1)	1.6 (0.1)	1.3 (0.1)	3	0.09	1.28	0.30
Dec 2000	2.2 (0.1)	2.1 (0.1)	2.5 (0.2)	2.1 (0.2)	3	0.43	1.19	0.32
Jan 2001	1.8 (0.1)	1.6 (0.1)	1.7 (0.1)	1.6 (0.1)	3	0.19	1.32	0.28
Leaf sugar content (µmol suc. equiv. g^{-1} FW)								
Oct 2000	101 (5)	146 (20)	91 (14)	119 (36)	3	3419	1.20	0.33
Nov 2000	41 (2)	73 (16)	65 (30)	45 (4)	3	1500	0.85	0.48
Dec 2000	37 (4)	74 (10)	92 (7)	83 (6)	3	9955	10.61	<0.01*

Table 3. *Zostera marina*. Linear regression for the effect of [CO_2(aq)] on biomass, specific growth, leaf area, flowering, and shoot abundance (only significant effects shown) at light-replete (33% E_0) and light-limited (5% E_0) treatments ($^*p \le 0.05$, $^{**}p \le 0.01$)

Dependent variable	Date (mm/dd/yy)	Slope	Intercept	r^2	ANOVA df	ANOVA MS	ANOVA *F*	ANOVA p
Light-replete (33% E_0)								
Total biomass (g FW shoot^{-1})	02/02/02	2.8	3.7	0.96	2	14.8	70.6	0.01**
Rhizome biomass (g FW shoot^{-1})	02/02/02	1.6	0.2	0.99	2	4.80	252	<0.01**
Internode length (mm)	09/01/01	5.7	0	0.97	2	62.5	105	<0.01**
	09/16/01	6.3	−0.7	0.99	2	77.3	770	<0.01**
	10/01/01	6.3	−0.05	0.98	2	76.2	188	<0.01**
	10/16/01	6.4	0.6	0.96	2	78.4	82.1	0.01**
	11/01/01	6.8	0.4	0.92	2	88.3	35.1	0.03*
Annual internode extension rate (cm yr^{-1})		13.7	6.0	0.92	2	359	37.0	0.03*
Flowering (no. of shoots)	05/29/01	5.2	3.2	0.98	2	52.0	135	<0.01**
Shoot abundance (no. of shoots)	12/07/01	45	−22	0.96	2	3960	74.5	0.01**
	12/21/01	45	−21	0.94	2	3830	51.4	0.02*
	01/07/02	42	−28	0.99	2	3400	334	<0.01**
	01/24/02	24	1.9	0.90	2	1060	26.6	0.04*
	02/01/02	24	−2.5	0.93	2	1080	40.6	0.02*
Absolute growth (cm^2 d^{-1})	05/29/01	1.2	4.7	0.86	2	2.60	18.9	0.05*
Leaf sugar content (µmol suc. equiv. g^{-1} FW)	03/10/01	70	−46	0.91	2	9340	30.4	0.03*
	10/12/01	45	1.4	0.99	2	3930	464	<0.01**
Light-limited (5% E_0)								
Leaf area (cm^2)	3/10/01	48	180	0.93	2	4370	40.1	0.02*
	12/07/01	62	−25	0.92	2	7290	34.6	0.03*
Absolute growth (cm^2 d^{-1})	3/10/01	2.1	2.2	0.96	2	8.31	75.3	0.01**
	07/25/01	1.8	2.3	0.87	2	5.90	21.6	0.04*
	01/07/02	0.2	0.5	0.93	2	0.04	39.8	0.02*
Specific growth (% d^{-1})	02/23/01	0.1	1.5	0.97	2	0.03	99.3	0.01**
	03/10/01	0.3	1.7	0.91	2	0.14	33.0	0.03*
Leaf sugar content (µmol suc. equiv. g^{-1} FW)	3/10/01	31	−5.7	0.98	2	1790	125	<0.01**

Shoots growing under light-replete conditions had larger internodes (greater length and biomass) than corresponding shoots growing under light limitation at all manipulated CO_2(aq) concentrations (Table 3, Figs. 4 & 5). Internodes produced in summer were larger than those produced in winter, especially at the highest CO_2 enrichment. Shoots grown under light limitation without CO_2(aq) enrichment had longer internode lengths but the same biomass as shoots in light-replete treatments (Table 5). The diameter of the first internode was greater in light-replete than in light-limited conditions for shoots growing in the 16, 36, and 85 µM CO_2(aq) treatments. However, diameters of the first internodes were not different between light treatments grown under the highest (1123 µM) CO_2 enrichment (Table 4).

Rhizome extension rates of light-replete shoots were strongly affected by CO_2(aq) enrichment (Fig. 6a). They did not show a statistically significant response to CO_2(aq) enrichment for shoots growing under light limitation (Fig. 6b).

Table 4. *Zostera marina*. Student's *t*-test for the impact of light level on biomass (g FW) allocation to different tissues: leaf, rhizome, root, first internode (mm). Data are mean (SE). $^*p \le 0.05$, $^{**}p \le 0.01$

[CO_2(aq)] (µM)	Tissue	Light-limited	Light-replete	*t*	df	p
16	Leaf	1.3 (0.2)	4.8 (1.0)	−2.8	10	0.02*
	Rhizome	2.3 (0.2)	2.1 (0.5)	0.23	10	0.82
	Root	0.6 (0.1)	1.0 (0.3)	−1.1	10	0.31
	Internode	3.1 (0.2)	4.5 (0.3)	−2.4	11	0.03*
36	Leaf	0.6 (0.1)	4.3 (3.0)	−2.6	15	0.02*
	Rhizome	1.4 (0.5)	2.8 (0.7)	−1.4	15	0.17
	Root	0.4 (0.1)	0.7 (0.2)	−0.92	15	0.37
	Internode	2.6 (0.2)	4.9 (0.3)	−3.5	12	<0.01**
85	Leaf	2.1 (0.6)	5.2 (1.1)	−1.8	13	0.10
	Rhizome	1.4 (0.3)	3.3 (0.7)	−1.8	13	0.08
	Root	0.5 (0.2)	1.2 (0.3)	−1.3	13	0.20
	Internode	2.8 (0.7)	5.0 (0.4)	−2.6	11	0.02*
1123	Leaf	1.7 (0.5)	5.2 (0.6)	−4.1	16	<0.01**
	Rhizome	2.5 (0.8)	4.7 (0.6)	−2.1	16	0.05*
	Root	0.4 (0.1)	1.3 (0.1)	−3.5	16	<0.01**
	Internode	4.5 (0.6)	5.4 (0.2)	−2.0	14	0.07

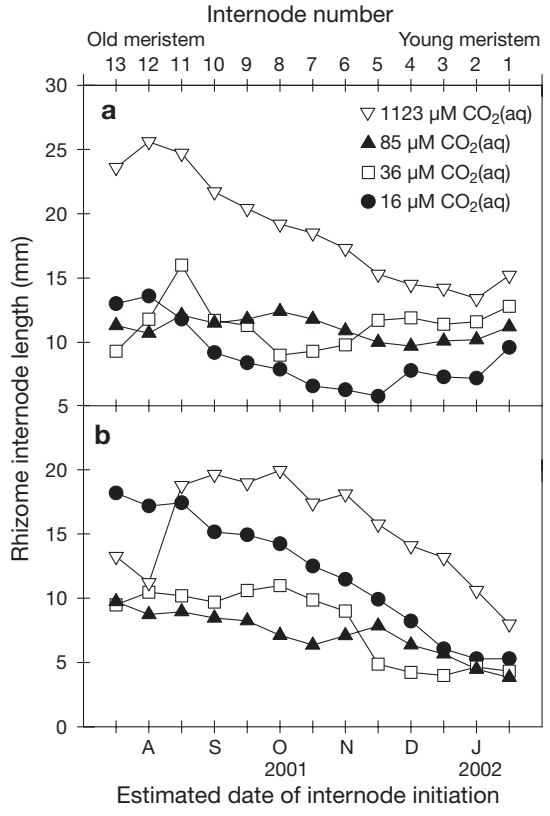

Fig. 5. *Zostera marina*. Average internode length plotted as a function of internode number, which increased away from the meristem. The date of each internode initiation was calculated assuming a 15 d plastochrone interval. (a) light-replete condition, (b) light-limited condition

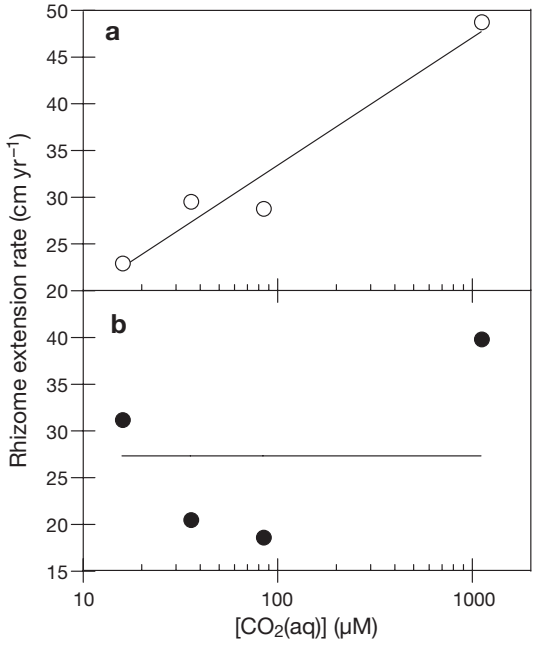

Fig. 6. *Zostera marina*. Calculated rhizome extension rate for (a) light-replete and (b) light-limited treatments

Flowering shoot production

The proliferation of flowering shoots responded positively to CO_2(aq) enrichment in the light-replete treatments (Table 3, Fig. 7a). Flowering shoots appeared earlier in the year and matured more quickly in proportion to [CO_2(aq)]. At 1123 µM CO_2(aq) in May 2001, 22% of the shoots differentiated into flowers, more than twice the flowering output of the other treatments at this light level (Fig. 8). Flowering output was very low under light limitation, and CO_2(aq) enrichment had no significant effect (Table 3, Fig. 7b). No flowering occurred in the light-limited, 36 µM treatment.

Vegetative shoot abundance

Shoot abundance was stable in the 16, 36, and 85 µM CO_2(aq) treatments under light-replete conditions through summer 2001 (Fig. 9a). Abundance in the 1123 µM treatment dropped in late spring as flowering shoots matured and then died. However, the shoot population of this highest CO_2(aq) treatment recovered subsequently through late spring and summer as a result of vegetative proliferation. Shoot numbers declined in all treatments in winter. Shoot numbers in all CO_2(aq) treatments grown under light limitation declined throughout the experiment (Fig. 9b). Unlike the light-replete treatments, there was no period of

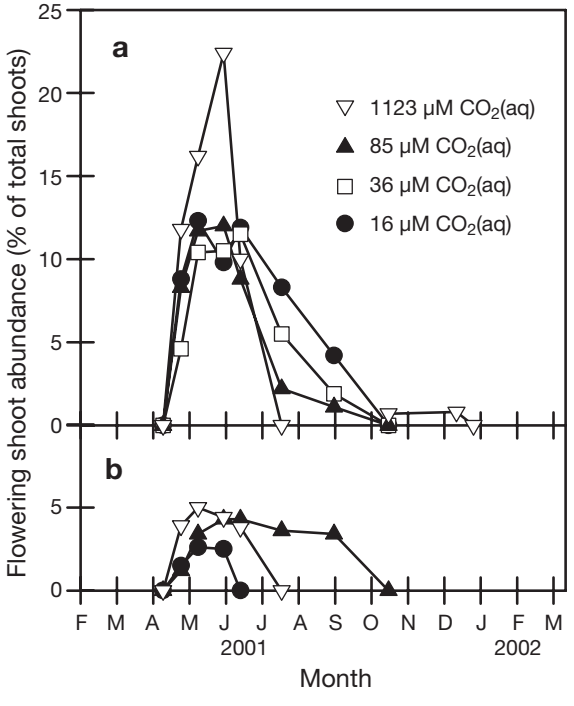

Fig. 7. *Zostera marina*. Flowering shoot abundance over time in (a) light-replete and (b) light-limited treatments

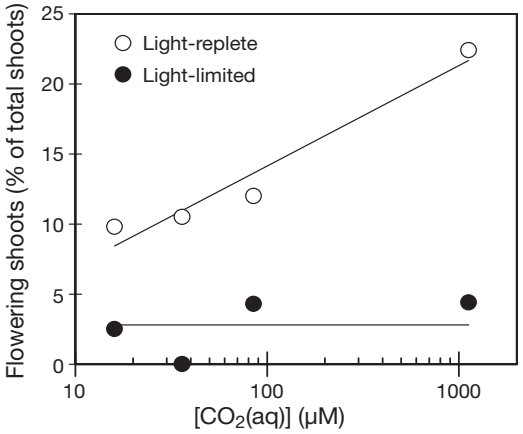

Fig. 8. *Zostera marina*. Flowering shoot abundance at peak flowering (May 2001) for light-replete and light-limited treatments plotted as a function of CO_2(aq) enrichment

summer stability or vegetative shoot proliferation in the light-limited treatments (Table 3, Fig. 9b). Further, the steady decline in shoot numbers under light limitation was due to vegetative shoot death, not the maturation and senescent death of flowering shoots.

CO_2(aq) enrichment enhanced shoot survival into the winter in the light-replete treatments (Table 3, Fig. 10). Shoot numbers in the 1123 μM treatment were double those of shoots growing at light-replete levels without CO_2(aq) enrichment (Fig. 10). Shoot numbers were low in the light-limited treatment at the end of the experiment, and CO_2(aq) enrichment did not impact shoot survival (Fig. 10).

Individual shoot leaf area, leaf growth rates and leaf sugar content did not respond to CO_2(aq) enrichment in either light treatment. We consider the few significant differences in individual shoot performance in each CO_2(aq) and light treatment to be spurious occurrences of Type I error, given the number of measurements performed and tested. No other statistically significant trends were detected for a CO_2(aq) enrichment effect on above-ground shoot morphometrics or sugar content. Shoot performance data were pooled across CO_2(aq) enrichment treatment, excluding significant treatments, for determination of Time × Light effects using 2-way ANOVA.

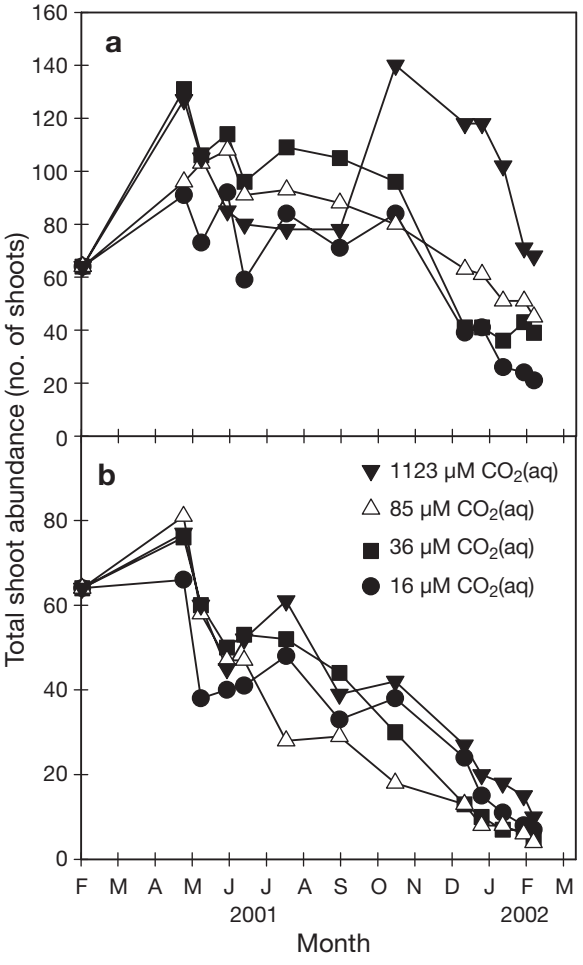

Fig. 9. *Zostera marina*. Shoot abundance over time in (a) light-replete and (b) light-limited treatments

Table 5. *Zostera marina*. Student's *t*-test of the impact of light level on eelgrass internode length. Length data are mean (SE). Only significant results are shown (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001). Dates given as mm/dd/yy

[CO_2(aq)] μM	Internode No.	Date	Length (mm) Light-limited	Light-replete	*t*	df	p
16	1	01/16/02	5.3 (0.9)	9.6 (1.1)	−2.6	14	0.021*
	5	11/16/01	9.9 (0.6)	5.8 (0.9)	3.5	12	0.004**
	6	11/01/01	11.5 (0.8)	6.3 (1.0)	4.0	11	0.002**
	7	10/16/01	12.5 (0.9)	6.6 (0.5)	6.0	11	<0.001***
	8	10/01/01	14.2 (1.0)	7.9 (0.7)	5.1	11	<0.001***
	10	09/01/01	14.9 (1.4)	8.4 (0.8)	4.4	10	0.001***
	11	08/16/01	15.2 (1.0)	9.2 (0.7)	5.0	9	0.001***
	12	08/01/01	17.4 (0.8)	11.8 (1.6)	3.1	8	0.016*
36	1	01/16/02	4.3 (0.5)	12.8 (2.2)	−2.8	15	0.014**
	3	12/16/01	4.0 (0.5)	11.4 (2.1)	−2.6	15	0.022*
	4	12/01/01	4.2 (0.4)	11.9 (2.3)	−2.4	15	0.028*
85	1	01/16/02	3.8 (0.4)	11.1 (1.5)	−3.1	15	0.007**
	2	01/01/02	4.5 (0.4)	10.2 (1.4)	−2.5	15	0.024*
1123	1	01/16/02	8.0 (1.4)	15.2 (1.1)	−4.0	18	0.001***
	13	07/16/01	11.2 (0.0)	25.6 (1.9)	−3.1	4	0.036*

Light regulation of eelgrass productivity

Seasonal light availability significantly affected the leaf area, growth rate, and sugar content of above-ground biomass

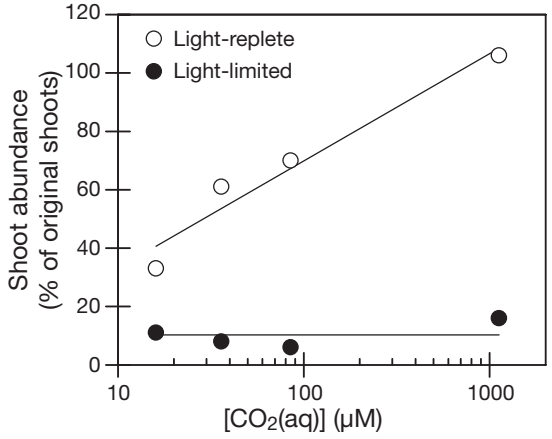

Fig. 10. *Zostera marina*. Shoot abundance at the termination of the experiment (February 2002) in light-replete and light-limited treatments plotted as a function of CO_2(aq) enrichment

independently of the CO_2(aq) treatment (Table 6). Differences existed between light treatments primarily during the winter (Fig. 11). The light treatment had significant effects on growth rate and leaf sugar content, but not on leaf area. There was no significant interaction of Time × Light for individual leaf area and shoot growth rate, but there was for leaf sugar, which indicates no strong evidence of synergy between time and light in this experiment. Calculated leaf area, absolute growth, and specific growth values were based on the same leaf width and length measurements and showed similar seasonal patterns.

Table 6. *Zostera marina*. 2-way ANOVA for the effects of time and light treatment on eelgrass leaf area, absolute growth, specific growth and leaf sugar content in both light treatments (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001)

Effect	df	MS	*F*	p
Leaf area				
Time	9	41440	12.18	<0.001***
Light	1	12640	3.72	0.058
Time × Light	9	6844	2.01	0.053
Within	60	3401		
Absolute growth				
Time	7	41.90	26.85	<0.001***
Light	1	35.52	22.76	<0.001***
Time × Light	7	1.75	1.12	0.366
Within	48	1.56		
Specific growth				
Time	9	1.66	14.28	<0.001***
Light	1	3.03	26.10	<0.001***
Time × Light	9	0.10	0.90	0.535
Within	60	0.12		
Leaf sugar				
Time	9	20350	16.41	<0.001***
Light	1	21080	16.99	<0.001***
Time × Light	9	3404	2.75	0.009**
Within	60	1239		

Growth rates of shoots in both light treatments were greater in summer than winter. Absolute growth rate in the light-replete treatments doubled from February to March 2001, steadily declined in late May and June, and then decreased through fall and into the winter (Fig. 11b). In the light-limited treatments, absolute growth initially doubled from February to March 2001,

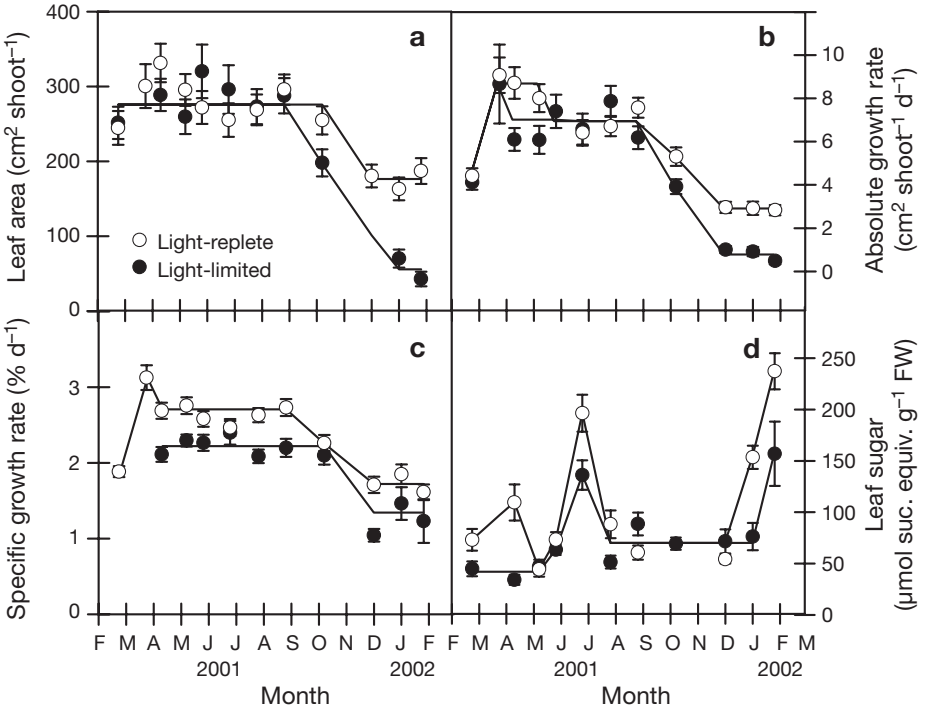

Fig. 11. *Zostera marina*. (a) Average leaf area, (b) absolute growth, (c) specific growth and (d) leaf sugar, over time for CO_2(aq) treatments pooled into light-replete and light-limited groups. Lines represent significant means, determined using ANOVA, and error bars represent ±1 SE

Table 7. Simple linear regression with 1-way ANOVA for the effect of duration of saturating irradiance (H_{sat}, no. of h d^{-1}) on the variables listed ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$). Leaf sugar content of eelgrass showed no statistically significant relationship to H_{sat}

Variable	Slope	Intercept	r^2	df	MS	*F*	p
Leaf area	12.0	160	0.20	86	136200	20.95	<0.01**
Absolute growth	0.5	2.0	0.35	78	195.1	42.03	<0.01**
Specific growth	0.1	1.4	0.41	86	12.4	59.36	<0.01**

then declined gradually through fall and winter. At the end of the experiment in February 2002, absolute growth of shoots in the light-limited treatments was only 17% of that observed for shoots in the light-replete treatment. Specific growth followed a similar pattern. These rates were the same for both light treatments at the end of the experiment (Fig. 11c). The duration of H_{sat} during the growth period was weakly correlated with leaf area, absolute growth, and specific growth (Table 7). Though seasonal light availability influenced leaf area, absolute growth rate and specific growth rate, there was no strong evidence of synergy between the effects of Time × Light on these growth parameters.

Leaf sugar content was significantly higher in the shoots growing in the light-replete treatments during April 2001, July 2001, and January 2002 (Fig. 11d). These increases preceded periods of increased growth, suggesting that growth may not be simply a function of light level, but may involve an endogenous seasonal component that requires a series of processes not clearly distinguished by analyzing month to month growth parameters.

DISCUSSION

Individual shoot parameters, such as leaf growth rate and sugar content, show significant responses to different environmental conditions (e.g. Durako 1993, Lee & Dunton 1997, Zimmerman et al. 1996, Zimmerman et al. 1997). More specifically, brief laboratory exposures to CO_2(aq) enrichment, ranging from a few hours to 45 d, lead to increased leaf sugar content (Zimmerman et al. 1995), higher growth rates and dramatically reduced H_{sat} requirements (Zimmerman et al. 1997). Eelgrass grown in the light-limited treatment of this experiment, however, showed no significant responses to CO_2(aq) enrichment, which appears at first to contrast with earlier work demonstrating a significant reduction in the H_{sat} requirement of laboratory grown eelgrass (Zimmerman et al. 1997). Although the duration of the daily photoperiod was manipulated in that experiment, the instantaneous irradiance was well above that required to saturate photosynthesis of unenriched leaves in normal seawater. Consequently, the addition of CO_2(aq) in that study significantly increased photosynthesis rates during the shortened photoperiod. However, the natural illumination cycle provided by the sun in this study meant that instantaneous photosynthesis of the eelgrass growing under 5% irradiance was limited by light, not carbon, for most of the day, and H_{sat} periods were well below 4 h throughout much of the year. Under these conditions, the CO_2 subsidy provided no benefit, as Liebig's Law would predict.

The shoots grown under light repletion and CO_2(aq) enrichment underwent a transient period of significantly higher growth rates and leaf sugar accumulation in March and April of 2001, consistent with previous studies, and this transient pulse of carbon accumulation subsequently gave way to a period of enhanced rhizome growth, flowering shoot production and vegetative proliferation that lasted throughout the summer. Like pine (DeLucia et al. 1999, LaDeau & Clark 2001, Woodward 2002) and wild radish (Chu 1992), eelgrass responds to CO_2 enrichment by increasing growth that benefits survival of the clone and/or population in ways that are not necessarily manifested at the level of individual shoots. Although the long-term integrated response of other seagrass species remains an open question, CO_2 limitation of photosynthesis appears to be a common feature (Durako 1993, Invers et al. 2001, but see Schwarz et al. 2000). Thus, rising concentrations of CO_2(aq) may increase vegetative propagation and seed production of other seagrass populations besides eelgrass.

The consistently significant responses to light and CO_2(aq) availability expressed by the eelgrass in this study involved the allocation of biomass to below-ground rhizomes, wintertime shoot survival, maturation of flowering shoots in early summer and proliferation of vegetative shoots. Except for below-ground biomass, temporal and/or spatial differences in these properties are detectable at the level of populations, but not at the level of individual shoots. Light-limited shoots never increased in abundance and less than 4% of the light-limited population flowered under any of the CO_2(aq) treatments. The fact that growth rate and leaf area were different in the 2 light treatments only during the short photoperiods of winter suggests that productivity parameters of individual shoots may be poor indicators of population responses to environmental stress. When exposed to severe grazing pressure from an epiphytic limpet, eelgrass shoot parameters (growth rate, size, and sugar content) declined precipitously, but in concert with losses in shoot

density (Zimmerman et al. 2001). Tracking the decline of shoot abundance, however, provides a poor tool for managing or monitoring seagrass populations, because they are extremely difficult to reverse. Approximately 10% of the shoots produce flowers under light-replete conditions in natural eelgrass populations (Hemminga & Duarte 2000). Thus, the reduction or complete lack of flowering exhibited by the light-limited treatments here may be an important indicator of light stress prior to the decline of vegetative shoot density.

How are the CO_2-stimulated increases in productivity likely to affect the distribution and abundance of eelgrass populations in the field? Atmospheric CO_2 levels predicted for the year 2100 (Zeebe & Wolf-Gladrow 2001), which correspond to the 36 µM CO_2(aq) enrichment in this study, may permit a doubling of vegetative shoot abundance in light-replete environments; this could have a positive feedback on properties of these systems. This study showed that increased [CO_2(aq)] is capable of increasing eelgrass reproductive output via flowering, and area-specific productivity via vegetative shoot proliferation under naturally replete light regimes. The resulting increases in eelgrass meadow density may initiate a positive feedback loop that facilitates the trapping of sediments and prevents their resuspension (Koch 1994), thereby reducing turbidity and increasing light penetration in coastal habitats. The increased light penetration may allow seagrass colonization depths to increase even further. The lack of stimulation under low light conditions, however, indicates that CO_2 enrichment will not permit eelgrass to survive at light levels approaching 1% of surface irradiance that can be tolerated by macrophytic algae (Markager & Sand-Jensen 1992). Whether rising atmospheric CO_2 can offset or keep pace with the effects of deteriorating water quality on eelgrass distributions remains an open question. It is clear, however, that efforts to expand and protect seagrass resources through improved water quality should benefit from the responses of eelgrass to CO_2 enrichment observed here.

Whether due to climate change or deliberate injection, rising CO_2(aq) concentrations may have consequences for seagrass ecosystems on a global scale. Where water quality is not compromised, elevated CO_2(aq) may increase seagrass productivity, enhancing fish and invertebrate stocks as well. Deliberate injection of CO_2 to seawater may facilitate restoration efforts by improving the survival rates of recently transplanted eelgrass shoots. Although CO_2(aq) enrichment does not appear to offset the effects of light starvation, it can buffer the negative effects of transplant shock by increasing rhizome reserve capacity and promoting shoot proliferation in light-replete environments. It may also facilitate eelgrass survival in environments where conditions are periodically limiting, such as long dark winters or usually warm summers that produce unfavorable productivity to respiration (P:R) ratios (Evans et al. 1986, Zimmerman et al. 1989). CO_2 injection may also promote flowering and seed production necessary for expansion and maintenance of healthy eelgrass meadows (Orth et al. 2006).

CO_2 increases, however, may not produce positive effects on all organisms associated with seagrass meadows that provide important habitat for fish and invertebrate species and are occupied by 42% more species than adjacent bare sand (Hemminga & Duarte 2000). Many of these species are juveniles that seek refuge among the shoots. Carbonate saturation state will decline as seawater CO_2(aq) rises (Zeebe & Wolf-Gladrow 2001), potentially stressing carbonate precipitating organisms such as mollusks, corals, and foraminifera (Kleypas et al. 1999). Rising CO_2(aq) concentrations may also stimulate nuisance algal blooms such as *Ulva* spp., which efficiently switch from HCO_3^- to CO_2(aq) as the primary source of inorganic carbon for photosynthesis (Beer 1989, Raven et al. 1995) in eutrophic estuaries. Prolific growth of these algae competitively excludes eelgrass populations. Finally, the continued deterioration of coastal water quality may overwhelm the positive effects of elevated atmospheric CO_2 on seagrass productivity, further limiting the space available for seagrass colonization. Nonetheless, eelgrass photosynthesis is severely carbon-limited in present day oceanic waters and that limitation plays a major role in determining the distribution, density, and reproductive success of this important coastal macrophyte.

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