EELGRASS RESPONSE TO CARBON DIOXIDE ENRICHMENT

A Thesis

Presented to

The Faculty of the Department of Biology

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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December 2003

ABSTRACT

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Projected increases in dissolved aqueous carbon dioxide concentrations, [CO_2(aq)], from fossil fuel combustion may have significant impacts on photosynthesis of CO_2-limited organisms, including seagrasses. This study examined the impacts of long-term CO_2(aq) enrichment on the performance of eelgrass (*Zostera marina* L.) growing under light replete and light limited conditions for one year. Eelgrass shoots were grown at four CO_2(aq) concentrations in outdoor flow-through seawater aquaria bubbled with industrial flue gas containing 10% CO_2. Vegetative and flowering shoot counts, and rhizome biomass increased in proportion to CO_2(aq) enrichment in light replete conditions. CO_2(aq) enrichment did not affect eelgrass performance in light limited conditions. CO_2(aq) enrichment did not alter growth rates, leaf size, or leaf sugar of individual shoots in either light treatment. Thus, CO_2(aq) enrichment had a significant positive impact on shoot populations, but not on individual shoot performance. Increased CO_2 availability may enhance productivity, density, and distribution of seagrass meadows.

ACKNOWLEDGMENTS

Many thanks go to those who guided and influenced me in my time at Moss Landing Marine Laboratories. I am indebted to Richard C. Zimmerman for believing in me when others had given up hope. Because of him I was able to do this research and present my work at several conferences around the world. G. Jason Smith was an excellent sounding board and always gave me valuable insight, especially whenever I got stuck. Kerstin Wasson, my NOAA-NERRS Graduate Research Fellowship supervisor, helped me stay focused on the practical aspects of why we need to monitor marine vegetation in Elkhorn Slough. Heidi Dierssen assisted me by writing MatLab programs to process the environmental data. Mike Foster kept me honest. I have Sally Wittlinger, Nicolas Ladizinsky, Clare Dominik, Aimee Bullard, Laura Bodensteiner, Don Kohrs, Judah Goldberg, and Tim Shaadt in the Environmental Biotechnology Laboratory to thank for intellectual stimulation, moral support, and hacky-sack playing.

One person cannot implement this type of project alone. Thanks must go especially to Steven Abbott at Duke Energy North America for making it possible to use the space and effluent gas from the power plant. Barry Giles, William Cochran, James Cochran, and Ralph Dzuro assisted me with maintaining the seawater system. Lisa Gilbain, Jenny Rhoem, Matt Huber, Heather Spalding, Laurie McConnico, Eli Landrau, Clare Dominik, Aimee Bullard, and Heidi Dierssen provided field and laboratory assistance. Joan Parker, Gail Johnston, and Donna Kline were always prompt and professional with helping me in the library and filing paperwork.

This project was funded by: The Japan New Energy Development Organisation, NOAA-NERRS Graduate Research Fellowship (NA07OR0259), Duke Energy North America (in-kind donation), Dr. Earl H. Myers and Ethel M. Myers Oceanographic and Marine Biology Trust, The Packard Foundation, Sigma-Xi, Richard S. Pasetto, American Society for Limnology and Oceanography (travel award), and The Estuarine Research Federation (travel award).

I am grateful to my husband, Rick Pasetto, for supporting me intellectually, emotionally, and financially throughout this process. I thank my in-laws, Marcella and Richard A. Pasetto, for being so supportive and tactful about the time it has taken me to finish. My family: Gloria Palacios, Julio Palacios, Nancy Heilig, and Dr. Monica Palacios-Boyce I thank for giving me the drive to pursue my dreams. Finally, I thank my adoptive parents/friends Rob and J Rosella Myers for being my west coast family and for enthusiastically listening to me talk about mud.

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INTRODUCTION

Anthropogenic activity has increased the carbon dioxide concentration of the Earth's atmosphere by 30% since the pre-industrial era, when CO_2 concentrations averaged 270 ppm (Keeling et al. 1976; Trenberth 1996). The CO_2 concentration of the atmosphere is expected to rise to 450 ppm by 2065 and 650 ppm by 2100 (Trenberth 1996; O'Neill and Oppenheimer 2002), levels not reached since the Cretaceous Period (Retallack 2001). These CO_2 increases may have dramatic impacts on global climate (Revelle and Suess 1957; Keeling 1997), global carbon cycle (Post et al. 1990), ocean circulation (Manabe and Stouffer 1994; Sarmiento et al. 1998), biotic diversity (e.g. Kleypas et al. 1999; Ehleringer et al. 2001), and marine ecosystem function (Denman 1996). As atmospheric CO_2 concentrations rise, ocean temperatures are predicted to increase 1-3°C. Polar ice is expected to melt, freshening surface waters at high latitudes and raising sea level by 0.5m (Trenberth 1996). These temperature changes will affect heat sensitive organisms, downwelling of seawater in high latitudes such as the Southern Ocean, and currents that transport marine larvae. Ocean chemistry also will change as a result of elevated atmospheric CO_2. The proportion of dissolved aqueous CO_2 [CO_2(aq)] in seawater will represent a larger fraction of the total dissolved inorganic carbon (DIC) pool (Zeebe and Wolf-Gladrow 2001). The resulting drop in seawater pH may cause widespread decline of calcium carbonate accreting systems such as coral reefs (Kleypas et al. 1999). Although increased [CO_2(aq)] is not expected to dramatically alter the photosynthetic performance of marine phytoplankton or macroalgae, increased [CO_2(aq)]

may significantly elevate the productivity and proliferation of CO_2-limited macrophytes such as seagrasses (Zimmerman et al. 1997).

Climate change and rising atmospheric CO_2 have been predicted to increase plant fecundity (Koch and Mooney 1996; DeLucia et al. 1999) and water use efficiency (Taiz and Zeiger 1998; Retallack 2001), alter biomass partitioning between sources and sinks (Chu et al. 1992), and decrease the nutritive value of plant material by diluting essential elements (N, Fe, etc.) with carbon (O'Neill and Norby 1996). Additionally, changes in population structure are predicted to favor C_3 over C_4 plants that may alter species assemblages with a correlated change in 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).

It is unclear how marine ecosystems will respond to long term CO_2(aq) enrichment. Most marine algae are not CO_2 limited, as they derive 80-90% of required DIC for photosynthesis from the efficient dehydration of HCO_3^- (Beer 1996). In seawater, bicarbonate represents about 88% of the total DIC pool (~2 mM), carbonate (CO_3^{2-}) represents about 11%, and CO_2(aq) only 0.5 % (Sverdrup 1942; Zeebe and Wolf-Gladrow 2001). The efficient utilization of HCO_3^- for photosynthesis contributes to the relatively low minimum light requirements for macroalgal growth (< 1% of surface irradiance) (Luning and Dring 1975). In contrast, seagrasses have high light requirements (11% of surface irradiance) (Dennison and Alberte 1985; Duarte 1991; Kraemer and Alberte 1995) which is limited at irradiances below 27 μmol photons m^{-2} s^{-1}

(Zimmerman et al. 1997) and this light limitation is linked to carbon limitation of photosynthesis (Zimmerman et al. 1995; Beer and Koch 1996; Zimmerman et al. 1996; Beer and Rehnberg 1997; Zimmerman 1997; Invers et al. 2001). Seagrasses inefficiently dehydrate HCO_3^-, (Durako 1993; Beer and Koch 1996;) and rely on CO_2(aq) for 50% of the carbon used in photosynthesis (Beer and Koch 1996; Beer and Rehnberg 1997). Short-term enrichment of eelgrass with CO_2(aq) in the laboratory under artificial illumination increased eelgrass productivity three-fold while simultaneously decreasing daily light requirements (Zimmerman et al. 1997). How seagrasses will respond to long-term CO_2(aq) enrichment under more natural light environments is not well understood.

Seagrasses enriched with CO_2(aq) may survive at previously light limiting levels. Long-term CO_2(aq) enrichment may decrease H_{sat} requirements and increase productivity as in short term studies (Zimmerman et al. 1997), and it may also decrease seagrass light requirements to below the 11% surface irradiance requirement. Therefore, long term enrichment with CO_2(aq) may supplement eelgrass shoots growing in turbid water. Seagrasses are vulnerable to deteriorated water quality that results from dredging, upstream non-point source sediment loading, and nuisance algal blooms caused by eutrophication (den Hartog 1970; Short and Wyllie-Echeverria 1996; Hemminga and Duarte 2000). Loss of submerged vegetation creates a positive feedback through sediment destabilization that further increases water column turbidity and accelerates seagrass loss (Orth and Moore 1983; Short and Wyllie-Echeverria 1996). Enrichment with CO_2(aq) may help mitigate these losses.

The main objective of this study was to determine if long-term CO_2(aq) enrichment permanently enhanced the productivity of eelgrass (*Zostera marina*) growing under natural irradiance regimes. The second objective was to determine whether changes in eelgrass productivity would be manifested at the level of individual shoots and/or populations. The third objective was to determine if industrial flue gas containing CO_2 derived from fossil fuel combustion could be injected deliberately into the water to promote eelgrass productivity. Understanding the impacts of CO_2(aq) availability on seagrasses will provide insight into potential responses of these ecologically important macrophytes to global climate change, and possible remediation techniques useful for promoting seagrass restoration in turbid coastal waters.

METHODS

Experimental Design

This study was performed using outdoor aquaria located at the Duke Energy-North America Power Plant at Moss Landing, CA (DENAPP). Combustion of natural gas for electric power generation by DENAPP produced industrial flue gas containing 10% CO_2, 58ppm NO_x, and 158 ppm CO. NO_x consisted of a mixture of NO, NO_2, NO_3, and NO_4 with NO comprising roughly 90% of the total NO_x pool and NO_2 1-7% of NO_x (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 25 p.s.i. Most of the water in the flue gas was

removed by condensation traps along the pipeline, raising the final [CO_2] of the dry flue gas to approximately 11%. Four [CO_2(aq)] treatments were chosen to (i) represent the present day atmosphere [15 μM CO_2(aq), (pH 8.2)], (ii) a 2.4-fold increase projected for 2100 [37 μM CO_2(aq), (pH 7.8)], (iii) a 4.5-fold increase projected for 2200 [68 μM CO_2(aq), (pH 7.5)], and (iv) a 100-fold increase [1433 μM CO_2(aq), (pH 6.2)] known to increase photosynthesis three-fold in eelgrass (Zimmerman et al. 1997). These concentrations were calculated using the dissociation constants of Hansson (1973), and the CO_2 solubility equations of Weiss and Price (1980) assuming full strength seawater (salinity = 35 ‰, alkalinity = 2500 μ equiv. kg^{-1}, temperature = 15° C).

Source Population

Eelgrass shoots (512 shoots) were collected by hand in September 2000 from a subtidal population located at Seal Bend in Elkhorn Slough, California, USA (36.8153 N, 121.7658 W). Care was taken to separate whole shoots from the mud with intact leaves, root bundles, and as many intact 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 4 L plastic nursery pots lined with plastic bags. Four eelgrass shoots were transplanted to each pot. The pots were divided equally among the four outdoor flowing seawater aquaria (Fig. 1). Each aquarium was eventually assigned to one of the four [CO_2(aq)] treatments. Seawater was pumped from Moss Landing Harbor into a 20,000 L storage silo and gravity fed into the four fiberglass open top aquaria (4000 L

each). Outflow from the aquaria was fed into the power plant's seawater outfall and transported offshore, more than 1km away from the source water. Seawater volume within the aquaria turned over approximately ten times per day. The pot-grown shoots were maintained for five months without CO_2(aq) enrichment to permit recovery from transplant effects (if any) and to evaluate the existence of any tank-specific effects that might confound the CO_2(aq) and light treatments. Light availability in all aquaria was reduced to 33% of ambient (in air) light using neutral density screens to simulate the natural submarine light environment in Elkhorn Slough, from which the shoots were collected, and to prevent any photoinhibition.

Manipulation of CO_2(aq) and Light Availability

Manipulation of CO_2(aq) concentration and light availability was initiated after the five-month recovery period in February 2000. The 32 pots in each tank were randomly segregated into light replete (33% of surface irradiance) and light limited (5% of surface irradiance) treatments of 16 pots each. Light was reduced to 5% of surface irradiance by the addition of more neutral density non-reactive screening. The light limited treatment was designed to be below the 11% of surface irradiance light level thought necessary for long term eelgrass survival (Duarte 1991).

New shoots created by vegetative proliferation were carefully removed and transferred to a new pot when shoot density exceeded four per pot. Shoots growing out of the pots were carefully removed and replanted as necessary to keep roots and rhizomes buried in the sediments. Three of the aquaria were enriched with flue gas from the power

plant regulated by pH-controlled solenoid valves and Cole-Parmer LED pH/ORP controllers (Model 05656-00) (Cole-Parmer Instrument Company, Vernon Hills, IL), that maintained seawater pH within ±0.1 unit. The pH electrodes were calibrated weekly to within 0.01 pH unit using Fisher™ standardized pH buffers and then submerged in each growth tank 30cm below the surface near the seawater outlet, at the end of the tank opposite the water input. The fourth (control) aquarium did not receive effluent gas and had an average pH of 8.2, equivalent to an average CO_2(aq) concentration of 15 μM. When the solenoid valves were open, flue gas was delivered to each tank via two 6 m loops of weighted Tygon® tubing running through the bottom of each tank. 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, the pH served as proxy for controlling the concentration of CO_2(aq) in each aquarium. Salinity was measured approximately once every two weeks using a refractometer, baselined with deionized water. The time series of CO_2(aq) concentration and the total DIC distribution in each tank, were calculated from pH, temperature, salinity, and alkalinity (assumed to be 2500 microequivalents kg^{-1}) using the dissociation constants of Hansson (1973) and the CO_2 solubility equations of Weiss and Price (1980) (Table 1).

Environmental Conditions

Environmental conditions (tank pH, temperature, irradiance) at the site were recorded every 15 minutes using a BASIC programmable microprocessor-controlled data logger (Tattletale Model 4A). Temperature was monitored in each growth tank using

YSI 44033 precision thermistors (Yellow Springs Instrument Co., Yellow Spring, OH) encased in Flexane 80 epoxy filled tubes. Temperature probes were calibrated (to 0.1°C precision) from 5°C to 25°C in a temperature-controlled water bath prior to the experiment. Downwelling plane irradiance, measured as photosynthetically available radiation (PAR=400-700nm) was measured in air using a factory calibrated LI-190SA Quantum Sensor™ (LI-COR Biosciences, Lincoln, NE). The on-site irradiance data contained gaps caused by occasional equipment failure, and data from the nearby Moss Landing Marine Laboratories Weather Station were used to fill those gaps. This weather station used the same LI-COR PAR sensor, and collected data each minute. The daily-integrated irradiances from the MLML Weather Station and the DENAPP site were plotted against each other. A test of simple linear regression and a Student's t test were used to determine if the light sensor measurements differed from a slope of 1 or had a y-intercept different from 0. The slope of the line of the two daily irradiances was 0.996 ± 0.003 with $r^2 = 0.99$ and a t value of -1.202 at 91 degrees of freedom which resulted in a p value greater than 0.05, therefore the slope did not differ from 1. The y-intercept of the line was -3.330 ± 0.041 and had a t value of 80.665 at 91 degrees of freedom that resulted in a p value less than 0.001, therefore the y-intercept did differ from zero. Daily-integrated irradiances measured by the two sensors were statistically identical for the slope, but differed for the y-intercept which was probably due to differences in sensor calibration. The MLML Weather Station had approximately 8% higher estimate of daily-integrated irradiance than the DENAPP site, but this difference was negligible with reference to photosynthetic requirements and relative to the total

daily irradiance. The number of hours per day that irradiance reached photosynthetically saturating levels (27 μmol photons m^{-2} s^{-1} in seawater) (Zimmerman et al. 1997), H_{sat}, was calculated using the MLML Weather Station data because the DENAPP site PAR data contained gaps.

Shoot Abundance, Growth Rates and Biomass Allocation

All vegetative shoots and flowering shoots (when present) were counted in September 2000 and then each month from February 2001 to February 2002. All abscised leaves and floating dead shoots were removed from the aquaria every three days. Nine shoots in each treatment 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 estimations two weeks prior to measurement using the hole-punch method of Zieman (1974) as modified by 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 digital calipers. Photosynthetic shoot size, or leaf area (cm^2 $shoot^{-1}$), was calculated by summing the area (leaf length x leaf width) of all leaves of the shoot. Absolute growth (cm^2 $shoot^{-1}$ d^{-1}) was calculated as:

[Eq. 1]
$$\text{Absolute Growth} = \frac{\text{new leaf area}}{\text{number of days from hole punch to measure}}$$

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

[Eq. 2] $$\text{Specific Growth} = \frac{\text{Absolute Growth}}{\text{Total Leaf Area}}$$

Biomass allocation among shoots, rhizomes, and roots requires destructive sampling. Consequently, it was measured only three times during the experiment: 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. Biomass (g FW) of the roots, rhizome, and leaves was measured using an analytical balance (0.001g). Lengths of individual internodes along each rhizome (4-18 internodes each) were measured at the end of the experiment to the nearest 0.1 mm using digital calipers. The date of internode creation was calculated assuming an average plastochrone interval of 15 days (Marba and Duarte 1998).

Leaf Sucrose Content

Each month, a segment of leaf #3 (#1 is the youngest leaf) was collected from each of the nine shoots marked for growth. The leaf samples were dried, ground in liquid nitrogen, and sucrose was extracted from the ground tissue three times using hot (80°C) ethanol (Zimmerman et al. 1989). The three 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 (Roe 1934, Huber and Israel 1982).

Statistical Analyses

Tank specific effects were tested during the pre-enrichment period from September 2000 to January 2001. Tank specific effect on eelgrass leaf area, absolute growth, specific growth, leaf sucrose content, and December 2000 biomass allocation was tested using a one-way ANOVA. All results were reported.

Statistical tests for the manipulative portion of the study included linear regression to test the relationship of CO_2(aq) enrichment to eelgrass productivity, and when there was no relationship to enrichment, light treatments were pooled across CO_2(aq) treatment to test for the effect of light and time on eelgrass productivity using a two-way ANOVA. Mean values were calculated for each light and CO_2(aq) enrichment treatment for the productivity variables of biomass allocation, flowering shoot abundance, total shoot abundance, leaf area, growth, and leaf sucrose content. The relationship of CO_2(aq) enrichment to the mean values for biomass allocation, flowering shoot abundance, total shoot abundance, leaf area, growth, and leaf sucrose content was evaluated at each sampling interval using linear regression for the two light treatments separately. A model I linear regression was used to test the effect of the fixed variable, [CO_2(aq)], on each of the productivity variables. The null hypothesis was that CO_2(aq) enrichment would have no relationship to biomass allocation, growth rate, shoot numbers, leaf area, or leaf sucrose content (H_o: slope = 0). Eelgrass productivity was expected to rise across the CO_2(aq) enrichment range and therefore only when slope > 0, significant effects ($p \leq 0.05$) were reported. Because the model used was testing for a rise in productivity across CO_2(aq) enrichment, in those cases where there

was no relationship, data for each light treatment were pooled across CO_2(aq) treatment for between light comparisons over time (Sokal and Rohlf 1981). Leaf area, absolute growth, specific growth, and leaf sugar means were pooled across CO_2(aq) treatment to test for light effects over time using a two-way ANOVA (light x time) and LSD multiple comparison (Zar 1996). The LSD multiple comparison was used in this study because the comparisons were planned comparisons through time (Sokal and Rohlf 1981). Additionally, the effect of light level on biomass allocation to each tissue compartment and internode length was tested using a Student's t test with significant effects ($p \leq 0.05$) reported. Each CO_2(aq) treatment was tested separately for these biomass allocation and internode length t tests. The effect of time, used as a proxy for light availability, on internode length at each [CO_2(aq)] in the light replete treatments was tested using a one-way ANOVA and a least squares difference, LSD, multiple comparison. The response of eelgrass pooled leaf area, absolute growth, and specific growth to H_{sat} was tested for each growth period using linear regression. As with the CO_2(aq) regression tests, the null hypothesis (H_o: slope = 0) was tested using regression ANOVA with significant effects ($p \leq 0.05$) reported when slope > 0.

RESULTS

Environmental Conditions

Daily-integrated irradiance followed a typical sinusoidal pattern across seasons (Fig. 2A). The maximum daily irradiance ranged almost three-fold from winter to

summer. Day-to-day fluctuations in irradiance resulting from weather (fog, clouds) during the summer months were almost as great as the seasonal amplitude. The daily H_{sat} period for the light replete treatment was consistently above the 5-hour minimum light requirement (Zimmerman et al. 1996) for 92% of the days of the study period regardless of season (Fig. 3). Irradiance in the light limited treatment was always below E_k from October to February. In summer (March through September), the minimum H_{sat} of 5 hours was exceeded only 47% of the time, and only 31% of the time for the total duration of the study.

Salinity ranged from 34 ‰ to 37 ‰ throughout the experiment and an average of 35 ‰ was used for the CO_2 solubility equations. Seasonal variation in ambient seawater temperature ranged from 9°C in winter to 17°C in summer (Fig. 2B). At any one time, however, the temperature fluctuation among tanks was less than 1°C throughout the experiment. The [CO_2(aq)] of the control tank averaged 18μM CO_2(aq), with transient excursions ranging from 8.0 to 45.7 μM CO_2(aq). Concentration of CO_2(aq) in the manipulated tanks averaged 35μM, 78μM, and 1235μM CO_2(aq) beginning in February 2001 (Fig. 2C). The data presented here were smoothed to 20 day running averages.

Evaluation of Tank Specific Effects

There were no significant aquarium-specific effects on eelgrass productivity during the non-manipulative four-month period from October 2000 through January 2001 (Table 2). Biomass allocation was the same across all aquaria. The two incidents of an aquarium effect, absolute growth in January 2001 and leaf sugar content in December

2000, occurred only once for each growth measure and in each case it was aquarium number 4 (what would later be the control aquarium) that was different. The specious significant effects noted for these two measures represent approximately 5% of all samples taken and were likely due to Type I error (Zar 1996).

Shoot Size and Biomass Allocation

Total plant biomass of light-replete treatments showed a significant positive relationship to CO_2(aq) enrichment at the end of the experiment (Table 3) (Fig. 4A). This increase resulted exclusively from an increase in biomass allocated to the rhizome as leaf and root biomass were unaffected by CO_2(aq) enrichment. These allocation results yielded a biomass increase of 25% for shoots growing at 37μM CO_2(aq), 50% at 68μM CO_2(aq) and 100% for shoots at 1433μM CO_2(aq). There was no significant effect of CO_2(aq) enrichment on any shoot biomass measurements in the light limited treatment at the termination of the experiment (Fig. 4B). Light availability affected biomass allocation most dramatically in the 1433 μM CO_2(aq) treatment with root and leaf biomass greater in the light replete than light limited treatment (Table 4). Shading decreased leaf biomass allocation in the 15 and 37 μM CO_2(aq) enrichment levels (Table 4).

Internode length was proportional to CO_2(aq) enrichment for some of the light replete shoots, but never for the light limited shoots (Table 3) (Fig. 5). Internodes are the site of sucrose reserve storage and new shoot initiation. Internodes were consistently longest on shoots grown at 1433 μM CO_2(aq) and shortest in shoots grown at 15 μM

CO_2(aq) in the light replete treatments. Internodes produced between September and October 2001, calculated using a 15-day plastochrone interval, ranged from 6.3 to 9.2 mm in the unenriched treatment to a range of 17.3 to 21.7 mm for the 1433 μM CO_2(aq) treatment in the light replete treatments. (Table 3) (Fig 5A). Shoots growing at 1433 μM CO_2(aq) had internode lengths up to 2.7 times longer than shoots growing at normal CO_2(aq) under light replete conditions.

Internodes were affected by time, which was used as a proxy for seasonal light availability, in the 15 and 1433 μM CO_2(aq) enrichment levels. Shoots growing in the control tank July through August 2001 had mean internode lengths of 11.45 ± 1.00 mm, followed by internode lengths of 7.16 ± 0.28 mm for early September 2001 through early January 2002, and then internode lengths that increased to 9.56 ± 1.14mm in late January 2002 (ANOVA results: $F = 4.08$, d.f. = 12, MS = 28.94, $p << 0.001$). Internodes were longest during July and August 2001 at 23.42 ± 0.86mm, followed by those produced between September and mid-November 2001 at 19.22 ± 0.63mm, and shortest as day length declined into the winter months at 14.52 ± 0.42 mm in the 1433 μM CO_2(aq) enrichment level (ANOVA results: $F = 8.53$, d.f. = 12, MS = 138.26, $p << 0.001$). The intermediate CO_2(aq) treatments produced variable internode lengths that were not significantly different from each other or through time ($p > 0.05$). Most internode lengths were longer in the light limited treatments than the light replete treatments at 15 μM CO_2(aq), when there was a significant difference (Table 5). Though internode lengths in the light limited treatments were longer than light replete treatments, biomass remained the same between the two treatments. Internode lengths of shoots in the light

replete treatments were greater in length than the light limited treatments in the 37, 68, and 1433 μM CO_2(aq) enrichment levels.

Calculated annual rhizome extension rates were strongly affected by CO_2(aq) enrichment for shoots at light replete levels. Rates ranged from 23cm yr^{-1} in the 15μM CO_2(aq) treatment to 48cm yr^{-1} in the 1433μM CO_2(aq) treatment (Fig. 6A). Rhizome extension rates did not respond to CO_2(aq) enrichment for shoots growing at light limited levels (Fig. 6B).

Flowering Shoot Production

The production of flowering shoots also responded positively to CO_2(aq) enrichment in the light replete treatment (Table 3, Fig. 7A). Flowering shoots appeared earlier in the year, and matured more quickly as a function of [CO_2(aq)]. Twenty-two percent of the total shoot population differentiated into flowers under 1433 μM CO_2(aq), which was more than twice the flowering output of the other CO_2(aq) enrichment treatments at this light level (Fig. 8). Flowering output was very low for all treatments under light limitation, and there was no significant effect of CO_2(aq) enrichment on flowering output (Table 3, Fig. 7B). No flowering occurred in the light limited, 37μM CO_2(aq) treatment.

Vegetative Shoot Abundance

Shoot abundance responded positively to elevated CO_2(aq) in the light replete treatment (Fig. 9A). All treatments began the study with the same number of shoots in September 2000. Following enrichment with CO_2(aq), shoot numbers in the 37 and 68 μM CO_2(aq) treatments were usually greater than the 15 μM CO_2(aq) (control) shoot numbers. Shoot abundances at 37 and 68 μM CO_2(aq) fluctuated through time but there was no consistent trend of one concentration having greater abundance (Fig. 9A) similar to the trend found with internode length. The 1433μM CO_2(aq) treatment shoot abundance was initially equal to the 68μM CO_2(aq) treatment but declined precipitously during May and June 2001 due to the natural loss of the exhausted flowering shoots. Vegetative shoot abundance in this treatment, however, rebounded via vegetative propagation after the flowering season and increased 75% over the June nadir by the end of the summer and remained higher than the other treatments throughout the duration of the experiment. Shoot abundances in all light replete CO_2(aq) treatments declined through the winter until the termination of the experiment in February 2002. Shoot abundances in the light limited treatment showed no response to [CO_2(aq)] and declined steadily throughout the experiment, with no summer plateau or increase in shoot numbers as in the light replete treatment (Table 2, Fig. 9B).

Shoot abundance was significantly related to CO_2(aq) enrichment in the light replete treatment at the termination of the experiment in February 2002 (Table 3, Fig. 10). Shoot abundance followed a rising linear trend across the [CO_2(aq)] gradient. The 15 μM CO_2(aq) (control) treatment consisted of 21 shoots followed by

increases of 85%, 114%, and then 223% over the control in the 37, 68, and 1433 μM CO_2(aq) enrichment treatments. Shoot numbers in the light limited treatment at the termination of the experiment were very low and showed no elevation in numbers due to enrichment (Fig. 10).

There was no consistent trend of a relationship of CO_2(aq) enrichment to individual shoot leaf area, growth rates, or leaf sugar content in either light treatment. A few instances of a significant difference in individual shoot performance in each CO_2(aq) and light treatment occurred, but this effect was fleeting (Table 3). Aside from these exceptions, no other statistically significant trends were detected for a CO_2(aq) enrichment effect on individual shoot morphometrics or sugar content. Consequently, shoot performance data were pooled across CO_2(aq) enrichment treatment, excluding significant treatments, for determination of light x time effects using two-way ANOVA.

Light Regulation of Eelgrass Productivity

Light availability had a significant effect over time on individual leaf area, shoot growth rate, and leaf sugar content that was independent of the CO_2(aq) treatment (Table 6). However, the differences between light treatments occurred primarily during the extreme light limitation of winter (Fig. 11). During the summer, growth rates and leaf area were not different between the light treatments (Fig. 11). Calculated leaf area, absolute growth, and specific growth values were based on the same leaf width and length measurements and show similar seasonal patterns.

Leaf area (cm^2 $shoot^{-1}$) in the light replete treatment averaged 277 cm^2 $shoot^{-1}$ in March 2001, increased to 299 cm^2 $shoot^{-1}$ in April 2001, then returned to 277 cm^2 $shoot^{-1}$ during the summer and early fall of 2001. Leaf area declined 37% to 174 cm^2 $shoot^{-1}$ in the winter and remained at that level until the end of the experiment (Fig. 11A). In the light limited treatments, leaf area averaged 277 cm^2 $shoot^{-1}$ throughout the spring, summer and early fall of 2001. Leaf area declined by 80% to 53.8 cm^2 $shoot^{-1}$ in the winter and was 61% smaller than the leaf area in the light replete treatments at this time.

Absolute growth rate (cm^2 $shoot^{-1}$ d^{-1}) in the light replete treatments increased 109% from 4.3 to 9.0 cm^2 $shoot^{-1}$ d^{-1} in March 2001 and then declined 10% through the spring to 8.1 cm^2 $shoot^{-1}$ d^{-1} in late May 2001, and then declined 19% to 6.5 cm^2 $shoot^{-1}$ d^{-1} in June 2001 (Fig 11B). Absolute growth increased to 6.7 cm^2 $shoot^{-1}$ d^{-1} in July 2001, but then decreased by 51% through the fall and into the winter. In the light limited treatments, absolute growth initially rose 42% from 4.3 to 6.2 cm^2 $shoot^{-1}$ d^{-1} in March 2001, it remained at this level increasing in late spring to 7.3 cm^2 $shoot^{-1}$ d^{-1}. Absolute growth dropped from 7.3 cm^2 $shoot^{-1}$ d^{-1} rate through the end of summer to 5.7 cm^2 $shoot^{-1}$ d^{-1} and then plunged by 86% to 0.8 cm^2 $shoot^{-1}$ d^{-1} the following winter in the light limited treatment. At the end of the experiment in the winter, absolute growth in the light limited treatment was 2.6 times less than in the light replete treatment. Absolute growth rate followed a seasonal trend of greater rates in the summer and lower in the winter in both light treatments.

Specific growth (% d^{-1}) in the light replete treatment initially rose from 1.8 to 3.1% d^{-1} in March 2001 it then fluctuated between 2.4 and 2.6 % d^{-1} through the summer

and early fall, then declined by 38% to 1.6 % d^{-1} in the winter (Fig. 11C). In the light limited treatment, specific growth fluctuated between 2.2 % d^{-1} and 2.4 % d^{-1} during the summer, declined by 54% through the fall to a rate of 1% d^{-1} in December and then rose by 60% to 1.6 % d^{-1} through the winter. Specific growth rates were the same at each light level at the end of the experiment. Light availability strongly influenced leaf area, absolute growth rate and specific growth rate. The duration of H_{sat} during the growth period was also strongly correlated with leaf area, absolute growth, and specific growth (Table 7).

Leaf sucrose content was greater in the light replete than in the light limited treatment during those times when it differed (Fig. 12). Leaf sucrose content for the shoots growing in the two pooled light treatments was the same for much of the experiment except during three sampling periods: April 2001, July 2001, and January 2002 when sucrose content in the light replete treatment was greater. Leaf sucrose in the light replete treatment in April 2001 was 112 μmol suc. equiv. gFW^{-1} and then declined by 51% to 54 μmol suc. equiv. gFW^{-1} in May 2001. Leaf sucrose remained at this level until July 2001 in the light replete treatments when it increased 2.6-fold to 198 μmol suc. equiv. gFW^{-1}, but then declined to 81 μmol suc. equiv. gFW^{-1} where it remained for the fall and early winter. In January 2002, leaf sucrose in the light replete treatment increased 90% to 154 μmol suc. equiv. gFW^{-1} and then increased again in February 2002 to 236 μmol suc. equiv. gFW^{-1}. Leaf sucrose in the light limited treatment was 54 μmol suc. equiv. gFW^{-1} through the spring with a 1.5-fold increase to 135 μmol suc. equiv.

gFW^{-1} in July 2001. Leaf sucrose decreased to 81 μmol suc. equiv. gFW^{-1} after July 2001 where it remained through the end of the experiment in the light limited treatment.

DISCUSSION

Eelgrass shoots growing in light replete conditions and CO_2(aq) enrichment in this study produced larger rhizomes and more vegetative and flowering shoots, but showed little to no change in individual shoot growth rates or leaf sugar content. These CO_2(aq)-stimulated increases in shoot propagation and flowering may increase reproductive success and area-specific productivity, but not the growth or productivity of individual shoots. Thus, through increased seed production and dispersal, seagrass populations may increase in density and distribution and expand habitat range as seawater CO_2(aq) concentrations climb in the next century-- as long as light availability is not limiting. This population-level response to CO_2 enrichment is similar to that observed for terrestrial species, including *Pinus* spp. (DeLucia et al. 1999; LaDeau and Clark 2001; Woodward 2002). The mechanism of producing greater sucrose reserves in the rhizome and new shoots when exposed to abundant carbon, ultimately promoted the survival of the eelgrass clone (Chu 1992; Farrar 1992).

CO_2(aq) enrichment increases eelgrass productivity when light is not limiting, but not at light starvation as originally hypothesized. Light limited shoots never increased in abundance and flowering was only 0-4% of the entire population at any of the CO_2(aq) enrichment levels. Conversely, flowering in natural populations is approximately 10% of the total in light replete conditions (Hemminga and Duarte 2000). Lack of flowering in

the light limited treatments in this study or in natural populations may be an indication of light stress, and is an example of a population scale warning that the local seagrass population may be in distress.

An index of seagrass health is needed to monitor seagrass population survival in low light environments or in other resource limiting or abundant conditions. Previous work on seagrass response to carbon enrichment or light limitation has focused primarily on individual shoot responses (Durako 1993, Morris and Tomasko 1993; Lee and Dunton 1997; Zimmerman et al. 1995; Zimmerman et al. 1996; Zimmerman et al. 1997). In this study when there was a difference between light treatments, individual shoot growth rates and leaf area were greater at the light replete level. However, growth rates and leaf area were often the same in each light treatment throughout the year despite the steady decline in shoot abundance and lack of flowering in the light limited treatments. Only during the short day-lengths of winter were growth rate or leaf area in the two light treatments different. Growth rates are generally used to compare seagrass "health" in light replete and light limited populations in the field. As evidenced here, these productivity parameters may be insufficient to herald the decline of a population before it is too late.

Measuring changes in population structure in concert with particular individual responses may be more appropriate to assess if a seagrass population is in decline. Eelgrasses respond to abundant light and CO_2(aq) by producing larger rhizomes, longer internodes, and more vegetative and flowering shoots. An index of seagrass survival could be developed using these productivity parameters. However, these productivity measurements could appear contradictory. Internode lengths were longer at light limited

levels than at light replete levels in the control CO_2(aq) treatment. Longer internodes in the low light treatment may have been a mechanism to increase the distance between shoots to alleviate the stress of self-shading (Hemminga and Duarte 2000, Marba and Duarte 1998). Therefore, seagrass internode length response to light limitation may be misleading and a more intimate knowledge of particular seagrass populations may be required in order to develop an index of seagrass health and survival. Ultimately, water quality is the best index for seagrass survival because it is light availability that regulates seagrass productivity.

As atmospheric CO_2 rises, eelgrass and other CO_2(aq) limited seagrass species (Invers et al. 2001) can be expected to increase in density and distribution. This population scale change in productivity may dramatically alter coastal habitats as seagrass range increases. Atmospheric [CO_2] is expected to triple over 1992 levels by 2100 (Keeling 1997). In this study, eelgrass shoot abundance doubled in the intermediate CO_2(aq) treatments that correspond to seawater concentration in the year 2100. Therefore, as [CO_2(aq)] increases to tripling by 2100, eelgrass density may double in coastal waters. The resulting greater leaf density in coastal waters could trap more sediment in the water column (Koch 1994), improving water clarity. Enrichment, therefore, may cause a positive feedback in these coastal waters, improving water column light penetration and increasing potential new seagrass habitat. However, anticipated development pressure in the coastal zone expected with rising human population would likely reduce water clarity to below seagrass light requirements, destroying any advantage that CO_2(aq) enrichment may have.

Deliberate injection of CO_2 to seawater as a method to restore eelgrass populations may improve the survival rates of recently transplanted eelgrass shoots. Although CO_2(aq) enrichment does not offset light starvation, it can buffer the negative effects of transplant shock by increasing rhizome reserve capacity, which may increase the chances of surviving turbidity events. However, CO_2 injection into seagrass beds as a restoration method requires further study. If implemented, it must be in light replete conditions in order to promote enhancement. Also, managers must determine if seagrass restoration using short term "boosts" of CO_2(aq) enrichment is economically feasible and not harmful to other seagrass meadow occupants.

If seagrass habitat increases due to rising CO_2 in the next century or through deliberate CO_2 injection into seawater, high ambient CO_2(aq) concentrations may affect seagrass meadow inhabitants as well seagrasses themselves. Seagrass beds provide vertical habitat for fish and invertebrate species and are occupied by 42% more species than adjacent bare sand (Hemminga and Duarte 2000). Many of these species are juveniles that seek refuge among the shoots. As seawater [CO_2(aq)] increases, with an attendant rise in eelgrass density and distribution, fish and invertebrate stocks may be enhanced as well (Duarte 2002). CO_2 increases, however, may not positively affect all organisms. Carbonate saturation state will decline as seawater CO_2(aq) rises (Zeebe and Wolf-Gladrow 2001), potentially stressing shell-forming organisms, such as mollusks, corals, and formanifera (Kleypas et al. 1999). Rising CO_2(aq) concentrations may also stimulate nuisance algae blooms such as *Ulva* sp. and *Enteromorpha* sp. 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, competitively excluding eelgrass populations. Further studies are needed to determine the deleterious effects of CO_2(aq) enrichment on other eelgrass meadow organisms.

Changing atmospheric CO_2 concentrations may already be providing an advantage to the survival of CO_2(aq) limited seagrasses, particularly since the 1970's when over half of total global CO_2 emissions since the beginning of the industrial revolution have occurred (Marland et al. 2002). Human activity emits 6.4 Gt C yr^{-1} (Marland et al. 2002), of which only half is absorbed into the ocean through chemical reactions with seawater (Keeling 1997) or photosynthetically fixed by terrestrial and aquatic macrophytes (Smith 1981). Atmospheric [CO_2] continues to rise because of this imbalance of emissions and absorption. Seagrass productivity may be enhanced by the rise in atmospheric [CO_2], and may remove excess CO_2 from the atmosphere through carbon fixation. Globally, seagrasses photosynthetically fix an estimated 0.6 Gt C yr^{-1}, of which 0.16 Gt C yr^{-1} is buried and removed from the global carbon cycle (Hemminga and Duarte 2000). As [CO_2(aq)] triples over the next century, seagrass density could double, increasing global seagrass sink capacity to 0.32 Gt C yr^{-1}, or approximately 4.5% of total annual CO_2 emissions.

If CO_2 stimulation increases colonization potential in seagrasses via greater flower output, seagrass habitat may expand, further increasing the sink capability of this taxonomic group as well locally improving water clarity. However, development of the coastal zone is expected to co-occur with rising human population and atmospheric CO_2 resulting in poorer water clarity and available seagrass colonization space. Any positive

effect of CO_2(aq) stimulation will therefore be overwhelmed by light starvation. CO_2 stimulation of eelgrass productivity due to climate change or as a restoration method will only enhance eelgrass survival if humans improve water quality in the coastal zone.

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Table 1. Equilibrium distribution of dissolved inorganic carbon in seawater under the treatment conditions 15, 37, 68, and 1433 μM CO_2(aq) based on dissociation constants of Hansson (1973) and the CO_2 – solubility equations of Weiss and Price (1980). Calculation assumptions: salinity=35 ‰; alkalinity = 2500 microequivalents kg^{-1}; and temperature = 15° C.

Parameter	**Un-enriched**	**Enriched Seawater**		
	Seawater (normal)	3x Atmospheric	6x Atmospheric	Photosynthetically Saturating
pH	8.1	7.75	7.5	6.2
Dissolved $[CO_2(aq)]$ (μM)	14.6	36.6	68.0	1433.4
$[HCO_3^-]$ (μM)	2020.8	2260.8	2359.7	2493.2
$[CO_3^{2-}]$ (μM)	198.9	99.4	58.3	3.1
Total $[CO_2]$ (μM)	2234.3	2396.8	2486.0	3929.7

Table 2. Mean, standard error, and one-way ANOVA results for the effects of aquarium on leaf area, absolute growth, specific growth, leaf sugar content, and biomass allocation (December 2000) prior to CO_2(aq) enrichment.

Effect	Mean (S.E.) Aquarium 1	Aquarium 2	Aquarium 3	Aquarium 4	df	MS	F	P
Leaf Area (cm^2)								
Dec-00	220.9 (21.5)	275.6 (21.3)	243.7 (25.9)	249.6 (24.3)	3	9071.97	0.92	0.43
Jan-00	272.1 (20.0)	236.2 (26.4)	281.6 (27.7)	290.9 (33.5)	3	10168.44	0.78	0.51
Absolute Growth ($cm^2\ d^{-1}$)								
Dec-00	31.6 (3.1)	34.4 (2.7)	30.5 (3.3)	27.7 (2.7)	3	138.90	0.90	0.45
Jan-00	29.2 (2.4)	18.8 (1.9)	20.1 (1.9)	17.1 (1.9)	3	473.28	6.46	0.00
Specific Growth (% d^{-1})								
Oct -00	2.6 (0.1)	2.5 (0.2)	2.3 (0.1)	2.7 (0.1)	3	0.22	1.40	0.26
Nov-00	1.4 (0.04)	1.4 (0.1)	1.6 (0.1)	1.3 (0.1)	3	0.09	1.28	0.30
Dec-00	2.2 (0.1)	2.1 (0.1)	2.5 (0.2)	2.1 (0.2)	3	0.43	1.19	0.32
Jan-01	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 (□mol sucrose equiv. gFW^{-1})								
Oct -00	101.1 (5.2)	145.8 (19.6)	91.4 (13.8)	118.8 (35.9)	3	3419.40	1.20	0.33
Nov-00	40.8 (2.4)	73.1 (15.9)	65.3 (29.9)	44.6 (3.8)	3	1500.07	0.85	0.48
Dec-00	37.3 (3.9)	74.4 (10.3)	92.1 (7.0)	82.6 (6.1)	3	9955.66	10.61	0.00
Biomass Allocation (g FW)								
leaves	12.5 (1.4)	15.8 (2.1)	17.6 (5.4)	16.1 (1.5)	3	37.90	0.28	0.84
roots	2.1 (0.4)	2.4 (0.4)	1.6 (0.3)	1.5 (0.2)	3	1.77	2.14	0.11
rhiz	6.3 (0.9)	7.5 (0.9)	5.9 (0.9)	5.9 (1.0)	3	4.53	0.50	0.68
total	20.9 (2.4)	25.7 (2.9)	25.2 (6.2)	23.4 (2.7)	3	37.18	0.19	0.90

Table 3. Results of simple linear regression with one-way ANOVA for the effect of CO_2 (aq) (15, 37, 68, and 1433 μM) on the variables; specific growth, leaf area, flowering, and shoot abundance (only significant effects shown) at light replete and light limited treatments. Equation: y = slope x log[CO_2(aq)] + y-intercept

Variable	Date	Slope	y-intercept	r^2	MS	d.f.	F	P
Light Replete								
Leaf Area								
	none							
Absolute Growth								
	5/29/01	1.03	5.00	0.93	2.61	2	25.05	0.04
Specific Growth								
	none							
Leaf Sugar								
	3/10/01	61.01	-27.30	0.91	9037.57	2	19.65	0.05
	10/12/01	40.05	12.80	0.99	3894.40	2	155.04	<0.01
Flowering								
	5/29/01	6.35	1.56	0.94	98.11	2	34.09	0.02
Shoot Abundance								
	12/7/01	39.93	-10.60	0.95	3872.09	2	40.20	0.02
	12/21/01	39.28	-9.36	0.94	3746.74	2	32.58	0.03
	1/7/02	37.26	-17.03	0.99	3371.50	2	136.92	<0.01
	1/24/02	20.98	7.40	0.94	1068.60	2	31.36	0.03
	2/1/02	21.19	2.98	0.97	1091.03	2	57.85	0.02
Biomass Allocation: 2/2/02								
	rhizome	1.48	0.67	0.97	5.33	2	87.76	0.01
	total	2.43	5.26	0.92	14.37	2	22.21	0.04
Internode Length								
	9/1/01	6.41	1.13	0.97	90.96	2	76.10	0.01
	9/16/01	6.00	1.34	0.99	79.76	2	277.64	<0.01

10/1/01	5.83	0.86	0.98	75.24	2	82.82	0.01
10/16/01	5.88	0.18	0.99	76.55	2	160.16	<0.01
11/1/01	5.31	0.76	0.98	62.51	2	103.89	<0.01
Annual Internode Extension Rate							
	13.22	7.67	0.96	363.66	2	49.67	0.02
Light Limited							
Leaf Area							
3/10/01	41.72	190.37	0.92	4226.60	2	23.75	0.04
12/7/01	54.67	-9.48	0.94	7257.93	2	32.37	0.03
Absolute Growth							
3/10/01	1.99	2.51	0.97	8.31	2	65.50	0.02
7/25/01	1.69	2.49	0.92	5.98	2	23.86	0.04
1/7/02	0.13	0.49	0.94	0.04	2	30.85	0.03
Specific Growth							
2/23/01	0.10	1.52	0.97	0.03	2	68.57	0.01
3/10/01	0.23	1.81	0.90	0.13	2	18.68	0.05
Leaf Sugar							
3/10/01	26.87	2.22	0.97	1753.18	2	56.97	0.02
Flowering							
none							
Shoot Abundance							
none							
Biomass Allocation							
none							
Internode Length							
none							
Annual Internode Extension Rate							
none							

Table 4. Results of a Student's t-test analysis of the impact of light level on biomass allocation to tissue compartments (types): leaf, rhizome, root. Means (±SD) are presented by CO_2(aq) enrichment level. Only significant results shown ($p<0.05$).

[CO_2(aq)]	Tissue Type	Mean light limited	Mean light replete	t	d.f.	P
15 μM	Leaf	1.30 (0.54)	4.38 (2.79)	-2.40	11	0.035
37 μM	Leaf	0.68 (0.18)	5.16 (3.02)	-3.25	12	0.007
68 μM	none					
1433 μM	Leaf	6.14 (3.18)	12.60 (4.36)	-3.21	16	0.006
	Rhizome	2.09 (1.04)	5.94 (2.30)	-3.86	16	0.001

Table 5. Results of a Student's t-test analysis of the impact of light level on eelgrass internode length. Means (±SD) are presented by CO_2(aq) enrichment level. Only significant results shown ($p<0.05$).

[CO_2(aq)]	Internode No.	Date	Mean light limited	Mean light replete	t	d.f.	P
15 μM	1	01/16/02	5.29 (2.21)	9.56 (3.62)	-2.60	14	0.021
	5	11/16/01	9.90 (1.57)	5.81 (2.49)	3.52	12	0.004
	6	11/01/01	11.46 (1.94)	6.26 (2.60)	4.03	11	0.002
	7	10/16/01	12.49 (2.12)	6.62 (1.35)	6.04	11	0.000
	8	10/01/01	14.23 (2.62)	7.93 (1.97)	5.05	11	0.000
	10	09/01/01	14.92 (3.07)	8.37 (2.16)	4.36	10	0.001
	11	08/16/01	15.15 (2.14)	9.17 (1.81)	5.04	9	0.001
	12	08/01/01	17.42 (1.89)	11.84 (3.61)	3.06	8	0.016
37 μM	1	01/16/02	4.32 (1.13)	12.80 (7.30)	-2.79	15	0.014
	3	12/16/01	3.98 (1.10)	11.38 (6.95)	-2.55	15	0.022
	4	12/01/01	4.23 (0.99)	11.85 (7.52)	-2.43	15	0.028
68 μM	1	01/16/02	3.83 (0.86)	11.13 (5.13)	-3.11	15	0.007
	2	01/01/02	4.47 (0.87)	10.15 (4.93)	-2.51	15	0.024
	7	10/16/01	6.35 (0.86)	10.59 (4.24)	-2.17	12	0.051
1433 μM	1	01/16/02	7.99 (3.68)	15.17 (3.94)	-3.97	18	0.001
	13	07/16/01	11.20 (0.00)	25.64 (4.23)	-3.12	4	0.036

Table 6. Two-way ANOVA results for the effects of time and light treatment on specific growth, absolute growth, leaf area, and leaf sugar content in both light treatments.

Effect	df	MS	F	P
Leaf Area				
Time	9	41438.05	12.18	<0.001
Light	1	12639.50	3.72	0.058
Time x Light	9	6843.83	2.01	0.053
Within	60	3400.82		
Absolute growth				
Time	7	41.90	26.85	<0.001
Light	1	35.52	22.76	<0.001
Time x 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 x Light	9	0.10	0.90	0.535
Within	60	0.12		
Leaf sugar				
Time	9	20345.05	16.41	<0.001
Light	1	21078.69	16.99	<0.001
Time x Light	9	3404.28	2.75	0.009
Within	60	1239.98		

Table 7. Results of simple linear regression with one-way ANOVA for the effect of duration of saturating irradiance (H_{sat}, # of hours d^{-1}) on the variables; pooled leaf area, pooled absolute growth, and pooled specific growth.

Variable	slope	y-intercept	r^2	d.f.	MS	F	P
Leaf Area	11.95	160.55	0.20	86	136225.4	20.95	<0.01
Absolute Growth	0.49	1.95	0.35	78	195.07	42.03	<0.01
Specific Growth	0.11	1.41	0.41	86	12.38	59.36	<0.01
Leaf Sugar	no relationship to H_{sat}						

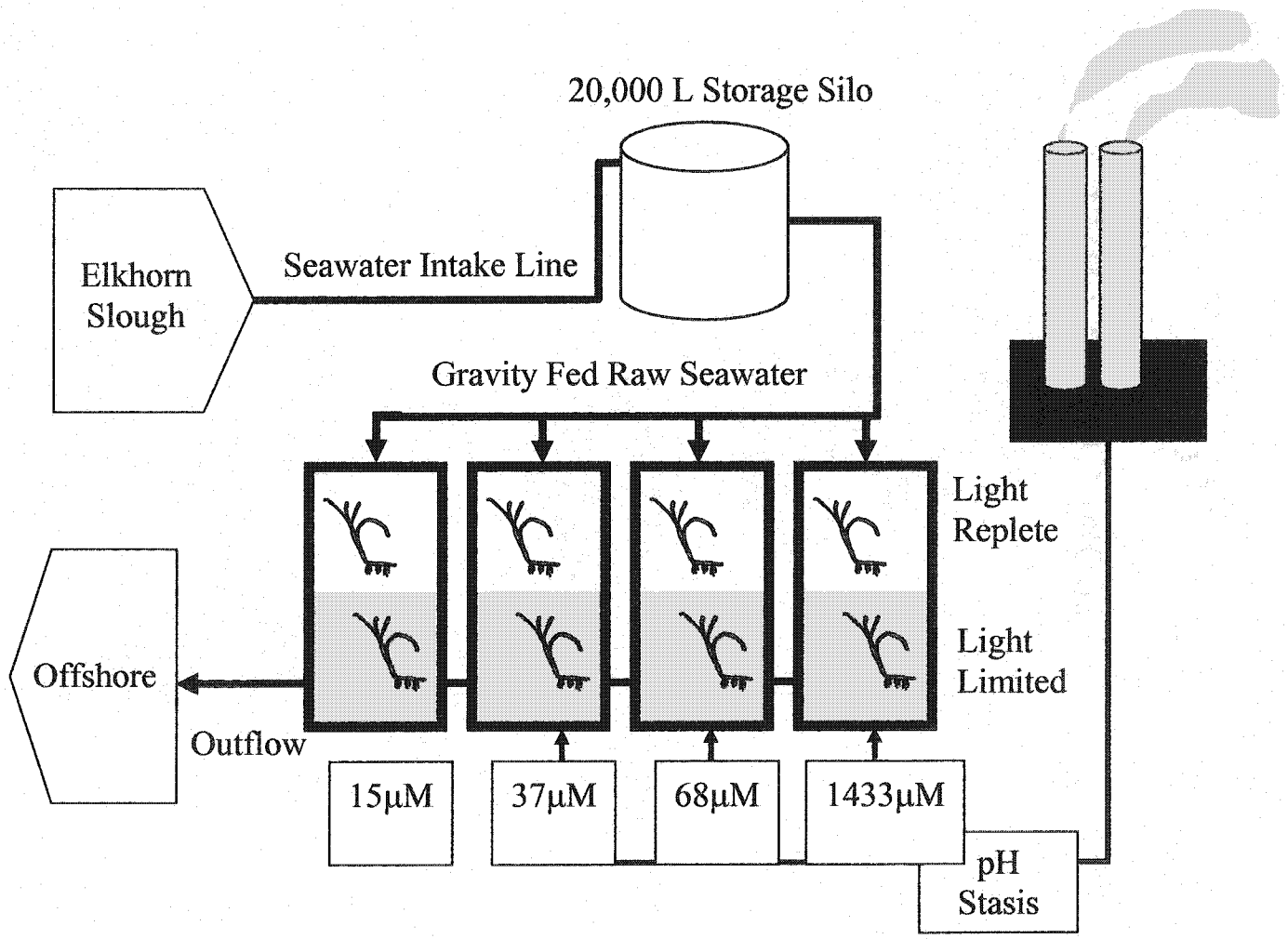

Figure 1. Schematic diagram illustrating the flow through seawater system, CO_2(aq)-enrichment incubation chambers constructed at the Duke Energy North America, Moss Landing Power Plant.

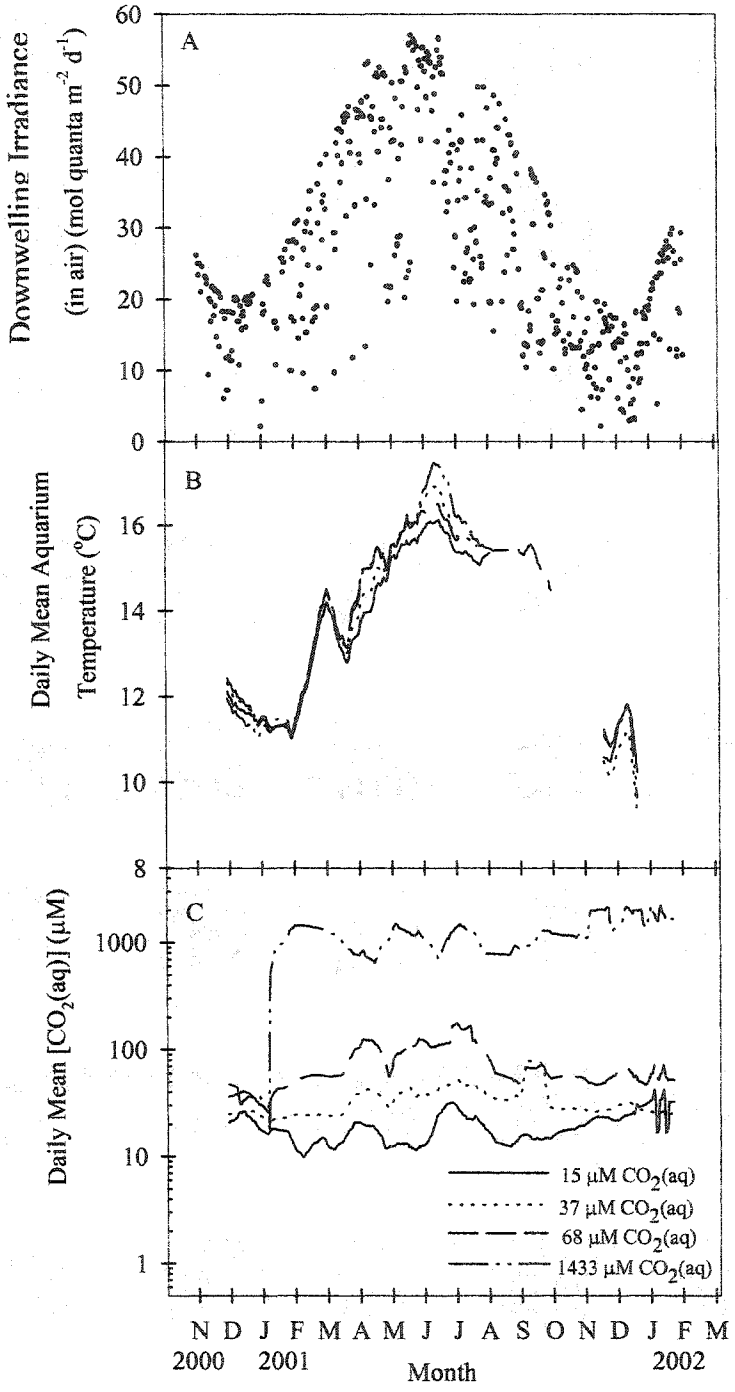

Figure 2. Environmental conditions during the study period (A) Daily integrated downwelling irradiance (PAR), in air; (B) calculated CO_2(aq) concentration in each tank based on pH, temperature, salinity, and alkalinity; and (C) water temperature in each tank. The gap from October 2001 – November 2001 was due to equipment failure.

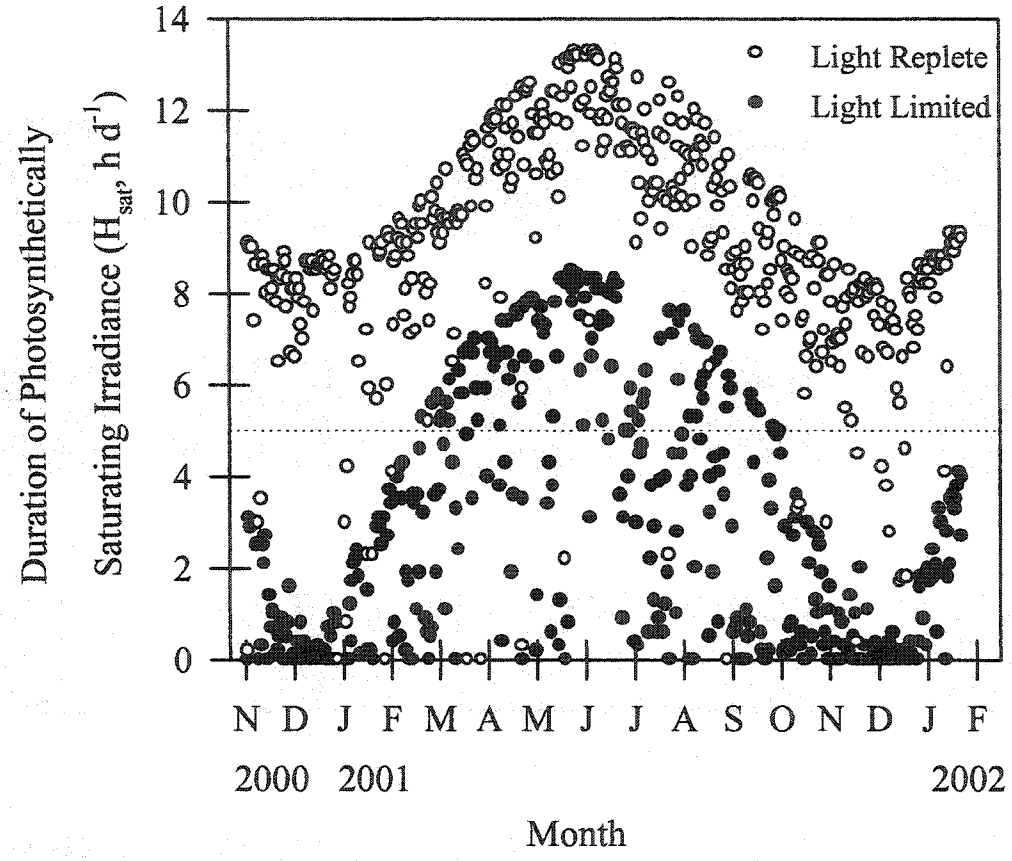

Figure 3. Daily period of irradiance saturated photosynthesis for the light replete and light limited treatments in normal seawater calculated from in-air light measurements from Moss Landing Marine Laboratories weather station.

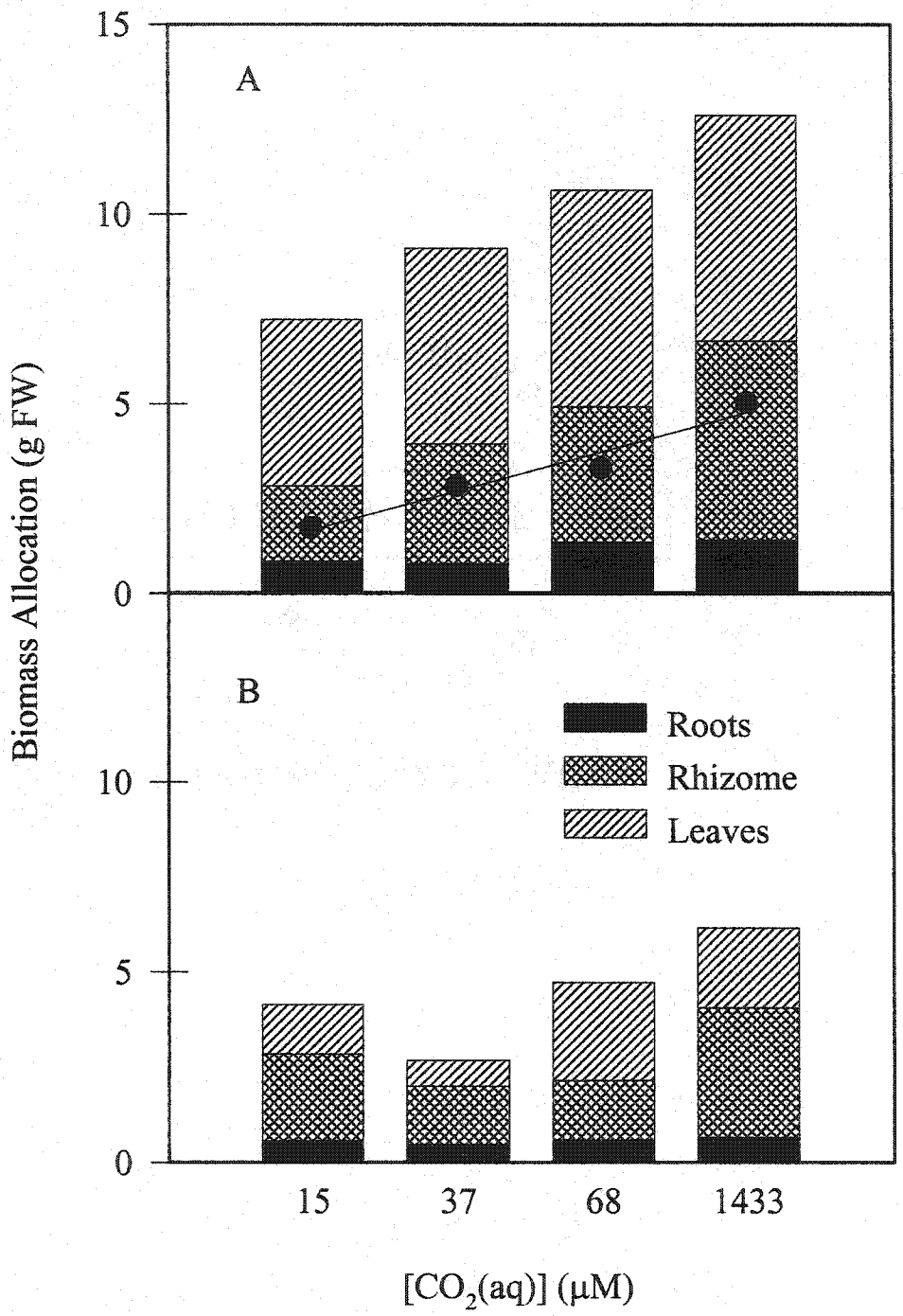

Figure 4. Biomass allocation among roots, rhizomes and leaves after one year plotted as a function of CO_2(aq) enrichment. Enrichment increased allocation to the rhizome in the light replete treatment (A) but not in the light limited treatment (B).

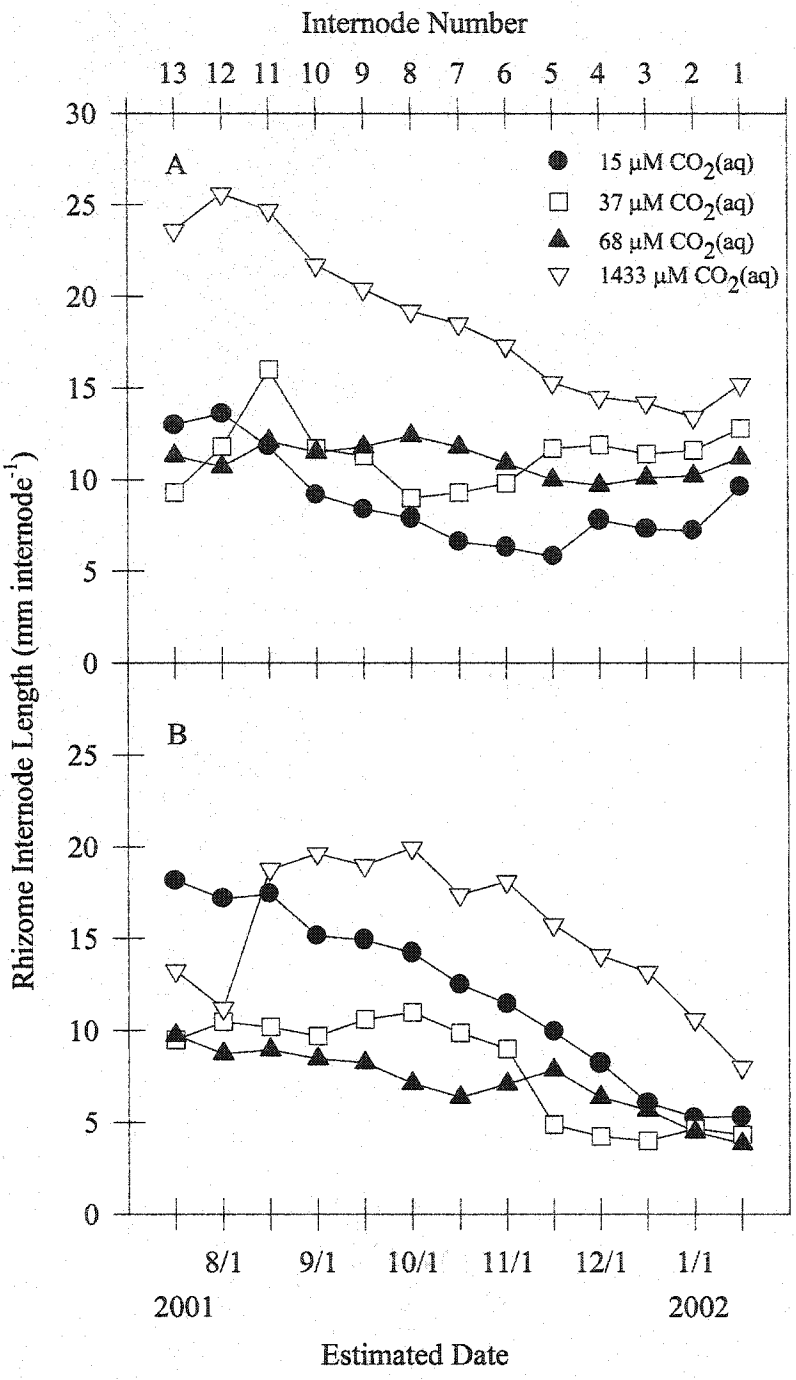

Figure 5. Average internode length plotted as a function of internode number, which increased away from the meristem. The date of internode formation was calculated using a 15-day plastochrone interval. Error bars represent ± 1 S.E. (A) light replete condition. (B) light limited condition.

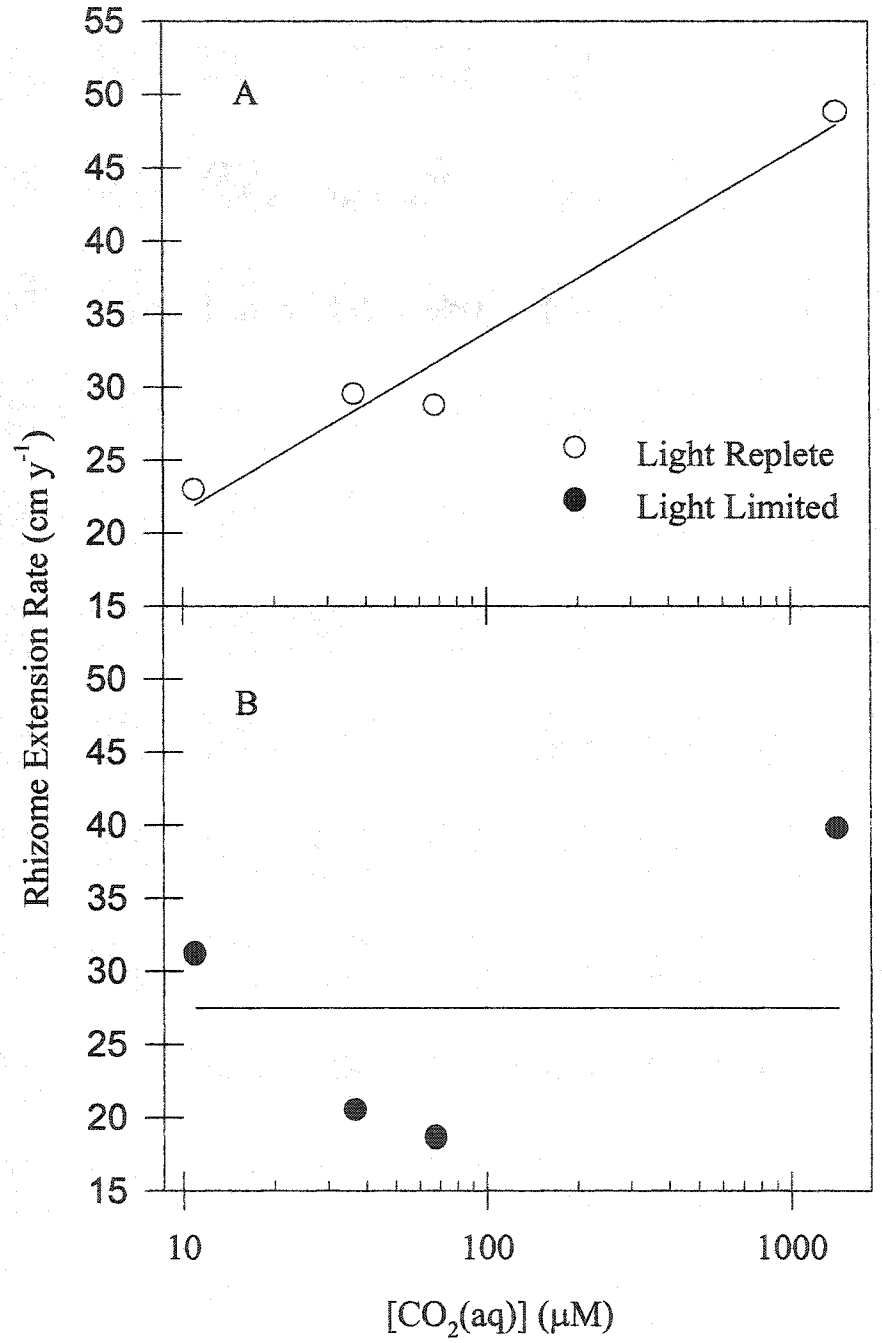

Figure 6. Calculated rhizome extension rate for (A) light replete and (B) light limited treatments plotted through time for each CO_2(aq) enrichment treatment.

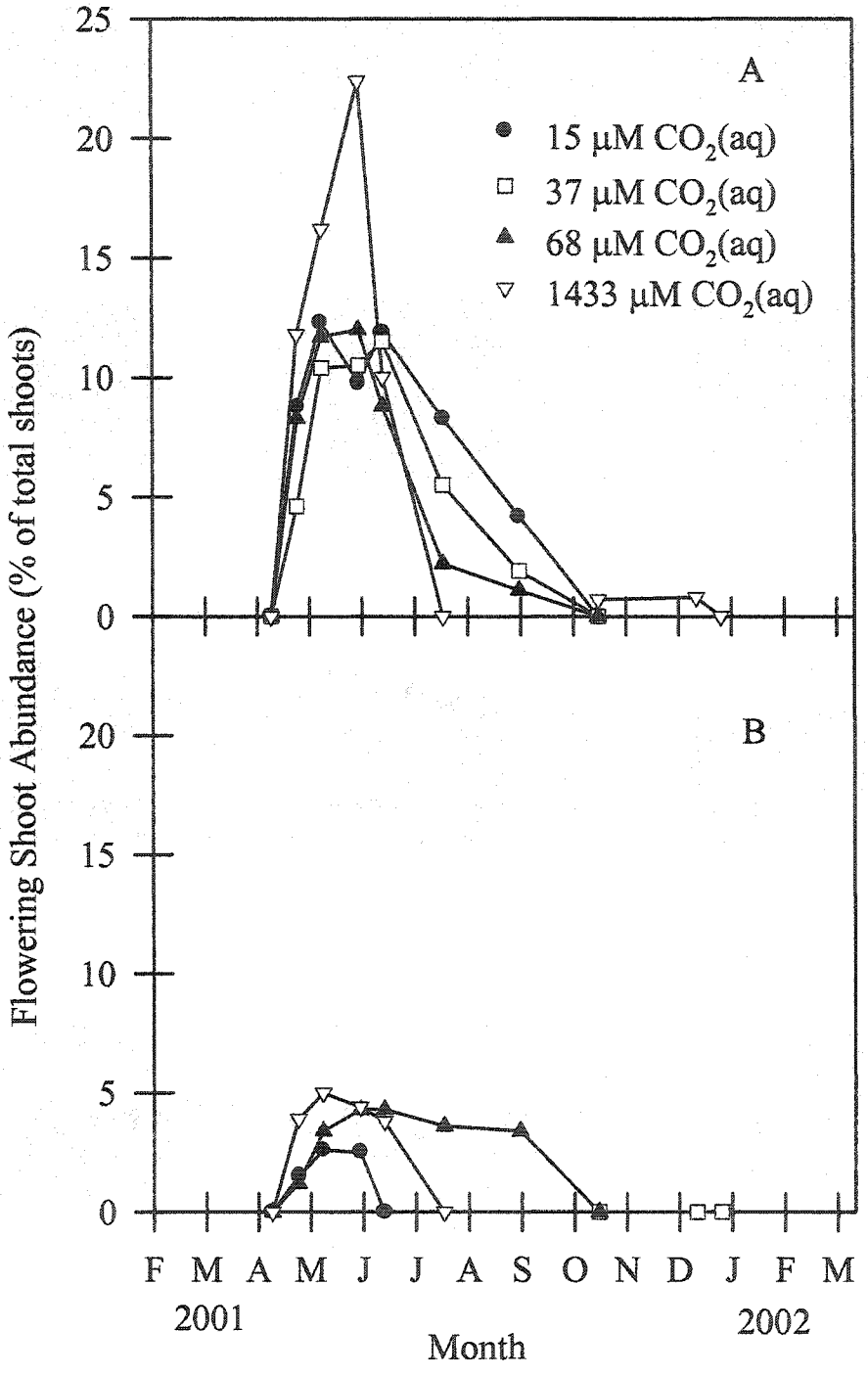

Figure 7. Flowering shoot abundance over time at (A) light replete and (B) light limited treatments for each CO_2(aq) treatment.

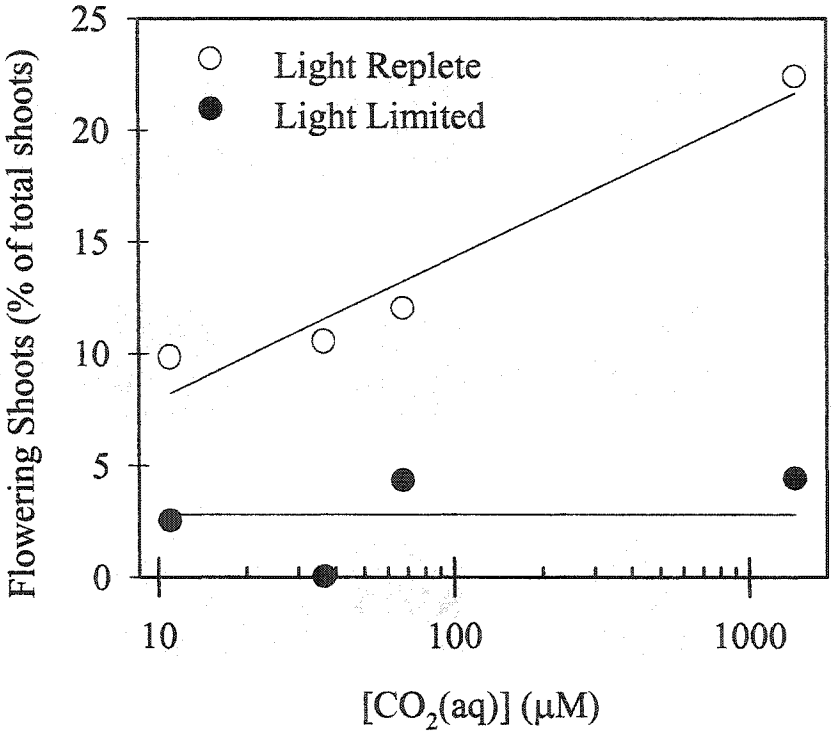

Figure 8. Flowering shoot abundance at peak flowering (May 2001) for light replete and light limited treatments plotted as a function of CO_2(aq) enrichment.

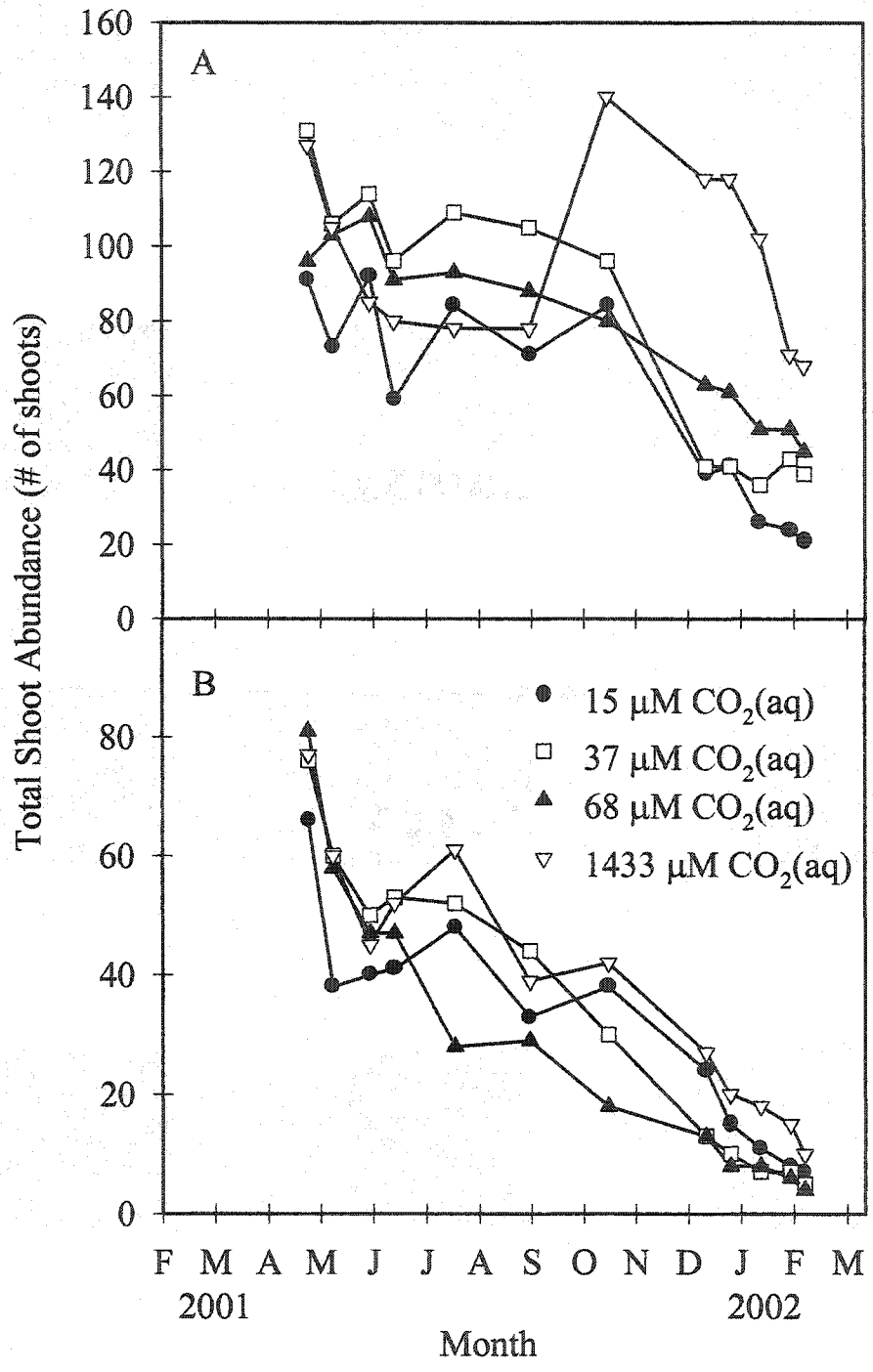

Figure 9. Shoot abundance over time at (A) light replete and (B) light limited treatments for each CO_2(aq) treatment.

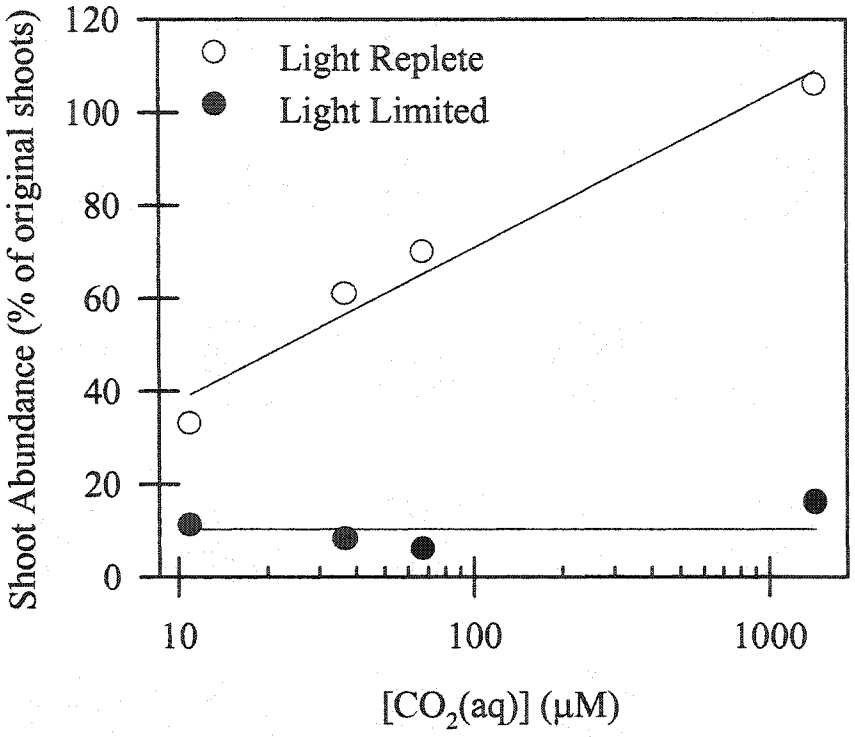

Figure 10. Shoot abundance at the termination of the experiment, February 2002, at light replete and light limited treatments across the CO_2(aq) enrichment range.

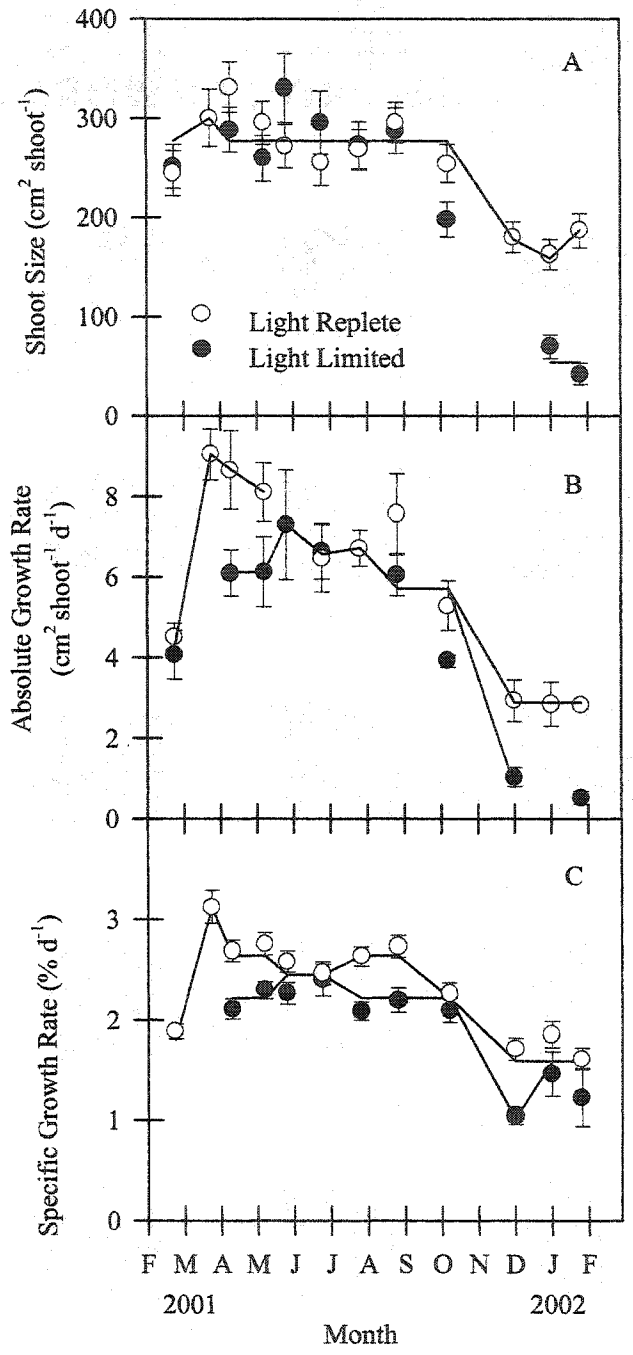

Figure 11. Average (A) leaf area (cm^2) (B) absolute growth (cm^2 d^{-1}), and (C) specific growth (% d^{-1}) over time for CO_2(aq) treatments pooled into light replete and light limited groups. Lines represent significant means and error bars represent ± 1 S.E.

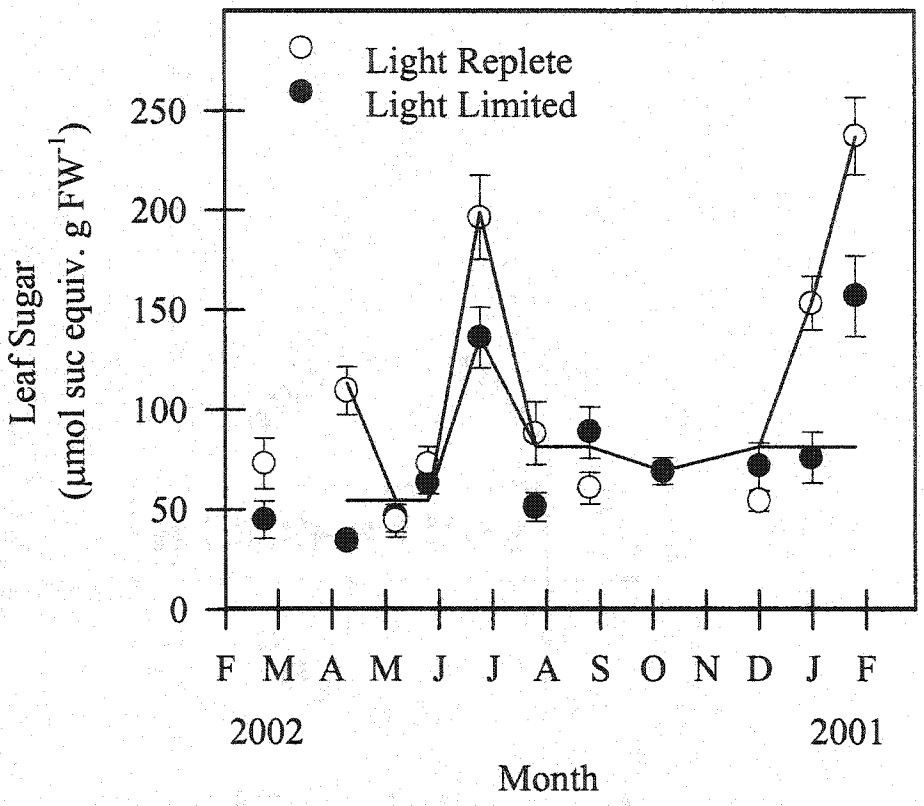

Figure 12. Average leaf sugar (µmol sucrose equivalents g FW^{-1}) over time CO_2(aq) treatments pooled into light replete and light limited groups. Lines represent significant means and error bars represent ± 1 S.E.