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Resource allocation and sucrose mobilization in light-limited eelgrass *Zostera marina*

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ABSTRACT: This study evaluated the ability of *Zostera marina* L. (eelgrass) to balance the daily photosynthetic deficit by mobilization of carbon reserves stored in below-ground tissues during a period of extreme winter light limitation. A quantitative understanding of the mobilization process and its limitations is essential to the development of robust models predicting minimum light levels required to maintain healthy seagrass populations. Plants were grown in running seawater tanks under 2 light regimes. One treatment was provided with 2 h irradiance-saturated photosynthesis (H_{sat}) to produce severe light limitation, while control plants were grown under 7 h H_{sat}, simulating the typical wintertime condition in Monterey Bay, California, USA. Although plants maintained under 2 h H_{sat} were more severely carbon limited than plants grown under 7 h H_{sat}, whole-plant carbon balance calculated from metabolic needs and growth rates was negative for both H_{sat} treatments. The eelgrass studied here responded to negative carbon balances by suppressing the production of new roots, depleting sucrose reserves, and effecting a gradual decrease in growth rate and an increase in the activity of sucrose synthase (SS, E.C. 2.4.1.13) in sink tissues in the terminal stages of carbon stress. The 7 h H_{sat} plants survived the 45 d course of the experiment while the plants grown under 2 h H_{sat} died within 30 d, even though one-third of their carbon reserves remained immobilized in the rhizome. Thus, extreme light limitation can prevent full mobilization of carbon reserves stored in below-ground tissues, probably through the effects of anoxia on translocation. Metabolic rates, particularly photosynthesis and respiration of the shoot, were unaffected by prolonged carbon limitation in both treatments. The patterns observed here can provide useful indices for assessing the state and fate of seagrass ecosystems in advance of catastrophic declines.

KEY WORDS: Seagrass · Carbon balance · Resource allocation · Photosynthesis · Light

INTRODUCTION

Resource limitation impacts the resulting growth form and pattern of resource allocation to above- and below-ground tissues of all plants. Although nutrient limitation frequently results in the proliferation of roots at the expense of above-ground growth, light or carbon limitation mobilizes stored reserves to support shoot or leaf proliferation at the expense of below-ground growth (Pierson et al. 1990, Perez et al. 1994, Sims & Pearcey 1994, Canham et al. 1996, Zimmerman et al. 1996, 1997, Clabby & Osborne 1997). Among marine macrophytes, seagrasses (marine angiosperms) are particularly vulnerable to light limitation, especially in temperate and subpolar waters (Backman & Barilotti 1976, Dennison & Alberte 1982, 1985, 1986, Pirc 1989, Tomasko & Dawes 1989, Duarte 1991, Zimmerman et al. 1991, Dunton & Tomasko 1994, Zimmerman et al. 1995b, Moore et al. 1997). Healthy eelgrass requires 5 to 6 h of irradiance-saturated photosynthesis (H_{sat}) each day to maintain positive carbon balance and vigorous growth (Zimmerman et al. 1995b, 1996, Zimmerman & Mobley 1997), although external factors, including leaf grazing from the commensal limpet *Tectura depicta* (Berry), can significantly increase that

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light requirement (Zimmerman et al. 1996). Given that winter light levels in temperate latitudes may hover near or below the 6 h H_{sat} threshold during winter months (McRoy 1969, Carruthers & Walker 1997), seagrass survival may depend on the utilization of carbon reserves accumulated during summer. The effects of light limitation on resource allocation and reserve mobilization in seagrasses, however, are poorly understood.

Periods of light-limited photosynthesis may change carbon allocation strategies which can significantly impact eelgrass growth and survival. Roots are critical for nutrient acquisition and stabilization of seagrass shoots within unconsolidated sediments (Harlin & Throne-Miller 1981, Iizumi & Hattori 1982, Zimmerman et al. 1987), but the maintenance of healthy roots in permanently flooded anoxic sediments depends on photosynthetically derived oxygen for daytime aerobiosis and sufficient reserves of reduced carbon to support anaerobic metabolism at night when translocation is blocked by anoxia (Smith 1989, Zimmerman & Alberte 1996). Winter is a period of maximum turbidity in water columns of temperate estuaries and nearshore environs. Storm-driven sediment loading combines with short daylengths to generate periods of extreme light limitation which prevent translocation of reduced carbon from eelgrass leaves to the roots and rhizomes and may prevent the mobilization of reserves accumulated in below-ground tissues during the summer period of high light availability (Pennock & Sharp 1994, Zimmerman et al. 1994, 1995b, Hillman et al. 1995, Zimmerman & Alberte 1996, Fetweis et al. 1998).

The objective of this study was to evaluate the ability of *Zostera marina* L. (eelgrass) to balance the daily photosynthetic deficit by mobilizing carbon reserves stored in below-ground tissues during a period of extreme winter light limitation. A quantitative understanding of the mobilization process and its limitations is essential to the development of robust models predicting minimum light levels required to maintain healthy seagrass populations, as reliance on simple carbon balance arguments may seriously overestimate the potential for seagrasses to survive periods of extreme light limitation, particularly in temporally variable habitats.

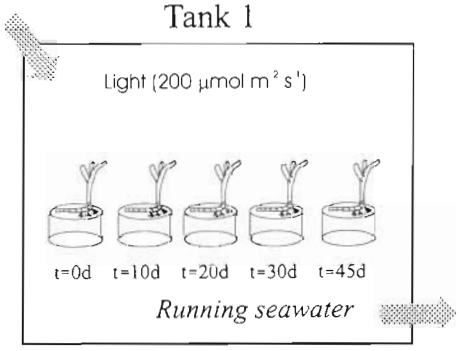

Fig. 1. Experimental design of 1 tank with running seawater and the 5 cores for each sampling time. There were 5 tanks per treatment (5 replicates) and 2 treatments (2 h and 7 h)

METHODS

Experimental design. In total, 50 *Zostera marina* plants growing at the deep edge (10 m depth) of an eelgrass meadow near Del Monte Beach, Monterey Bay, California (36°30'40" N, 121°52'30" W) were collected with SCUBA in late November 1993 using the rhizosphere core method (Dennison & Alberte 1982) and transported to the laboratory within 2 h of collection. Each core contained an intact single shoot with 10 to 12 rhizome internodes and associated roots with intact sediment. The leaf grazing limpet *Tectura depicta* was present on all plants collected, and their numbers were not otherwise manipulated as part of this experiment. Five separate cores were placed into each of the ten 150 l tanks plumbed with running seawater flowing at 3 turnovers h^{-1} at a temperature of 12°C (Fig. 1). Illumination was provided from above by timer-activated 300 W quartz-halogen lamps producing 200 µmol quanta m^{-2} s^{-1} of photosynthetically active radiation (PAR) to the bottom of each tank, well above the photosynthesis saturating irradiance (E_k) of 30 to 50 µmol quanta m^{-2} s^{-1} required for plants growing *in situ* during December 1993 and January 1994 (Zimmerman unpubl. data). A total of 25 cores distributed among 5 replicate tanks were illuminated for only 2 h each day (H_{sat} = 2 h) to induce severe light limitation, while the other 5 replicate tanks (25 cores) received 7 h of illumination to simulate the mean daily H_{sat} period recorded at the collection site during December 1993 and January 1994 (Zimmerman unpubl. data).

One core was harvested from each replicate tank every 10 d (5 samples per H_{sat} treatment) and analyzed for biomass distribution (shoots, rhizomes and roots), number of internodes, growth rate, maximum net photosynthesis (P_m) and respiration (R), the activity of sucrose-mobilizing enzymes sucrose phosphate synthase (SPS, E.C. 2.4.1.14) in leaves and sucrose synthase (SS, E.C. 2.4.1.13) in roots, protein content and total carbohydrate reserves (TCR).

Metabolic rates and leaf chlorophyll. Leaf P_m, and leaf, root and rhizome R were measured at ambient growth temperature (15°C) using temperature-controlled polarographic O_2 electrodes. Respiration rates of rhizomes (first [= youngest] internode) and roots

(youngest bundle on each rhizome) were measured in O_2-enriched seawater to simulate the lacunal O_2 tension generated by fully illuminated intact plants and to prevent O_2 limitation of respiration (Zimmerman et al. 1989). Leaf P_m and R were measured during 20 min around noon on 2 cm segments removed from the middle portion of Leaves 2, 3 and 4. Illumination (PPF = 500 µmol photons m^{-2} s^{-1}) was provided by 35 mm slide projectors. Leaf respiration was measured in the dark using air-saturated seawater. The newly emerged leaf (1) was generally too small to sample, and the oldest leaf (5), if present, was generally senescent. After metabolic rate determinations, each leaf segment was ground in cold 90% (v/v) acetone for spectrophotometric determination of total chlorophyll (a + b) content using the extinction coefficients of Jeffrey & Humphrey (1975).

The ability to estimate whole-plant metabolic activity from the middle segments of Leaves 2, 3 and 4 was compared to a more detailed estimate based on measurements of metabolic rates at 4 positions on each leaf. Five shoots were collected from Del Monte Beach, Monterey Bay in January 1994. Segments were cut from the sheath (that portion that still sequestered with the leaf sheath), base (the youngest emergent third of each leaf), middle (middle one-third), and tip (upper one-third) of each leaf, and metabolic rates measured polarographically as above. Metabolic rates of individual leaves were then calculated by integrating the measured rates of these compartmentalized segments relative to their proportion of biomass in each leaf. Metabolic rates of individual shoots were calculated by scaling the integrated rates of Leaves 2, 3 and 4 according to their biomass distribution in the shoot. These estimates were then contrasted with integrals calculated using only the middle segments of each leaf.

Enzyme activity. The capacity for sucrose formation and export from leaves was evaluated by measuring the maximum velocity (V_{max}) activity of SPS in crude extracts (Zimmerman et al. 1995a). Sink strength of translocation-dependent roots, as measured by SS activity, was assayed in the youngest root bundle emerging from each shoot (Zimmerman et al. 1995a). Protein content of the enzyme extract was determined by dot-blot analysis using a dye-binding assay (Winterbourne 1986).

Total carbohydrate reserves. Sugars were extracted from ground samples of leaf, root and rhizome in hot (80°C) ethanol (Zimmerman et al. 1989). The extracts were evaporated to dryness at room temperature under a stream of compressed air, redissolved in distilled water and analyzed spectrophotometrically using a resorcinol assay standardized to sucrose (Huber & Israel 1982). Starch was extracted from ethanol-insoluble residue overnight in 1N KOH and analyzed spectrophotometrically using an anthrone assay standardized to sucrose (Yemn & Willis 1954). Sucrose and starch contents were added to obtain the TCR.

Growth rates and biomass allocation. Five days prior to harvest, shoots were marked above the meristem with a hypodermic needle and left to grow. Growth was determined on the harvest day by measuring the total length of each leaf and the length of new tissue below the punch mark on each leaf (Zieman 1974, Zimmerman et al. 1996). Young leaves without punch marks were assumed to have been produced entirely after marking. Biomass-specific growth was calculated by normalizing the length of new leaf tissue (below the punch marks) to the total length of all leaves on each shoot. In addition, fresh weight of leaves, rhizomes, roots and rhizome lengths (number of internodes) were measured. Growth of subterranean tissue was not measured but was assumed proportional to leaf growth rates for calculating carbon budgets.

Statistical analyses. Statistical significance of treatment effects were determined by 2-way ANOVA (H_{sat} × Time) or 3-way ANOVA (H_{sat} × Time × Tissue) for each variable measured. Only observations to 30 d were included in the analysis because none of the plants grown under 2 h H_{sat} periods survived to 45 d.

Whole plant carbon balance. Measured rates of P_m, R, and growth were used to calculate daily carbon balances. Metabolic rates were converted from units of O_2 to C using molar photosynthetic and respiratory quotients of 1.0 (O_2:CO_2). Daily net carbon gain (or loss) in the shoot was then calculated according to Zimmerman et al. (1996) as:

$$C_{gain} = [P_m \times H_{sat}] - [R_s\,(24 - H_{sat})] \quad (1)$$

where P_m was the light-saturated rate of net photosynthesis, R_s was the rate of dark respiration of the photosynthetic shoot, and H_{sat} was either 2 or 7 h. Aggregate metabolic rates of shoots for each sampling period were obtained by scaling the measured rates of P_m and R_s of the middle segments of Leaves 2, 3 and 4 to the relative biomass of those leaves in each shoot.

Carbon demand of root and rhizome (D_{R-R}) Internode 1 was calculated from measured rates of respiration. For the remaining internodes, D_{R-R} was obtained from the exponential relationship reported by Kraemer & Alberte (1993). The rate of carbon consumption by below-ground tissues during the dark (anaerobic) period was assumed to be 65% of the rate during the light (aerobic) period, as shown by Smith (1989):

$$D_{R-R} = [R \times H_{sat}] + [0.65 \times R \times (24 - H_{sat})] \quad (2)$$

where R was the respiration rate of roots and rhizomes measured at rate-saturating O_2 tensions (200% of air saturation). The biomass-specific rates were then

Table 1 Results of 3-way ANOVA examining the effects of Time, H_{sat} and leaf age on photosynthesis, respiration, photosynthesis:respiration (P:R) and chlorophyll ($a + b$) content. df: degrees of freedom for each treatment, MS: mean-square values, F: resulting F-ratio, p: probability that the independent variables had no effect on the dependent variables. (•) Treatments effects were considered statistically significant if $p < 0.05$, (•••) $p < 0.001$. ns: not significant

Dependent variable	Independent variable	df	MS	F	p	Significance
Photosynthesis	Time	3	0.009	0.96	0.414	ns
(µmol O_2 g^{-1} FW min^{-1})	Leaf age	2	0.234	24.42	<0.001	•••
	H_{sat}	1	0.013	1.34	0.249	ns
	Time × Leaf age	6	0.016	1.67	0.136	ns
	Time × H_{sat}	3	0.022	2.34	0.078	ns
	Leaf age × H_{sat}	2	0.004	0.45	0.640	ns
	Time × Leaf age × H_{sat}	6	0.006	0.66	0.683	ns
	Within	96	0.010			
Respiration	Time	3	0.003	1.13	0.341	ns
(µmol O_2 g^{-1} FW min^{-1})	Leaf age	2	0.008	2.71	0.072	ns
	H_{sat}	1	0.008	2.74	0.101	ns
	Time × Leaf age	6	0.006	2.04	0.068	ns
	Time × H_{sat}	3	0.007	2.65	0.053	ns
	Leaf age × H_{sat}	2	0.003	1.02	0.366	ns
	Time × Leaf age × H_{sat}	6	0.002	0.83	0.547	ns
	Within	96	0.003			
P:R	Time	3	9.19	3.05	0.032	•
	Leaf age	2	27.65	9.18	<0.001	•••
	H_{sat}	1	3.44	1.14	0.287	ns
	Time × Leaf age	6	4.18	1.38	0.227	ns
	Time × H_{sat}	3	0.76	0.25	0.858	ns
	Leaf age × H_{sat}	2	0.95	0.31	0.729	ns
	Time × Leaf age × H_{sat}	6	0.92	0.30	0.932	ns
	Within	93	3.01			
Chlorophyll ($a + b$)	Time	3	7.473	106.1	<0.001	•••
(mg chl g^{-1} FW)	Leaf age	2	0.027	0.38	0.679	ns
	H_{sat}	1	0.179	2.54	0.115	ns
	Time × Leaf age	6	0.111	1.57	0.165	ns
	Time × H_{sat}	3	0.038	0.53	0.659	ns
	Leaf age × H_{sat}	2	0.035	0.49	0.612	ns
	Time × Leaf age × H_{sat}	6	0.023	0.33	0.919	ns
	Within	89	0.070			

Table 2. Average of P_m, respiration, protein content and biomass for the different tissues. Standard deviations are given in brackets

	Leaf 2	Leaf 3	Leaf 4	Rhizome	Root
Biomass (g FW)				3.48 (1.30)	1.53 (0.85)
Maximum net photosynthesis (µmol O_2 g^{-1} FW min^{-1})	0.301 (0.122)	0.229 (0.093)	0.145 (0.083)		
P_q:R	3.71 (2.16)	3.50 (2.21)	2.39 (1.57)		
Respiration (µmol O_2 g^{-1} FW min^{-1})	−0.099 (0.053)	−0.088 (0.059)	−0.067 (0.051)	−0.031 (0.022)	−0.038 (0.037)
Protein (mg g^{-1} FW)		10.74 (3.81)			2.03 (1.05)

scaled to the proportion of root and rhizome biomass present in each plant.

Daily growth rates (g FW [fresh weight] d^{-1}) were converted to µmol C d^{-1} required for plant growth (G_p) using a ratio of 0.22 g DW g^{-1} FW, and a carbon content of 0.4 g C g DW^{-1} for new tissue (Alcoverro 1995). The resulting carbon required for growth was added to the metabolic rate calculations in order to determine whole-plant carbon balance (B_p):

$$B_p = C_{gain} - (D_{R-R} + G_p) \quad (3)$$

Positive values of B_p indicate that daily integrated photosynthesis fixed more carbon than was required to meet the daily demand of respiration and growth, leading to the accumulation of stored carbon reserves. Negative values of B_p indicate that photosynthesis was insufficient to offset daily carbon demand, requiring the mobilization of stored carbon.

RESULTS

Metabolic rates and leaf chlorophyll

P_m decreased significantly from Leaf 2 to 3 to 4 (Tables 1 & 2, Tukey test $p < 0.05$ for all pairwise comparisons of leaves). The mean photosynthetic capacity of Leaf 2 was 132% of Leaf 3 and 192% of Leaf 4. Respiration rates, in contrast, were not affected by leaf age (Tables 1 & 2). As a result of the differences in P_m, the instantaneous P_{gross}:R of Leaf 2 was 3.71, Leaf 3 was 3.50 and Leaf 4 was 2.39. Chlorophyll content of leaves within each age category declined significantly over time (Table 1, Fig. 2). Additionally, P_m declined significantly along the axis of Leaf 2 from 5 freshly collected plants (Fig. 3, ANOVA, $F = 7.41$, $p < 0.003$). Rates of respiration again showed no significant age-dependent effects within the leaf (ANOVA, $F = 1.46$, $p = 0.26$). Respiration rates of the youngest (Leaf 1) rhizome internode and root bundle were statistically identical and showed no significant effects of Time or H_{sat} (Tables 2 & 3).

Total carbohydrate reserves

Soluble sugar represented more than 95% of the TCR in all tissues sampled during the experiment, with the remaining 5% consisting of starch. TCR decreased through time in both treatments, but there was no significant effect of H_{sat} or the interaction term (Time × H_{sat}) on leaf TCR (Fig. 4, Table 4). Almost 70% of the TCR initially present in the leaves was depleted in both H_{sat} treatments over the course of the experiment. Rates of rhizome TCR depletion were internode dependent. TCR concentrations in the youngest internode (Internode 1) declined at equivalent rates in both that were equivalent in both H_{sat} treatments (Fig. 5, Table 3). TCR levels in Internode 3 declined by almost 50% over 30 d in the 2 h treatment, but remained unchanged in the 7 h treatment (Fig. 5, Table 3). There was no significant

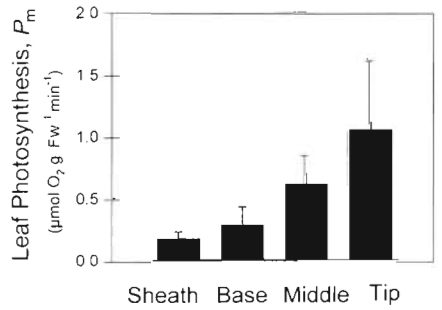

Fig. 3. Rates of light-saturated photosynthesis (P_m) in different portions of Leaf 2. Leaves were divided into sheath (that portion below the abscission line), base (the lower third of the leaf above the abscission line), middle (middle third of the leaf) and tip (upper third of the leaf). Error bars indicate standard error

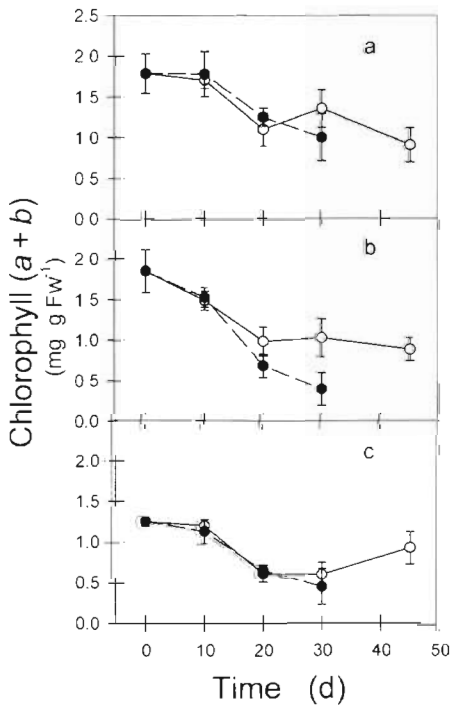

Fig. 2. Temporal changes in chlorophyll (a + b) content measured on middle segments of (a) Leaf 2, (b) Leaf 3 and (c) Leaf 4 growing under 2 (●) and 7 h (○) H_{sat} periods. Error bars indicate standard error of 5 replicate measures. FW: fresh weight

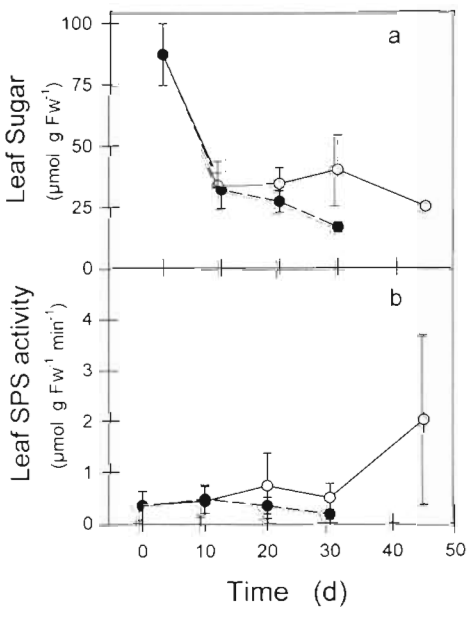

Fig. 4. Temporal changes in (a) sugar content of leaf No. 3 and (b) activity of SPS of Leaf 3 growing under 2 (●) and 7 h (○) H_{sat} periods. Error bars indicate standard error of 5 replicate measures

Table 3. Results of 2-way ANOVA testing the effects of Time and H_{sat} on rhizome biomass, root biomass, rhizome respiration (Internode 1), rhizome sugar content (Internodes 1, 3 and 6), root respiration, root sugar content, root SS and root protein. See Table 1 for explanations. (**) $p < 0.01$

Dependent variable	Independent variable	df	MS	F	p	Significance
Rhizome biomass (g FW)	Time	3	3.15	1.73	0.176	ns
	H_{sat}	1	0.24	0.13	0.713	ns
	Time × H_{sat}	3	0.18	0.09	0.959	ns
	Within	35	1.81			
Root biomass (g FW)	Time	3	0.32	0.46	0.715	ns
	H_{sat}	1	1.27	1.81	0.186	ns
	Time × H_{sat}	3	0.87	1.23	0.312	ns
	Within	35	0.70			
Internode 1 resp (μmol O_2 g^{-1} FW min^{-1})	Time	3	0.0003	0.53	0.666	ns
	H_{sat}	1	$5x10^{-6}$	0.01	0.897	ns
	Time × H_{sat}	3	0.0002	0.47	0.711	ns
	Within	35	0.0005			
Internode 1 sugar (μmol g^{-1} FW)	Time	3	25774	5.62	0.003	**
	H_{sat}	1	286	0.06	0.791	ns
	Time × H_{sat}	3	2240	0.49	0.696	ns
	Within	34	4597			
Internode 3 sugar (μmol g^{-1} FW)	Time	3	13487	3.33	0.03	*
	H_{sat}	1	23426	5.77	0.02	*
	Time × H_{sat}	3	7732	1.91	0.145	ns
	Within	35	4054			
Internode 6 sugar (μmol g^{-1} FW)	Time	3	23442	3.22	0.039	*
	H_{sat}	1	1454	0.19	0.658	ns
	Time × H_{sat}	3	1581	0.22	0.883	ns
	Within	26	7279			
Root respiration (μmol O_2 g^{-1} FW min^{-1})	Time	3	0.0027	1.90	0.146	ns
	H_{sat}	1	0.0002	0.11	0.733	ns
	Time × H_{sat}	3	0.001	0.72	0.552	ns
	Within	35	0.0015			
Root sugar (μmol g^{-1} FW)	Time	3	484.1	3.09	0.04	*
	H_{sat}	1	0.348	0.002	0.915	ns
	Time × H_{sat}	3	47.11	0.3	0.826	ns
	Within	32	156.8			
Root protein (mg g^{-1} FW)	Time	3	0.0013	0.001	0.922	ns
	H_{sat}	1	1.11	1.11	0.363	ns
	Time × H_{sat}	3	3	2.01	0.08	ns
	Within	32	1			
Root SS activity (μmol g^{-1} FW min^{-1})	Time	3	2.40	0.96	0.427	ns
	H_{sat}	1	0.56	0.22	0.645	ns
	Time × H_{sat}	3	7.54	3	0.044	*
	Within	32	2.51			

effect of H_{sat} on TCR levels of Internode 6, which declined to 1/3 of its initial level in both treatments (Table 3, Fig 5). Root TCR levels declined at approximately equal rates in both treatments (Fig. 6). Although the effect of Time on root TCR content was statistically significant, there was no effect of H_{sat} (Table 3).

Enzyme activity. There were no significant effects of Time or H_{sat} on leaf SPS activity for the first 30 d of the experiment (Fig. 4, Table 4). At 45 d, however, leaf SPS activity of plants exposed to 7 h H_{sat} increased significantly. All the plants grown under 2 h H_{sat} died before the 45 d sampling period. Root SS activity was consistently low in the 2 h treatment throughout the course of the experiment, but, like SPS activity, rose sharply at 30 d in the 7 h H_{sat} treatment and remained high through the end of the experiment at 45 d (Fig. 6). The synergistic effect of Time and H_{sat} on root SS activity is indicated by the statistically significant interaction term in the 2-way ANOVA (Table 3). Leaf and root protein content were not affected by H_{sat} or time (Tables 3 & 4).

Growth rates and biomass allocation. Plant biomass, absolute growth rates and shoot-specific growth rates declined in both 2 h and 7 h H_{sat} treatments throughout the 45 d course of the experiment (Table 4, Fig. 7). In both treatments, the reduction in shoot biomass resulted primarily from loss of the oldest leaf during

Table 4. Results of 2-way ANOVA examining the effects of Time and H_{sat} on different variables: shoot biomass, shoot growth rates, leaf carbohydrates, SPS activity and leaf protein. See Table 1 for explanations

Dependent variable	Independent variable	df	MS	F	p	Significance
Shoot biomass (g FW)	Time	3	227.4	11.82	<0.001	•••
	H_{sat}	1	11.1	0.52	0.46	ns
	Time × H_{sat}	3	3.3	0.17	0.911	ns
	Within	32	19.2			
Shoot growth (cm d^{-1})	Time	2	84.2	3.65	0.040	•
	H_{sat}	1	109.4	4.75	0.037	•
	Time × H_{sat}	2	12.3	0.53	0.597	ns
	Within	24	23.1			
Specific shoot growth (d^{-1})	Time	2	0.59	5.38	0.011	•
	H_{sat}	1	0.70	6.34	0.018	•
	Time × H_{sat}	2	0.08	0.75	0.484	ns
	Within	25	0.11			
Carbohydrates (µmol suc g^{-1} FW)	Time	3	1706	20.76	<0.001	•••
	H_{sat}	1	707	1.58	0.214	ns
	Time × H_{sat}	3	275	0.61	0.611	ns
	Within	34	445			
SPS activity (µmol suc g^{-1} FW min^{-1})	Time	3	0.094	2.08	0.125	ns
	H_{sat}	1	0.032	0.70	0.41	ns
	Time × H_{sat}	3	0.090	1.99	0.14	ns
	Within	28	0.045			
Leaf protein (mg g^{-1} FW)	Time	3	1.19	0.20	0.89	ns
	H_{sat}	1	0.30	0.05	0.81	ns
	Time × H_{sat}	3	6.42	1.08	0.37	ns
	Within	27	5.94			

the first week of the experiment. By Day 20, growth rates of the 2 h plants were significantly lower than the 7 h plants, and biomass was significantly lower by Day 30 (Fig. 7, Table 4). In total, 7 of the 25 plants grown under 2 h H_{sat} treatment died within the first 30 d, before they could be harvested for analysis. None of the 2 h plants survived to be sampled at 45 d. In contrast, only 2 of 25 plants from the 7 h H_{sat} treatment died before the experiment was terminated at 45 d, despite similar TCR concentrations and depletion rates. Plants produced new leaves (between 0 and 1) throughout the course of the experiment, but new roots were not initiated in either treatment. Plants in both treatments were devoid of roots on the first 2 to 3 nodes, and existing roots were generally long, fibrous and gray rather than the bright yellow to white colors typical of new rapidly growing roots.

Whole plant carbon balance

Whole plant carbon budgets calculated using only the middle section of each leaf showed a strong linear relationship to the more detailed calculation integrating different leaf segments (r^2 = 0.97). The significant differences in P_m associated with leaf position and age (Fig. 3), however, resulted in a consistent under-estimate of the carbon budget in this case (slope = 1.41 ±

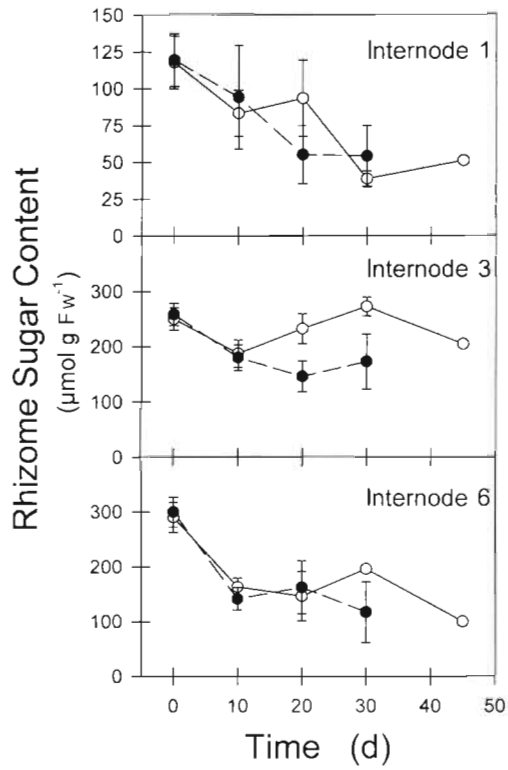

Fig. 5. Temporal changes in rhizome sugar content of Internodes 1, 3 and 6 growing under 2 (●) and 7 h (○) H_{sat} periods. Error bars indicate standard error of 5 replicate measures

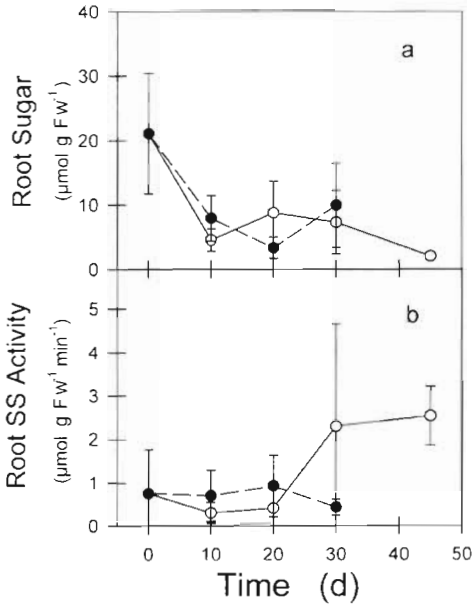

Fig. 6. (a) Temporal changes in root sugar content (first bundle) growing under 2 (●) and 7 h (○) H_{sat} periods. (b) Temporal changes in SS activity in the roots (first bundle) growing under 2 (●) and 7 h (○) H_{sat} periods. Error bars indicate standard error of 5 replicate measures

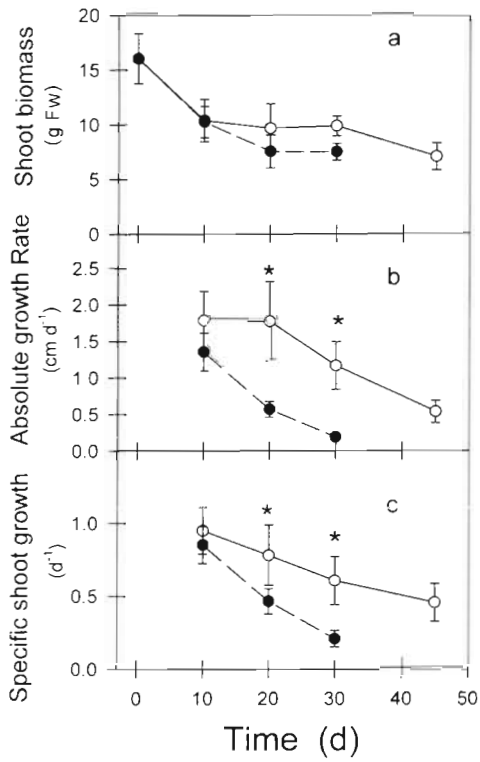

Fig. 7. Temporal changes in (a) shoot biomass, (b) shoot growth rates and (c) specific shoot growth growing under 2 (●) and 7 h (○) H_{sat} periods. Error bars indicate standard error of 5 replicate measures. (*) Values significantly different from zero ($p < 0.05$, t-test)

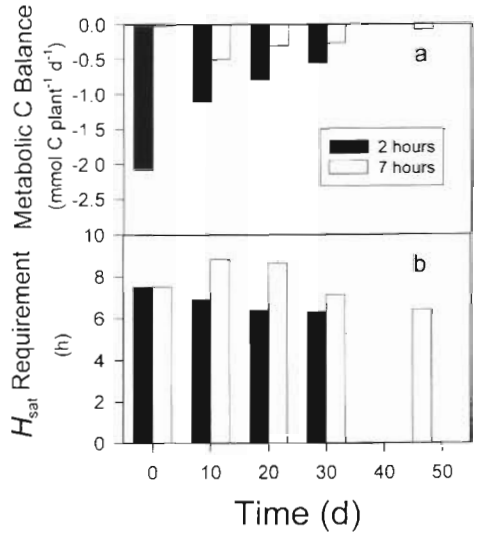

Fig. 8. (a) Estimates of daily metabolic carbon balance for plants growing under 2 and 7 h H_{sat} during the course of the experiment. (b) Estimated H_{sat} periods required to meet daily carbon demand, based on metabolic carbon balance calculations for plants growing under 2 and 7 h H_{sat}

0.12, t-test for difference from slope = 1: $t = 11.7$, df = 8, $p < 0.0001$, y-intercept = 162 ± 92, t-test for differences from y-intercept = 0: $t = -1.12$, $p = 0.30$). Consequently, the carbon balance estimates for the H_{sat} experiment were multiplied by 1.41 to correct for this difference caused by the high variation in leaf P_m.

Although plants maintained under 2 h H_{sat} were more severely carbon limited than plants grown under 7 h H_{sat}, whole-plant carbon balance calculated from the metabolic and growth rates was negative for both H_{sat} treatments throughout the course of this experiment (Fig. 8). Thus, growth and survival in both H_{sat} treatments required the mobilization of stored reserves to balance carbon demand. Metabolic carbon demand calculated from rates of photosynthesis, respiration and growth was able to account for almost all (79 ± 26%) the carbon depletion observed in both treatments (Fig. 9, $r^2 = 0.57$, slope = −0.79 ± 0.26, t-test for difference from slope = 1: $t = -2.98$, df = 6, $p = 0.03$, y-intercept = −196 ± 139, t-test for differences from y-intercept = 0: $t = -1.42$, $p = 0.21$). Given that these calculations did not include carbon lost by the sloughing of senescent older leaves in the first 10 d (roughly 20% of the shoot biomass), observed rates of carbon depletion and calculated rates based on metabolic demand appear to be essentially in balance. Carbon balance became less negative through time as plant size and growth rates of both treatments declined, but the H_{sat} period required to meet that demand remained relatively constant at about 7.4 h because metabolic rates remained constant (Fig. 8).

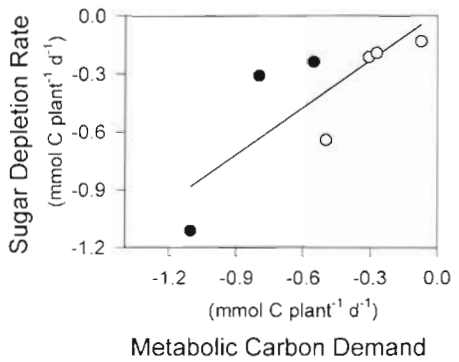

Fig. 9. Relationship between observed rates of sugar depletion and daily carbon balance calculated from the sum of metabolic rates and growth for plants under 2 (●) and 7 h (○) H_{sat}. Regression line was fit to the combined data set

DISCUSSION

Internal carbon reserves clearly provide an important buffer to extend eelgrass survival when photosynthesis is inadequate to maintain positive daily carbon balance. This mobilization of carbohydrate reserves accumulated during periods of abundant light availability represents an important strategy for survival of perennial seagrasses in temporally variable environments, and appears to be considerably more common than other strategies that include seasonal dormancy and annual life histories exhibited by populations found at the extremes ranges of eelgrass distribution (Keedy & Patriquin 1978, Gagnon et al. 1980, Phillips and Backman 1983, Robertson & Mann 1984, Harrison 1993). Although low rates of photosynthesis resulted in negative carbon balances for both treatments throughout this study, the 2 h H_{sat} treatment imposed a severely short period of daily photosynthesis and below-ground aerobiosis that led to plant death within 30 d despite consuming only 2/3 of the carbohydrate reserves stored in rhizomes of the dead plants. This inability to fully mobilize below-ground reserves demonstratesthat translocation was perhaps disrupted in the 2 h plants by the extended night time anoxia, as has been shown in a short-term experiment (Zimmerman & Alberte 1996). Thus, severe light limitation can lead to plant death before below-ground carbon reserves are completely exhausted.

Rates of TCR utilization by leaves, roots, and rhizomes observed here were consistent with the carbon demand calculated from growth and metabolism of tissue segments. Quantitative agreement between these approaches underscores the reliability of whole-plant carbon balance estimates derived from careful respirometry of leaf, rhizome and root segments when the light environment is carefully characterized or controlled (Zimmerman et al. 1995b, 1996, Zimmerman & Mobley 1997). Discrepancies between laboratory-based calculations and *in situ* respirometry of whole plants have been reported, particularly in turbid environments that produce large gradients in light availability through the canopy (Herzka & Dunton 1997). Unless these light gradients are measured and modeled accurately using radiative transfer theory, laboratory photosynthesis versus irradiance models are likely to over-estimate production if irradiance is assumed to be uniform over the entire canopy (Zimmerman & Mobley 1997). The *in situ* measures, however, are likely to produce site and event-specific correlations that are difficult to generalize to other periods, locations or seagrass populations.

The patterns of TCR depletion in leaves, roots and rhizomes provide insight into the buffering capacity of reserve mobilization in response to light-availability. The decline in shoot growth that began after 10 d was clearly a response to overall carbon balance, as has been observed in *Thalassia testudinum* and *Posidonia oceanica* (Tomasko & Dawes 1989, Alcoverro 1995). Continued leaf elongation and proliferation of new leaves up to the point of plant death in the absence of any root proliferation also demonstrates that the photosynthetic shoot is the primary sink for reduced carbon at the expense of the roots. Release from carbon limitation, however, results in the rapid re-direction of photosynthate into the roots of eelgrass (Zimmerman et al. 1996).

Total carbohydrate reserves of Internode 3 were least affected by the severe reduction in light availability imposed by the 2 h H_{sat} treatment, indicating that this internode acts as an important conduit for the transport of carbon reserves from older internodes to the meristem. Although TCR declined monotonically in Internodes 1 and 6 in both H_{sat} treatments, Internode 3 TCR declined significantly only in the 2 h H_{sat} treatment, and then only after 20 d. Furthermore, plants in the 2 h H_{sat} treatment died with more than 100 µmol sucrose equivalent g^{-1} FW remaining in Internode 3, roughly 75% of the TCR present in this internode at the beginning of the experiment. In contrast, TCR levels in Internodes 1 and 6 dropped almost 3-fold during the course of this experiment. The 2 h H_{sat} plants died shortly after equilibration of the sucrose gradient between Internodes 6 and 3 even though 1/3 of the TCR present at the beginning of the experiment remained unutilized. Thus, eelgrass may require a strong source-sink gradient to maintain adequate delivery of reduced carbon to the meristem when translocation is limited to short daily periods of aerobiosis.

The lack of healthy root growth even during the initial phase of this experiment indicates that negative carbon balance inhibits root production on new internodes of eelgrass, as it does in a variety of terrestrial plants (Pierson et al. 1990, Sims & Pearcy 1994). The

lack of new roots on the first 3 internode segments at the first sampling period revealed that plants probably had been carbon-limited at the time of collection. Thus, the proliferation of metabolically active roots may occur only when whole-plant carbon balance is positive (Zimmerman et al. 1996). The lack of roots, however, may provide eelgrass with a less secure hold on the sediment, leaving plants vulnerable to physical disturbance and erosion.

The final phase of carbon depletion was characterized by cessation of growth and changes in enzyme activity. The increased activity of root SS in the 7 h H_{sat} plants at 30 d may represent a stress response to increase the sink strength of severely carbon limited tissues, as has been described previously in eelgrass and maize in response to anoxia (Freeling & Bennett 1985, McCarty et al. 1986, Xue et al. 1991, Zimmerman et al. 1995b, 1996). Leaf SPS activity, which controls sucrose loading from photosynthetic sources (Huber et al. 1985), is unresponsive to shifts in light availability or photosynthetic rate in carbon-replete eelgrass, unlike many terrestrial plant species (Zimmerman et al. 1995a). However, the significant increase in SPS activity reported here for 7 h H_{sat} plants at 45 d may provide another indicator of severe carbon stress.

In contrast to the observed changes in carbon reserves and growth rates, metabolic rates were insensitive to the temporal increases in carbon limitation imposed by both light regimes in this study. Rates of P_m from both 7 h and 2 h H_{sat} treatments were 30 to 50% lower than previously reported for *Zostera marina*, but leaf R remained consistent with the literature (Dennison & Alberte 1986, Zimmerman et al. 1989, 1991). Rates of root respiration were lower than previously published values. Respiration rates of healthy young roots are typically about 50% of leaf R and 200% of rhizome R, but decline significantly with age (Zimmerman et al. 1989, Kraemer & Alberte 1993). Thus, the low rates of root respiration observed here probably reflect the metabolic activity of older roots and the lack of new root production in these carbon-limited plants.

The decline in photosynthetic capacity with age in leaf tissue also represents a significant contrast with earlier reports which showed relatively little variation in light-saturated photosynthetic capacity as a function of leaf age and had allowed the construction of accurate carbon budgets from measures performed on a few leaf segments (Zimmerman et al. 1995a, 1996, Zimmerman & Mobley 1997). In addition, the rates of P_m reported here were considerably lower than previously published results for *Zostera marina* from the same population and other locations (Dennison & Alberte 1995, Zimmerman et al. 1989, 1991, 1995a,b, 1996). The plants in this study, however, were heavily grazed by *Tectura depicta*. These effects are most evident in older leaves that have been grazed for longer periods of time and correlate well with a reduction in leaf chlorophyll content (Zimmerman et al. 1996). Such variations must be considered whenever calculating whole-plant metabolic carbon balances, as was done here by increasing the number and range of leaf segments measured.

Although the 7 h treatment provided a realistic winter H_{sat} period, it was not sufficient to maintain positive carbon balance in these plants that required about 7.4 h H_{sat}. The similarity of these observations to freshly collected plants from the field (Zimmerman unpubl. data) indicates that the experimental conditions did not significantly alter daily carbon requirements of the plants or their metabolic capacity for carbon assimilation relative to plants growing *in situ*. Clearly, winter conditions in temperate ecosystems can lead to negative carbon balance in eelgrass, especially when plants are stressed by other factors such as leaf grazing or high water column turbidity. The eelgrass studied here responded to negative carbon balances by suppressing the production of new roots, depleting of sucrose reserves, and effecting a slow decline in growth rate and an increase in SS activity in sink tissues in the terminal stages of carbon stress. These patterns may provide useful indices for assessing the state and fate of seagrass ecosystems in advance of catastrophic declines.

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