Botanica Marina Vol. 45, 2002, pp. 23–34

Seasonal Variations in Carbon and Nitrogen Constituents in Eelgrass (*Zostera marina* L.) as Influenced by Increased Temperature and Water-Column Nitrate

B. W. Touchette* and J. M. Burkholder

Department of Botany, Box 7612, North Carolina State University, Raleigh, NC 27695-7510, U.S.A.

* Corresponding author: brant_touchette@ncsu.edu

Fluctuations in nitrogen and carbon compounds were examined over an autumn growing season in the submersed marine angiosperm *Zostera marina* L. (eelgrass). The experimental design included replicated controls (ambient NO_3^-, typically < 2 μM), increased water-column nitrate (8 μM NO_3^- above ambient, pulsed daily), increased environmental temperature (3 to 4 °C above 20-year weekly means), and combined increased water-column nitrate and temperature. Above- and belowground tissues were collected weekly to biweekly and assayed for total soluble carbohydrates, non-reducing carbohydrates, starch, α-cellulose, lipids, free amino acids, total protein, tissue nitrate, tissue nitrite, and tissue ammonium. Tissue nitrate declined, and amino acids, proteins, lipids, and cellulose increased as the growing season progressed in both control and treated plants. In addition, there were seasonal quadratic responses for tissue ammonium, soluble carbohydrates, and non-reducing sugars, with maxima during periods of optimal plant growth (mid- to late September). Increased temperature promoted periodic increases in amino acids and soluble carbohydrates, but decreased accumulation of α-cellulose by the end of the experiment. Moreover, increases in water-column nitrate led to periodic increases in tissue ammonium and amino acids, as well as decreases in non-reducing sugars. Toward the end of the experiment, increases in soluble carbohydrates for plants grown under higher temperatures may have been associated with an extension of the growing season. In contrast, decreased non-reducing sugars in nitrate-enriched plants may have resulted from an increased carbon demand during nitrate assimilation/reduction, as well as a reallocation of carbon to enhance amino acid synthesis.

Introduction

The marine angiosperm *Zostera marina* L. (eelgrass) inhabits an environment that is characteristically harsh for many plant species (Thayer *et al.* 1984). Aquatic plants that reside in turbid, shallow-water coastal habitats often undergo periods of low radiation due to increased light attenuation caused by either phytoplankton growth or sediment loading, as well as increased epiphytic algal development on leaf tissues (Morris and Tomasko 1993). Additionally, the diffusion rate of carbon dioxide is 10^4-fold lower in aqueous media relative to diffusion under atmospheric conditions, and there is a tendency toward the formation of bicarbonate rather than dissolved carbon dioxide gas, which is more readily available for photosynthesis (Riley and Chester 1989). Other environmental parameters such as increased temperature (e.g., from global climate change) and salinity alterations also may affect carbon availability/utilization, and thereby alter internal demands for carbon resources (Riley and Chester 1989, Zimmerman *et al.* 1989, Touchette 1999). Under such conditions, some aquatic plant species may encounter carbon-limited photosynthesis/growth (Adams 1985, Levavasseur *et al.* 1991).

Eelgrass has been shown to undergo periods of carbon and/or nitrogen growth limitation, depending on the prevailing environmental conditions (Short 1983, Zimmerman *et al.* 1987, van Lent *et al.* 1995, Touchette and Burkholder 2000). Moreover, during periods of increased nitrate uptake, plants must divert energy and carbon toward nitrate reduction and ammonium assimilation, and toward amino acid synthesis (Turpin *et al.* 1991). These processes could promote an additional loss or 'drain' of carbon, thereby lowering soluble carbohydrate supplies that otherwise would have been allocated as food reserves in the plant. Such a response has been suggested as a mechanism for decreased productivity and survival for *Zostera marina* under water-column nitrate enrichment (Burkholder *et al.* 1992, 1994).

The morphological characteristics of this marine perennial require that it maintain adequate rhizomal carbohydrate reserves to survive extended periods of light attenuation, tissue loss (e.g., from herbivory, with associated carbon demands for tissue replacement), seasonally dependent growth (including dormancy periods), flower and fruit development, and auxiliary shoot and rhizome development. *Zostera marina* also requires carbon skeletons to produce organic compounds used in osmoregulation during hyper- and hyposaline periods (Lingle 1987, Souza and Silva 1987, Fougere *et al.* 1991). Thus, the accumulation and maintenance of non-structural carbohydrate re-

serves are vital for the survival of *Z. marina* and other closely related submersed marine angiosperms that rely upon rhizomatous carbon reserves during periods of low growth.

Little is known about seasonal variations in carbon and nitrogen compounds in seagrasses and environmental influences on these variations. Such information would provide insights about how these plants respond to various perturbations, especially temperature changes and nitrogen-linked eutrophication. The data would also be useful for coastal resource managers involved with establishment or replacement of seagrass meadows, by indicating suitable time frames for harvesting and transplanting. For example, plants with higher reserves of nitrogenous compounds and non-structural carbohydrates may have greater survival potential when transplanted into new locations. Therefore, the object of this study was to test the hypothesis that environmental perturbations – especially water-column nitrate enrichment and increased temperature – can influence the concentrations of internal carbon and nitrogen constituents in *Zostera marina* tissues. Plants experiencing nitrate enrichment would be expected to have higher levels of nitrogenous compounds (e.g., amino acids and proteins), whereas plants experiencing higher temperatures would be expected to have altered structural- and soluble-carbohydrate levels (e.g., cellulose and sucrose) due to increased respiration and decreased photosynthesis.

Materials and Methods

Study area

The research was conducted in North Carolina, which is the southernmost extension for eelgrass along the western Atlantic Ocean (Thayer *et al.* 1984). In this region eelgrass growth is stunted due to high temperature stress (Den Hartog 1970). Shallow waters in this area often reach temperatures as high as 33 °C from late spring through early autumn (Thayer *et al.* 1984). Temperatures above 30 °C have been shown to adversely affect more northern eelgrass populations by increasing respiration and impairing vital enzyme activities (Lambers 1985, Marsh *et al.* 1986, Zimmerman *et al.* 1989).

Mesocosm system

The mesocosm system consisted of 12 fiberglass tanks (2.0 m in diameter) that were sealed with nontoxic white gelcoat resin. The tanks were 1.0 m in height with a 60-cm working depth (including sediment and water levels) that was established by creating a raised floor. This raised floor was designed to accommodate the relatively small eelgrass plants (typically < 40 cm in length), and to eliminate wall-shading effects. The sediment consisted of clean dredge soils and sand mixed in a 3:1 ratio by volume. The substratum depth was adjusted to 30 cm in each tank. Prior to transplanting, the system was allowed to acclimate in running seawater for 4 months, thereby establishing natural chemical and biological gradients in the sediment and water column.

Temperature and water circulation (current 5 to 8 cm s^{-1}) were controlled using a chiller system that consisted of three 4.5-tonne condensing units, each capable of a maximum of 60,000 BTU h^{-1} cooling capacity. The chiller units had electronically controlled thermal expansion valves that regulated the flow of freon into each titanium heat exchanger. Each mesocosm had an independent heat exchanger, thereby providing independent temperature control. Each tank was also independently plumbed in both intake and outflow lines, so that there was no cross-contamination among mesocosms.

Light reduction from high tide was simulated in each mesocosm by using neutral density shades that decreased photosynthetically active radiation (PAR) by approximately 30 %. Shades were placed on each tank for 3 h intervals on a rotating schedule to simulate light reduction during high tide (0900–1200 h for 3 days, followed by 1200–1500 h for 3 days, then 3 days with no shading). Even under shaded conditions, light levels were still above light saturation values that have been reported for field populations of *Zostera marina* (> 350 μE m^{-2} s^{-1}; Dennison and Alberte 1985, Dennison 1987).

Experimental design

Eelgrass shoots were harvested during mid-April from natural populations in an unenriched site at Middle Marsh, near Beaufort, North Carolina, USA. Plants were maintained in running seawater while they were individually cleaned of fouled tissue (e.g., old leaf material), epiphytes (primarily diatoms and filamentous algae), and sediment. The cleaned plants were then transplanted into each tank at a density of 650–700 shoots m^{-2}. Plants were allowed to acclimate in the tanks with running seawater for 4 months prior to the initiation of treatments. The mesocosms and plants were cleaned of excessive macroalgal (primarily *Cladophora* and *Polysiphonia*) growth when necessary, and immediately prior to initiation of treatments.

The experiment followed a split-plot design, with condensing units defined as the plot variant. Although each tank had individual circulating systems, tanks (in groups of four) were attached to the same condensing/compressor unit, thereby making the split-plot design appropriate. The experiment began in early August, prior to the initiation of autumn growth. Replicated controls (n = 3) were maintained with ambient NO_3^- and 20-year weekly temperature means (NOAA 1994), and replicated treatments (n = 3) included (i) increased water-column nitrate (HN; 8.0 μM above ambient), (ii) increased temperature (HT; 3 to 4 °C

above 20-year weekly means for shallow bay waters [depth < 0.5 m]), and (iii) both increased nitrate and increased temperature (HTN). Nitrate additions were pulsed daily (at 0900 h), and nitrate was allowed to be taken up by the established eelgrass communities throughout the day. A 10 % water exchange was conducted at 1700 h to simulate conditions in poorly flushed, shallow-water embayments and lagoons. Plants were sampled by harvesting prior to the beginning of the experiment, and then harvesting weekly (August) to biweekly (September to October). A final sample was collected at 14 weeks in November. Once harvested, the plants were immediately frozen on dry ice and transported to the laboratory where they were stored at –70 °C until analysis. All samples were collected at the same time of day (between 1500 and 1600 h) to minimize variability due to daily physiological rhythms.

Tissue analyses

Samples were analyzed for total soluble non-structural carbohydrates, insoluble non-structural carbohydrates (starch), non-reducing carbohydrates (mostly sucrose), cellulose, lipids, free amino acids, total proteins, nitrate, nitrite, and ammonium in both the root-rhizome complex and shoot tissues. Total soluble carbohydrates (reported as sucrose equivalents), including non-reducing sugars, were determined by extracting compounds from tissue using 70 % hot ethanol with mortar and pestle. The extracts were centrifuged, and the supernatant was collected and allowed to dry *in vacuo*. The samples were resuspended in hot deionized water, cleared with aluminum hydroxide, and analyzed using anthrone (Yemm and Willis 1954, Van Handel 1968). This procedure involved adding 0.20 % anthrone in 71 % H_2SO_4 (4 °C), heating the sample for 10 min (100 °C), and measuring absorbance at 630 nm on a Milton Roy Spectronic 601 spectrophotometer (Ivyland, PA, USA). Determination of sucrose and other non-reducing carbohydrates was conducted using a similar anthrone procedure. However, interfering reducing sugars (e.g., glucose and fructose) were destroyed with hot concentrated alkali followed by the determination of non-reducing carbohydrates at low temperatures (40 °C; Van Handel 1968). Lipid analysis was conducted following the procedures described by Guisande and Serrano (1989), Vestal and White (1989), and Ahlgren and Uppsala (1991), wherein dried samples were extracted in chloroform-methanol (2:1 by volume) and then the solvent was evaporated at low pressure under a N_2 gas atmosphere. The samples were rehydrated in concentrated H_2SO_4 and heated at 100 °C for 10 min. After cooling (to room temperature), lipid content was assayed spectrophotometrically at 528 nm with vanilline reagent.

Starch analysis was conducted using the technique described by Lustinec *et al.* (1983) and Coombs *et al.* (1985). Tissue was ground in 32 % perchloric acid with mortar and pestle. Homogenates were filtered (Whatman GF/A glass fiber filter), and iodine solution was added. The precipitated starch-iodine complex was collected on a glass fiber filter, and was washed consecutively with perchloric iodine solution, ethanolic NaCl solution, and ethanolic NaOH solution (Lustinec *et al.* 1983). The filters with samples were dried and starch was determined using 5 % phenol-H_2SO_4 reagent, with absorbance from the colorimetric change read at 480 nm. Cellulose analysis (α-cellulose) was determined gravimetrically via the acid-detergent-fiber method, which compared mass changes before and after removal of all interfering inorganic and organic material including lignin from the sample (Southgate 1976).

Extraction of total proteins and free amino acids involved grinding tissue in 0.37 M trichloroacetic acid (TCA) as described by Clayton *et al.* (1988). The homogenate was transferred to a centrifuge tube, 3.6 mM deoxycholic acid (DOC) was added, and the mixture was maintained at room temperature for 20 min (Bensadoun and Weinstein 1976). The sample was then centrifuged at 3000 g for 15 min. The supernatant was collected and analyzed for free amino acids using a modified ninhydrin technique described by Kesner and Kirschenbaum (1970). The protein pellet was resuspended in 0.1N NaOH and 2 % sodium dodecyl sulfate (SDS) solution, and was maintained at room temperature for 30 min (Peterson 1977). Protein was assayed using the Smith BCA procedure (Smith *et al.* 1985; Pierce protein assay reagent, Prod. No. 23225), with concentrations reported as BSA equivalents.

Amino acid analysis additionally was performed using high-voltage electrophoresis to determine the major amino acid constituents in the plant tissues (Block *et al.* 1958, Rosenthal 1982). This semi-quantitative analysis was completed on plants that were collected during the maximum growth period (mid-September, which also corresponded with the period of maximum amino acid content). Amino acids were extracted in a 70 % EtOH solution and applied to Whatman 3 mm paper. Electrophoresis utilized a pH buffer consisting of 2 M acetic acid, adjusted to pH 1.8 with formic acid. Amino acid bands were removed based on the position of known standards, and were assayed for amino acid concentration using the ninhydrin procedure as described above.

Tissue ammonium (NH_4^+) levels were determined as described by Guerrero (1987), with extracts analyzed spectrophotometrically (using a Technicon Auto Analyzer, Chicago, IL, USA) at 640 nm following the formation of indophenol via a reaction with ammonium, hypochlorite, and phenol (Solórzano 1969). Although this method has been criticized because of reported color formation due to interfering amino acids (e.g., Ali and Lovatt 1995), preliminary evaluations using eelgrass tissues and standard amino acid compositions (mostly glutamine and glutamate at con-

centrations typical in eelgrass) revealed color interference values < 1 %.

Tissue nitrate was extracted according to Raab and Terry (1995), wherein nitrate samples were reduced to nitrite via cadmium reduction and assayed spectrophotometrically. Nitrite samples were assayed similarly, excluding the cadmium reduction step.

Data analyses

Statistical analyses were performed using Statistical Analysis Software (SAS). The data analysis included repeated-measures ANOVA with days from initiation of treatment as the repeated-measures factor. This analysis considered both linear and quadratic models to describe the associated response curves (Meredith and Stehman 1991). In addition, pre-planned comparisons were conducted for each individual sample date using two-way ANOVA (LS means and LSD) to assay treatment response that may not have been associated with time. All probability values (p) were considered significant at α less than 0.05.

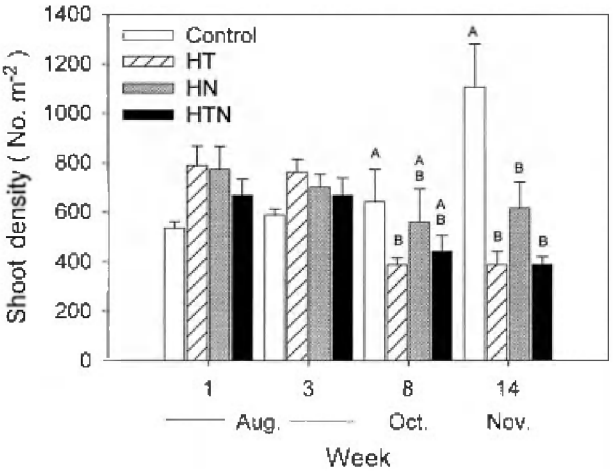

Fig. 1. Shoot density of *Z. marina* over the growing season, in control mesocosms, and in treatments including high temperature (HT), high nitrate (HN), and high temperature and high nitrate (HTN). Letters above the error bars represent the statistical relationship within each sample period (e.g., all bars with A are considered for that date). Data are given as means ± 1 standard error [SE].

Results

Shoot density, based on fixed 0.10 m^2 quadrats, increased over time for control plants, whereas shoot density declined in both nitrate and temperature treatments (alone or in combination; Fig. 1). The declines in shoot densities of treated plants occurred during the period of maximum shoot production in controls (September through mid-November). These declines were not associated with algal light attenuation, as indicated by a lack of significant treatment effects on macroalgal and epiphyte biomass ($p = 0.08$ and 0.10 for nitrate and temperature treatments, respectively).

Starch and nitrite were not detected in either above- or belowground tissues (minimum detection levels 2 ng mL^{-1} and 5 µg mL^{-1} extract for nitrite and starch, respectively). The lack of detectable nitrite in eelgrass tissues suggests that sensitivity to nitrite accumulation was not involved in the growth responses observed in

Table I. Summary of treatment effects on carbon and nitrogen constituents in *Zostera marina*.

Constituent	High temp.	High NO_3^-	High temp. and NO_3^-
Leaf tissue			
Ammonium	Decrease[6]	No change	Increase[6, 8]
Nitrate	Decrease[1–4, S]	No change	Increase[2, 3, S]
Amino acids	Increase[8, 14]	Increase[8]	Increase[8, 14, S]
Protein	Increase[14]	Decrease[2]	Increase[8, S]
Sucrose	Increase[14]	Decrease[14]	Increase[14]
Tot. carbohydrates	No change	No change	No change
Cellulose	No change	No change	No change
Lipids	No change	No change	Decrease[14]
Root/Rhizome tissue			
Ammonium	No change	Increase[2]	Increase[2, 6, 8, S]
Nitrate	No change	No change	Decrease[4]
Amino acids	Increase[3]	Increase[8]	Increase[2, 3, 4, 8, 14, S]
Protein	Increase[14, S]	No change	Increase[14]
Sucrose	Increase[14, S]	No change	Increase[14, S]
Tot. carbohydrates	Increase[3, 14]	No change	Increase[3, 8, 14]
Cellulose	Decrease[14]	No change	Decrease[14]
Lipids	No change	No change	No change

Tissue components include ammonium, nitrate, amino acid, protein, sucrose, total carbohydrate, cellulose, and lipid in leaf and root/rhizome tissue. Responses include significant increase, decrease, or no change relative to the control (significance as defined when $p < 0.05$). Numbers following response indicate the sample period (i.e., experimental week) where significance was detected, and the letter S represents a significant seasonal response (repeated measures ANOVA).

the nitrate treated plants (Shimazaki *et al.* 1992). However, significant changes were observed in other carbon and nitrogen compounds. Many of these compounds changed appreciably in concentration over the course of the growing season as a response to either environmental influences (e.g., temperature, nutrients) or growth status (Table I). These changes included a linear decline in tissue nitrate concentration over time ($p = 0.01$, leaf tissue), as well as linear increases in free amino acid concentrations ($p = 0.0001$, leaf; $p = 0.0001$, root-rhizome), total protein concentration ($p = 0.0083$, leaf; $p = 0.0001$, root-rhizome), and lipid concentration ($p = 0.0001$, leaf; $p = 0.0001$, root-rhizome) over the autumn. There were also quadratic responses with time for tissue ammonium concentration ($p = 0.0001$, leaf; $p = 0.0001$, root-rhizome), soluble carbohydrate concentration ($p = 0.0001$, leaf; $p = 0.0001$, root-rhizome), and sucrose levels ($p = 0.0001$, leaf; $p = 0.008$, root-rhizome), with maximum concentrations measured during September.

Early in the growing season, tissue nitrate concentrations were influenced primarily through an interaction between water-column nitrate and environmental temperature (TN; Fig. 2). At higher temperatures, increased water-column nitrate often was related to an increase in tissue nitrate. Such increases were observed in leaf tissue throughout the month of August ($p < 0.046$), and in root-rhizome tissue during early September ($p = 0.03$). However, toward the end of the season this trend was no longer maintained, and relatively low nitrate levels occurred in plant tissues from late September through the remainder of the experiment.

Significant treatment responses also were found in tissue ammonium concentrations (Fig. 3). During mid-August, there was a significant increase in below-ground tissue ammonium with elevated water-column nitrate (HN and HTN; $p = 0.025$). Moreover, there was a significant interaction between environmental temperature and water-column nitrate in affecting leaf and root-rhizome tissue ammonium by mid- to late September (HTN; $p = 0.033$, and $p = 0.003$, for leaf and root-rhizome tissue, respectively). On those dates, plants exposed to the combination of elevated water-column nitrate maintained significantly higher-tissue ammonium relative to plants in high temperature or high nitrate treatments alone.

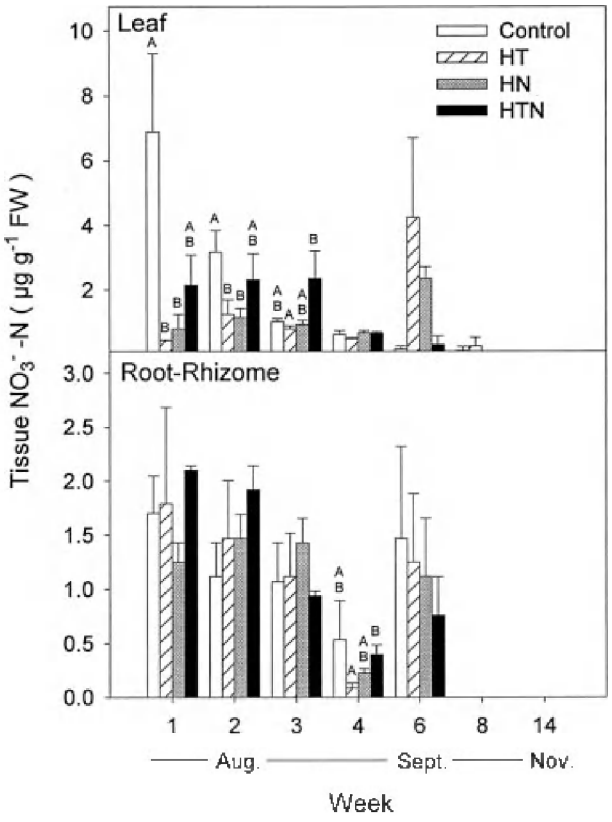

Fig. 2. Seasonal variation in tissue nitrate (fresh weight [FW] basis) for *Z. marina* leaf tissue (upper panel), and root/rhizome (lower panel), in control mesocosms, and in treatments including high temperature (HT), high nitrate (HN), and high temperature and nitrate (HTN). Letters above the error bars represent the statistical relationship within each sample period (e.g., all bars with A are considered similar for that date). Data are given as means ± 1 SE.

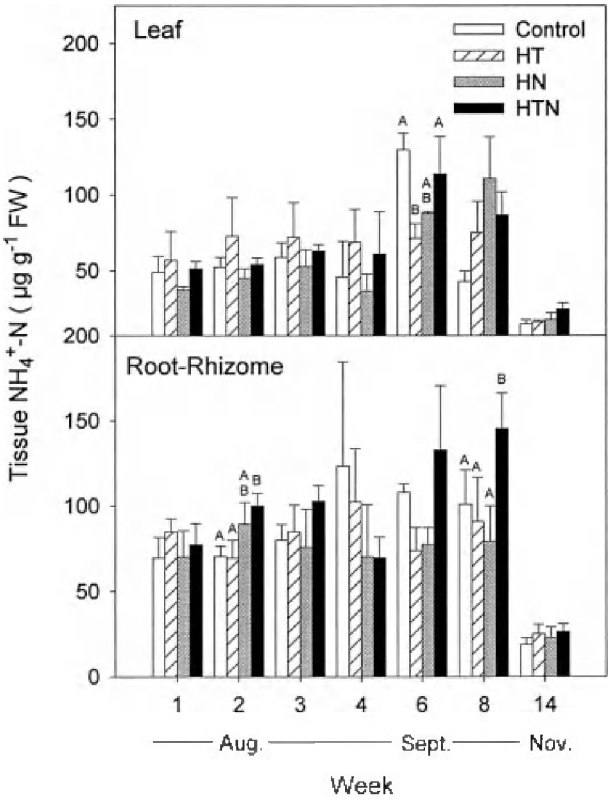

Fig. 3. Seasonal variation in tissue ammonium for *Z. marina* leaf tissue (upper panel) and root/rhizome tissues (lower panel), in control mesocosms, and in treatments including high temperature (HT), high nitrate (HN), and combined high temperature and nitrate (HTN). Letters above the error bars represent the statistical relationship within each sample period (e.g., all bars with A are considered similar for that date). Data are given as means ± 1 SE.

Concentrations of free amino acids were significantly altered under treatment conditions (Fig. 4). Increased temperatures were associated with significant increases in free amino acids of root-rhizome tissues by late August (HT and HTN; $p = 0.01$), and of leaf tissue by late September ($p = 0.025$). Nitrate-enriched plants maintained higher free amino acids in root-rhizome tissues by late September, relative to unen-

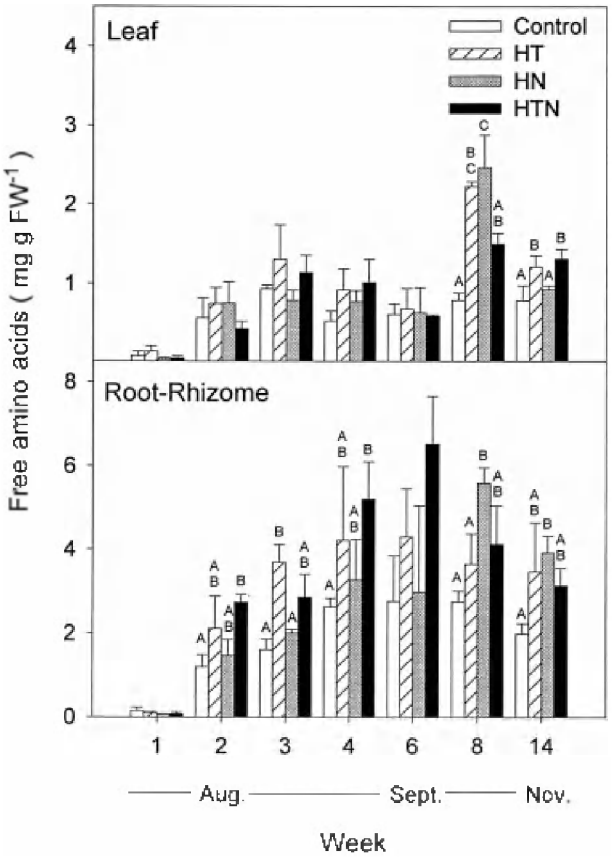

Fig. 4. Seasonal variation in free amino acids (glutamine equivalent) in *Z. marina* leaf- (upper panel) and root/rhizome-tissue (lower panel), in control mesocosms, and in treatments including high temperature (HT), high nitrate (HN), and high temperature and nitrate (HTN). Letters above the error bars represent the statistical relationship within each sample period (e.g., all bars with A are considered similar for that date). Data are given as means ± 1 SE.

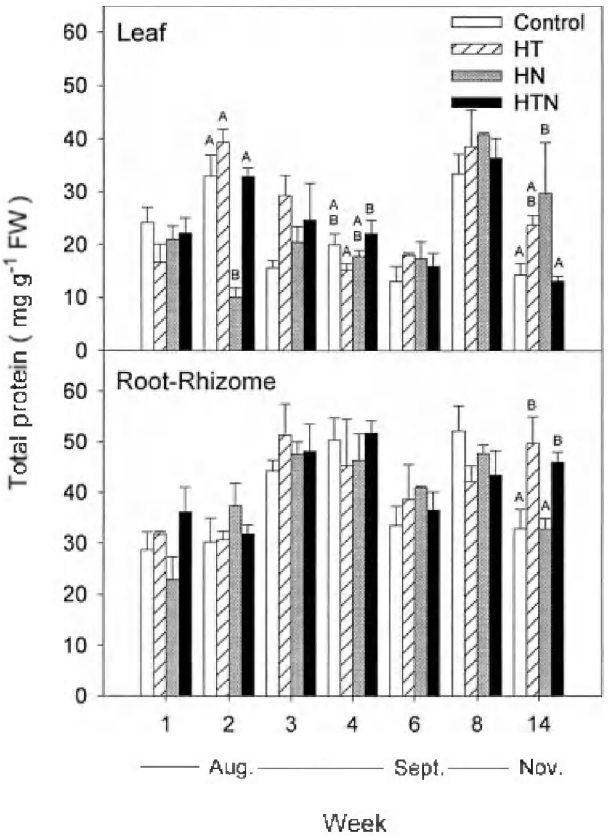

Fig. 5. Seasonal variation in total protein (BSA equivalent) in *Z. marina* leaf- (upper panel), and root/rhizome-tissues (lower panel), in control mesocosms, and in treatments including high temperature (HT), high nitrate (HN), and high temperature and nitrate (HTN). Letters above the error bars represent the statistical relationship within each sample period (e.g., all bars with A are considered similar for that date). Data are given as means ± 1 SE.

Table II. Amino acid composition (% of major constituent pool) in *Z. marina* tissues for control plants and treated plants in high temperature (HT), high nitrate (HN), and high temperature and nitrate (HTN; Sept., week 8; means ± 1 SE).

Tissue (amino acid)	Control	HT	HN	HTN
Leaf tissue				
Alanine	0.17 ± 0.15	ND	ND	ND
Aspartic acid	11.9 ± 4.1	19.1 ± 2.7	18.2 ± 5.4	15.9 ± 1.1
Glutamine/Glutamic acid	78.4 ± 5.5	69.8 ± 3.9	70.9 ± 2.7	78.6 ± 2.9
Valine	9.5 ± 1.7	11.0 ± 4.0	10.9 ± 8.1	5.5 ± 2.9
Root/Rhizome tissue				
Alanine	14.2 ± 1.6	22.6 ± 4.4	14.5 ± 6.1	14.1 ± 1.2
Aspartic acid	15.6 ± 1.6	5.5 ± 4.3	11.6 ± 5.9	18.1 ± 4.2
Glutamine/Glutamic acid	65.7 ± 3.4	66.8 ± 4.3	73.9 ± 0.1	60.1 ± 5.3
Valine	4.5 ± 3.3	5.0 ± 2.5	ND	7.8 ± 0.7

Note that alanine was not detected (ND) in all treatments.

riched plants (HN and HTN; $p = 0.04$). A nitrate-by-temperature interaction also was observed in leaf tissue of treated plants during the same period ($p = 0.002$), and in root-rhizome tissues by mid-November ($p = 0.049$). These interactive responses resulted in significantly lower free amino acid levels at higher temperatures under water-column nitrate enrichment. Amino acid analysis of *Zostera marina* tissues with high-voltage electrophoresis revealed that by mid-September, the most prevalent free amino acids consisted of alanine, aspartic acid, glutamine/glutamic acid (not separated using this technique), and valine (Table II).

Protein levels in leaf tissues were affected by an interaction between water-column nitrate and temperature during early September (HTN; Fig. 5; $p < 0.035$), wherein a significant increase in leaf protein content occurred at higher temperatures under nitrate enrichment. In contrast, significant increases in protein content of root-rhizome tissue were later observed under higher temperatures without nitrate enrichment (HT, November; $p = 0.01$).

For all growing conditions, lipid concentrations in aboveground tissues (range 5.7–17.2 mg g^{-1} dry weight) were higher than in belowground tissues (range 2.8–9.5 mg g^{-1} dry weight, based on three sample dates; Table III). However, no significant treatment effects on lipid content were observed.

Concentrations of total soluble carbohydrates were highly variable (Fig. 6). Nevertheless, there was a significant effect of high temperature on soluble carbohydrates in root-rhizome tissues by late August (HT and HTN; $p = 0.049$), followed by a significant nitrate-by-temperature interaction affecting soluble carbohydrates of the root-rhizome complex latter in the experiment (HTN; late September, $p = 0.048$; mid-November, $p = 0.011$).

There were significant treatment effects on non-reducing carbohydrate (primarily sucrose) concentrations in both above- and belowground tissues (Table III). In mid-November significant temperature and nitrate treatment effects on leaf tissue were observed relative to the controls, wherein increased temperature was associated with higher sucrose levels (HT and HTN; $p = 0.01$), and increased nitrate was associated with decreased sucrose (HN and HTN; $p = 0.039$). Moreover, non-reducing sugars significantly increased in root-rhizome tissues under higher temperatures (HT and HTN; $p = 0.017$).

Throughout this investigation, cellulose levels typically ranged between 10 and 20 % dry weight in both above- and belowground tissues. There was no significant change in cellulose of leaf tissue that could be related to either season or treatments (Table I). Nevertheless, both season ($p = 0.006$) and temperature (HT and HTN; $p = 0.026$) significantly affected cellulose content in belowground tissues. As the season progressed, root-rhizome cellulose content significantly

Table III. Sucrose, α-cellulose, and lipid concentrations in aboveground (above) and belowground (below) tissues of *Z. marina*.

Date	Tissue	Control	HT	HN	HTN
Sucrose (mg g^{-1} FW)					
Aug.	above	4.2 ± 0.7	8.7 ± 1.9	5.6 ± 1.6	4.5 ± 0.6
week-3	below	24.7 ± 2.3	18.8 ± 0.9	20.8 ± 5.3	29.3 ± 1.9
Sept.	above	24.9 ± 4.7	28.7 ± 7.9	23.4 ± 2.5	22.9 ± 6.2
week-8	below	27.0 ± 3.3	31.5 ± 9.2	18.6 ± 3.3	28.1 ± 2.8
Nov.	above	3.5 ± 0.2	8.7 ± 1.1^{T}	2.6 ± 1.0^{N}	5.0 ± 1.4$^{T\&N}$
week-14	below	12.0 ± 3.4	23.5 ± 5.4^{T}	8.8 ± 1.8	15.5 ± 1,8^{T}
α-Cellulose (% dry weight)					
Aug.	above	16.8 ± 3.0	26.1 ± 5.6	18.1 ± 1.6	16.7 ± 0.9
week-3	below	14.0 ± 1.3	14.8 ± 0.8	16.8 ± 2.8	13.5 ± 1.3
Sept.	above	22.2 ± 4.7	19.2 ± 1.1	28.6 ± 1.0	18.3 ± 1.0
week-8	below	15.5 ± 1.4	14.7 ± 0.9	14.4 ± 2.7	12.5 ± 0.6
Nov.	above	20.8 ± 0.02	17.2 ± 1.0	17.2 ± 5.2	21.7 ± 4.5
week-14	below	20.5 ± 1.1	16.6 ± 1.5^{T}	25.4 ± 5.7	16.3 ± 0.6^{T}
Lipids (mg g^{-1} dry weight)					
Aug.	above	7.0 ± 1.3	8.1 ± 0.3	6.8 ± 0.4	6.5 ± 0.5
week-3	below	3.7 ± 0.2	4.0 ± 0.5	3.9 ± 0.6	3.9 ± 0.3
Sept.	above	12.4 ± 2.4	8.2 ± 0.7	13.7 ± 1.9	8.3 ± 1.4
week-8	below	5.6 ± 1.9	5.9 ± 1.0	4.5 ± 0.7	4.4 ± 1.2
Nov.	above	14.1 ± 3.8	11.3 ± 0.8	8.7 ± 1.0	13.4 ± 3.5
week-14	below	5.6 ± 0.3	5.2 ± 0.9	7.0 ± 1.1	4.9 ± 1.0

Data are given as means ± 1 SE (n = 3). Significant differences are indicated by T (for temperature effects) and N (for nitrate effects). HT, high temperature; HN, high nitrate; HTN, high temperature and nitrate.

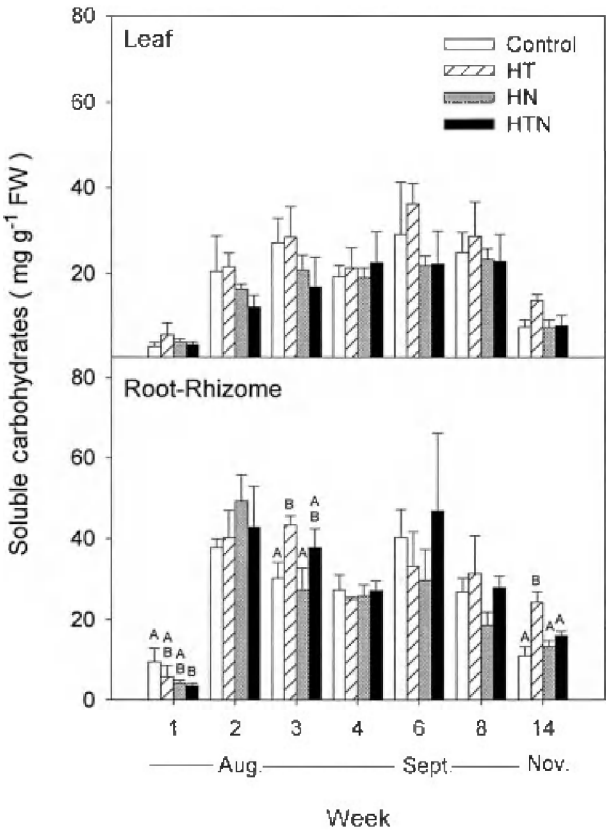

Fig. 6. Seasonal variation in total soluble carbohydrates (sucrose equivalent) in *Z. marina* leaf- (upper panel), and root/rhizome-tissues (lower panel), in control mesocosms, and in treatments including high temperature (HT), high nitrate (HN), and high temperature and nitrate (HTN). Letters above the error bars represent the statistical relationship within each sample period (e.g., all bars with A are considered similar for that date). Data are given as means ± 1 SE.

increased in both controls and treated plants. However, in high temperature treatments, cellulose accumulation in belowground structures was substantially less than for plants that were grown at ambient temperatures.

Discussion

In aquatic systems, due to the reduced nature of sediment pore water, ammonium generally is the dominant form of inorganic nitrogen available to belowground tissues (Riley and Chester 1989). Nitrate typically is localized within the water-column such that, for submersed plants, uptake and reduction of nitrate often are restricted to aboveground tissues. In this study, tissue nitrate concentrations were approximately twice as high in aboveground tissues as in belowground structures. Nevertheless, leaf nitrate levels tended to be low in comparison to values observed in certain terrestrial plants. For example, nitrate concentrations in *Zostera marina* ranged from below detection to 6.8 μg g^{-1} FW in aboveground tissue, whereas values ranging from 0.90 μg to 4.3 mg NO_3^- g^{-1} FW have been observed in certain crop plants (i.e., corn [*Zea mays* L.], tomato [*Lycopersicon esculentum* Mill.], spinach [*Spinacea oleracea* L.], and lettuce [*Lactuca sativa* L.]; Steingrover *et al.* 1982, Bellaloui and Pilbeam 1991, Teyker *et al.* 1991, Gaudreau *et al.* 1995). The low nitrate levels observed in *Zostera marina* suggest that under these growing conditions, nitrate tends to be reduced and assimilated relatively quickly, in contrast to plants that tend to accumulate nitrate in vacuoles prior to reduction (Miller and Smith 1992, Zhen *et al.* 1992). Our data demonstrated a strong seasonal trend in tissue nitrate content of eelgrass. Tissue levels tended to be highest early in the growing season (early August), and slowly declined to levels below detection by the end of September (< 2 ng mL^{-1} extract). The accumulation of nitrate during warmer periods may have reflected decreased activities of enzymes involved in nitrogen assimilation at higher temperatures (e.g., nitrate reductase and glutamine synthetase), especially for plants that inhabit cooler climates (Laurie and Stewart 1990, Gao *et al.* 1993, Touchette *et al.* 1996).

Like tissue nitrate concentrations, tissue ammonium levels were strongly dependent on season (via a quadratic response). Tissue ammonium content remained fairly stable (between 50 to 75 μg g^{-1} FW) until late September when levels began to increase (up to 150 μg g^{-1} FW), followed by a significant decrease in ammonium content by mid-November (near 25 μg g^{-1} FW). Little is known about ammonium accumulation and assimilation in seagrasses. Ammonium levels in these tissues were comparable to concentrations observed in terrestrial plants, which typically range from 8 to 790 μg g^{-1} FW (e.g., *Zea mays* L. and *Citrus* spp. tissues; Magalhaes and Huber 1991, Ali and Lovatt 1995).

Amino acid levels in *Zostera marina* were comparable to those observed in other seagrasses. In *Z. capricorni* Aschers., amino acid concentrations have been reported to range from 0.3 to 2.5 mg g^{-1} FW in leaf tissue (data converted to glutamine equivalents; Udy and Dennison 1997a and b). In *Halodule pinifolia* (Miki) den Hartog shoot tissue amino acids ranged from 1.6 to 2.6 mg g^{-1} FW (Longstaff and Dennison 1999). However, higher levels of free amino acids were reported in *Posidonia australis* Hook. F., approaching 15 mg g^{-1} FW (data converted to fresh weight by assuming 80 % water content; Augier *et al.* 1982). Amino acid levels in *Zostera marina* tissue (this study) ranged from 5.0 μg g^{-1} FW to 3.0 mg g^{-1} FW in leaves, and from 4.0 μg g^{-1} FW to over 7.0 mg g^{-1} FW in root-rhizome tissues. This study revealed strong seasonal variations in free amino acid concentrations. Prior to initiation of autumn growth, amino acid levels were < 1 mg g^{-1} FW. Amino acid concentrations steadily increased until mid–September, corresponding to periods of increased plant growth and development. There was a decrease in free amino acids toward the

end of the growing season as plant productivity began to subside. An apparent inverse relationship between leaf tissue nitrate and free amino acid concentrations was also observed throughout this study. That is, while tissue nitrate concentrations were high early in the growing season and then slowly decreased, amino acid levels were low initially and then slowly increased. If lower enzyme activity had limited tissue nitrate reduction during warmer periods, then lower activities of other enzymes involved in N assimilation similarly may have restricted the formation/accumulation of amino acids (Touchette 1999). Increased temperature and/or elevated water-column nitrate periodically affected free amino acid levels, often by elevating free amino acids in both above- and belowground tissues. The temperature response of free amino acids was strongest in belowground tissues during periods associated with rapid plant growth and development.

Total protein content in *Zostera marina* was influenced by season and tissue type. Compared to other seagrasses, these levels were relatively high. In *Phyllospadix scouleri* Hooker leaves, protein levels were as low as 3.8 mg g^{-1} FW (data converted to fresh weight by assuming 80 % water content; Neighbors and Horn 1991). However, in *Thalassia testudinum* König, total protein levels can range from 5 to 26 mg g^{-1} FW in leaf tissue and from 2 to 10 mg g^{-1} FW in belowground structures (Burkholder *et al.* 1959; Durako and Moffler 1985). Few previous studies have contributed seasonal observations on protein content in temperate seagrasses. However, in more tropical species (e.g., *Halodule wrightii* Aschers., *Ruppia maritima* L., *Syringodium filiforme* Kütz., and *Thalassia testudinum*), leaf protein levels were highest in winter or spring – periods associated with relatively high productivity (Lazar and Dawes 1991, Dawes 1986, Durako and Moffler 1985). In this study, protein levels in leaf tissues of *Zostera marina* were highly variable. As observed in other seagrasses, protein content in belowground tissues increased during periods of high growth, with little apparent effect from experimental treatments. By mid-November, there was significantly higher root-rhizome protein in plants grown at higher temperatures compared with control plants. Toward the end of the growing season, protein levels of control plants, like free amino acid concentrations, began to decline. This seasonal response may have been delayed for plants at higher temperatures (i.e., from extension of the growing season) and, thus, significant declines in protein concentrations were not observed in plants subjected to high temperature treatments.

Lipid content typically comprises less than 5 % dry weight in submersed aquatic angiosperms (Dawes *et al.* 1987, Dawes and Lawrence 1989, Lazar and Dawes 1991, Tomasko *et al.* 1993). In this study, eelgrass lipid content ranged from 0.6 to 1.5 % dry weight in leaf tissue, and from 0.4 to 0.7 % dry weight in the root-rhizome complex. There was a small but significant increase in lipid content of *Zostera marina* as the growing season progressed. However, lipid content, although comparatively low, was not significantly affected by either increased temperature and/or water-column nitrate enrichment.

Soluble carbohydrate levels in this southernmost *Zostera marina* ecotype were lower than those reported for more northern eelgrass populations. Kraemer and Alberte (1993), found soluble carbohydrates over 100 mg g^{-1} FW (sucrose equivalent) in *Z. marina* rhizome tissue. Moreover, in the European species *Zostera noltii* Hornem., seasonal variation in leaf tissue carbohydrate ranged from below detection to 150 mg g^{-1} dry weight (Vermaat and Verhagen 1996). Although the present study combined root and rhizome tissue for analysis, the bulk of the belowground tissue was rhizomatous (84.2 ± 3.0 %; on a dry weight basis). Therefore, the relatively low carbohydrate levels reported in root tissue alone (typically less than 7 mg g^{-1} FW; Kraemer and Alberte 1993) could not have accounted for the lower soluble carbohydrate measurements reported as total belowground structures for this study.

There was a strong seasonal response in total soluble carbohydrates of both control and treated eelgrass in this study. Initially, carbohydrate levels were relatively low. However, as plant growth and development progressed, tissue concentrations increased to levels approaching 50 mg g^{-1} FW in the root-rhizome complex and 40 mg g^{-1} FW in leaf tissue (by mid–September). Toward the end of the season (mid-November), carbohydrate concentrations once again declined, and were < 15 mg g^{-1} FW in leaf tissue, and < 30 mg g^{-1} FW for root-rhizome tissue. During this later period, a significant interaction between higher temperature and nitrate was observed in carbohydrate content of the root-rhizome complex. Soluble carbohydrates increased at higher temperatures, and decreased under elevated water-column nitrate. The temperature response likely occurred because of an extension of the growing season (as previously discussed), and/or an enhancement (up-regulation) of enzymes involved in carbon metabolism. The nitrate response, however, may have been associated with increased carbon demands for nitrate reduction and amino acid synthesis (Turpin *et al.* 1991). This association is further supported by similar observations on non-reducing sugars (primarily sucrose) in both above- and belowground tissues. That is, toward the end of the growing season, there was an increase in sucrose levels with increased temperature, and a decrease in sucrose levels under water-column nitrate enrichment relative to the sucrose content of control plants.

Observed levels of structural carbohydrates such as cellulose in *Zostera marina* (ca. 16 to 28 % dry weight in both above- and belowground tissues) were comparable to values reported for seagrass in other investigations. In *Halophila engelmannii* Aschers., Dawes *et al.* (1987) reported cellulose values ranging from 11.6 to 12.3 % dry weight in shoots, 20.5 to 25.0 % in rhi-

zomes, and 8.4 to 16.9 % in roots. In this study, treatment effects were observed only in belowground tissues; near the end of the autumn growing season, there was a significant decrease in cellulose content of experimental (higher temperature) plants. Lower cellulose content is uncharacteristic of cool-temperature plants during periods of elevated temperature (Ford *et al.* 1979). That is, temperate plants tend to increase cellulose content at higher temperatures, whereas tropical plants tend to decrease cellulose concentrations (Ford *et al.* 1979, Fales 1986, Roberts *et al.* 1992). The data from this experiment suggest that *Zostera marina* plants grown under normal temperatures have higher cellulose content than plants grown in warmer waters. This increase in cellulose coincides with a significant increase in new shoot productivity for these plants, suggesting that increasing plant structural integrity is important for new shoot development.

Overall, many of the carbon and nitrogen constituents in *Zostera marina* showed seasonal variations. Compounds such as amino acids, proteins, and carbohydrates generally increased during periods of maximum productivity. It is likely that ample supply of these compounds is necessary in order to achieve maximum growth. Another prominent trend in both above- and belowground tissues was increased amino acids, proteins, and carbohydrates toward the end of the growing season in plants subjected to elevated temperatures. This response may have occurred because of an effective extension of the growing season, wherein plants under elevated temperatures continued to maintain relatively high productivity relative to plants at ambient temperature. The influence of elevated temperatures on nitrogen compounds may, in part, result from decreased activity of important enzymes involved in nitrogen metabolism during periods of high temperature (e.g., nitrate reductase and glutamine synthetase; Touchette and Burkholder 2000). Such decreases in enzyme activity may account for the higher tissue nitrate and lower free amino acids in late summer. As temperature inhibition of these important enzymes was alleviated (later in the growing season), tissue nitrate levels began to decline and a concomitant increase in amino acid levels was observed. In this study relatively low levels of tissue NO_3^- were observed throughout the growing season, even in plants that were exposed to elevated water-column NO_3^-. These low tissue levels indicate that these plants were not storing NO_3^-, but rather, were immediately reducing NO_3^- upon entry into the plant. The immediate reduction of NO_3^- is consistent with observations by Burkholder *et al.* (1992, 1994), wherein *Zostera marina* has little feedback inhibition for NO_3^- reduction, thereby preventing tissue NO_3^- accumulation (Roth and Pregnall 1988). The decrease in leaf sucrose levels for NO_3^- enriched *Z. marina* by November (week-14) is also consistent with a "carbon drain" mechanism, wherein carbon compounds would be required to supply reducing energy for NO_3^- reduction and carbon skeletons for amino acid synthesis (Burkholder *et al.* 1992, 1994).

Acknowledgements

Funding support for this research was provided by the North Carolina Sea Grant College Program, the North Carolina General Assembly, the North Carolina Agricultural Research Service, and the North Carolina State University Department of Botany. We are grateful for the help provided by a number of individuals. Most of all, we would like to thank D. Briley, T. Brister, J. Compton, N. Deamer-Melia, E. Fensin, H. Glasgow, E. Hannon, M. Larson, M. Mallin, G. Morgan, J. Springer, and H. Williams for their assistance in harvesting and transplanting seagrass. T. Brister, G. Burrows, J. Colton, S. McCarthy, and D. Melanson assisted in sample preparation and collections. We also thank C. Brownie, S. Chilton, E. Davies, H. Glasgow Jr., J. Huber, D. Pharr, C. Proctor, D. Tomasko, and R. Volk for their scholarly counsel and / or review of the manuscript.

Accepted 1 October 2001.

References

Adams, M. S. 1985. Inorganic carbon reserves of natural waters and the eco-physiological consequences of their photosynthetic depletion: (II) Macrophytes. Chapter 27. *In*: (W. J. Lucas and J. A. Berry, eds) *Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms.* American Society of Plant Physiologists. Waverly Press, Baltimore, MD. pp. 421–435.

Ahlgren, G. and L. M. Uppsala. 1991. Lipid analysis of freshwater microalgae: a methods study. *Arch. Hydrobiol. 121*: 295–306.

Ali, A. G. and C. J. Lovatt. 1995. Evaluating analytical procedures for quantifying ammonium in leaf tissue. *J. Amer. Soc. Hort. Sci. 120*: 871–876.

Augier, H., H. Calvert, E. Wollaston and M. Santimone. 1982. A comparison of the C, H, N, protein and amino acid composition of *Posidonia australis* Hook. F. with that of *Posidonia oceanica* (L.) Delile and several other marine phanerogams. *Aquatic Bot. 12*: 69–80.

Bellaloui, N. and D. J. Pilbeam. 1991. Effects of nitrate withdrawal and resupply on the assimilation of nitrate in leaves of tomato. *J. Exp. Bot. 42*: 81–88.

Bensadoun, A. and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. *Anal. Biochem. 70*: 241–250.

Block, R. J., E. L. Durrum and G. Zweig. 1958. *A Manual of Paper Chromatography and Paper Electrophoresis.* 2nd ed., Academic Press, NY. pp. 710.

Burkholder, P. R., L. M. Burkholder, and J. A. Rivero. 1959. Chemical composition and yields of turtle grass (*Thalassia testudinum*). *Bull. Torrey Bot. Club 86*: 2.

Burkholder, J. M., K. M. Mason and H. B. Glasgow. 1992. Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: evidence from seasonal mesocosm experiments. *Mar. Ecol. Prog. Ser. 81*: 163–178.

Burkholder, J. M., H. B. Glasgow and J. E. Cooke. 1994. Comparative effects of water-column nitrate enrichment on eelgrass *Zostera marina*, shoalgrass *Halodule wrightii*, and widgeongrass *Ruppia maritima*. *Mar. Ecol. Prog. Ser. 105*: 121–138.

Clayton, J. R., Q. Dortch, S. S. Thoresen and S. I. Ahmed. 1988. Evaluation of methods for the separation and analysis of proteins and free amino acids in phytoplankton samples. *J. Plank. Res. 10*: 341–358.

Coombs, J., G. Hind, R. C. Leegood, L. L. Tieszen and A. Vonshak. 1985. Analytical Techniques. *In*: (J. Coombs, D. O. Hall, S. P. Long and J. M. O. Scurlock, eds) *Techniques in Bioproductivity and Photosynthesis*. 2nd ed. Pergamon Press, New York. pp. 219–228.

Dawes, C. J. 1986. Seasonal proximate constituents and caloric values in seagrasses and algae on the west coast of Florida. *J. Coastal Res. 2*: 25–32.

Dawes, C. J. and J. M. Lawrence. 1989. Allocation of energy resources in the freshwater angiosperms *Vallisneria americana* Michx. and *Potamogeton pectinatus* L. in Florida. *Fl. Sci. 52*: 58–63.

Dawes, C., M. Chan, R. Chinn, E. W. Koch, A. Lazar and D. Tomasko. 1987. Proximate composition, photosynthetic and respiratory responses of the seagrass *Halophila engelmannii* from Florida. *Aquatic Bot. 27*: 195–201.

Den Hartog, C. 1970. *The Seagrasses of the World.* North Holland, Amsterdam.

Dennison, W. C. 1987. Effects of light on seagrass photosynthesis, growth and depth distribution. *Aquatic Bot. 27*: 15–26.

Dennison, W. C. and R. S. Alberte. 1985. Role of daily light period in the depth distribution of *Zostera marina* (eelgrass). *Mar. Ecol. Prog. Ser. 25*: 51–61.

Durako, M. J. and M. D. Moffler. 1985. Spatial influences on temporal variations in leaf growth and chemical composition of *Thalassia testudinum* Banks ex König in Tampa Bay, Florida. *Gulf Research Reports 8*: 43–49.

Fales, S. L. 1986. Effects of temperature on fiber concentration, composition, and *in vitro* digestion kinetics of tall fescue. *Agron. J. 78*: 963–966.

Ford, C. W., I. M. Morrison and J. R. Wilson. 1979. Temperature effects on lignin, hemicellulose and cellulose in tropical and temperate grasses. *Aust. J. Agric. Res. 30*: 621–633.

Fougere, F., D. LeRudulier and J. G. Streeter. 1991. Effects of salt stress on amino acid, organic acid, and carbohydrate composition of roots, bacteroids, and cytosol of alfalfa (*Medicago sativa* L.). *Plant. Physiol. 96*: 1228–1236.

Gao, Y., G. J. Smith and R. S. Alberte. 1993. Nitrate reductase from marine diatom *Skeletonema costatum*: biochemical and immunological characterization. *Plant Physiol. 103*: 1437–1445.

Gaudreau, L., J. Charbonneau, L. P. Vezina and A. Gosselin. 1995. Effects of photoperiod and photosynthetic photon flux on nitrate content and nitrate reductase activity in greenhouse-grown lettuce. *J. Plant Nutrition 18*: 437–453.

Guerrero, M. G. 1987. Assimilatory nitrate reduction. *In*: (J. Coombs, D. O. Hall, S. P. Long and J. M. O. Scurlock, eds) *Techniques in Bioproductivity and Photosynthesis*, 2nd ed. Pergamon Press, New York. pp 165–172.

Guisande, C. and L. Serrano. 1989. Analysis of protein, carbohydrate and lipid in rotifers. *Hydrobiologia 186/187*: 339–346.

Kesner, L. and D. Kirschenbaum. 1970. Amino acids, polypeptides, and proteins. *In*: (C. A. Streuli and P. R. Averell, eds) *The Analytical Chemistry of Nitrogen and Its Compounds*, Vol. 2. Wiley-Interscience Press, New York. pp. 635–704.

Kraemer, G. P. and R. S. Alberte. 1993. Age-related patterns of metabolism and biomass in subterranean tissues of *Zostera marina* (eelgrass). *Mar. Ecol. Prog. Ser. 95*: 193–203.

Lambers, H. 1985. Respiration in intact plant tissues: its regulation and dependence on environmental factors, metabolism and invaded organisms. *In*: (R. Douce and D. A. Day, eds) *Encyclopedia of Plant Physiology, New Series, Higher Plant Cell Respiration*. Springer-Verlag, Berlin. pp. 418–474.

Laurie, S. and G. R. Stewart. 1990. The effects of compatible solutes on the heat stability of glutamine synthetase from chickpeas grown under different nitrogen and temperature regimes. *J. Exp. Bot. 41*: 1415–1422.

Lazar, A. C. and C. J. Dawes. 1991. A seasonal study of the seagrass *Ruppia maritima* L. in Tampa Bay, Florida. Organic constituents and tolerances to salinity and temperature. *Bot. Mar. 34*: 265–269.

Levavasseur, G., G. E. Edwaeds, C. B. Osmond and J. Ramus. 1991. Inorganic carbon limitation of photosynthesis in *Ulva rotundata* (Chlorophyta). *J. Physiol. 27*: 667–672.

Lingle, S. E. 1987. Sucrose metabolism in the primary culm of sweet sorghum during development. *Crop Sci. 27*: 1214–1219.

Longstaff, B. J. and W. C. Dennison. 1999. Seagrass survival during pulsed turbidity events: the effects of light deprivation on the seagrasses *Halodule pinifolia* and *Halophila ovalis*. *Aquatic Bot. 65*: 105–121.

Lustinec, J., V. Hadacova, M. Kaminek and Z. Prochazaka. 1983. Quantitative determination of starch, amylose, and amylopectin in plant tissues using glass fiber paper. *Analytical Biochem. 132*: 265–271.

Magalhaes, J. R. and D. M. Huber. 1991. Free ammonia, free amino acids, and enzyme activity in maize tissue treated with methionine sulfoximine. *J. Plant Nutrition 14*: 883–895.

Marsh, J. A., W. C. Dennison and R. S. Alberte. 1986. Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). *J. Exp. Mar. Biol. Ecol. 101*: 257–267.

Meredith, M. P. and Stehman. 1991. Repeated measures experiments in forestry: Focus on analysis of response curves. *Can. J. For. Res. 21*: 957–965.

Miller, A. J. and S. J. Smith. 1992. The mechanism of nitrate transport across the tonoplast of barley root cells. *Planta 187*: 554–557.

Morris, L. J. and D. Tomasko. 1993. *Proceedings and Conclusions of Workshops on: Submersed Aquatic Vegetation and Photosynthetically Active Radiation*. Special Publication SJ93–SP13. Palatka, FL.: St. Johns River Water Management District. pp. 244.

National Oceanic and Atmospheric Administration [NOAA]. 1994. *Local Climatic Data-Weekly and Monthly Summaries for Beaufort, North Carolina, and Water Temperature Data for Beaufort Inlet.* National Marine Fisheries Service (W. Heller), Beaufort, NC.

Neighbors, M. A. and M. H. Horn. 1991. Nutritional quality of macrophytes eaten and not eaten by two temperate-

zone herbivorous fishes: a multivariate comparison. *Mar. Biol. 108*: 471–476.

Peterson, G. L. 1977. A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. *Anal. Biochem. 83*: 346–356.

Raab, T. K. and N. Terry. 1995. Carbon, nitrogen, and nutrient interactions in *Beta vulgaris* L. as influenced by nitrogen source, NO_3^- versus NH_4^+. *Plant Physiol. 107*: 575–584.

Riley, J. P. and R. Chester. 1989. *Introduction to Marine Chemistry.* Academic Press. New York, NY. pp. 465.

Roberts, E. M., N. R. Rao, J. Y. Huang, N. L. Trolinder and C. H. Haigler. 1992. Effects of cycling temperatures on fiber metabolism in cultured cotton ovules. *Plant Physiol. 100*: 979–986.

Rosenthal, G. A. 1982. *Plant Nonprotein Amino and Imino Acids: Biological, Biochemical, and Toxicological Properties.* Academic Press, NY. pp. 273.

Roth, N. C. and A. M. Pregnall. 1988. Nitrate reductase activity in *Zostera marina. Mar. Biol. 99*: 457–463.

Shimazaki, K. S. W. Yu, T. Sakaki and K. Tanaka. 1992. Differences between spinach and kidney bean plants in terms of sensitivity to fumigation with NO_2. *Plant Cell Physiol. 33*: 267–273.

Short, F. T. 1983. The response of interstitial ammonium in eelgrass (*Zostera marina*) beds to environmental perturbations. *J. Exp. Mar. Biol. Ecol. 68*: 195–208.

Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia and F. H. Gartner. 1985. Measurement of protein using bicinchoninic acid. *Anal. Biochem. 150*: 76–85.

Solórzano, L. 1969. Determination of ammonium in natural waters by the phenol-hypochlorite method. *Limnol. Oceanogr. 14*: 799–801.

Southgate, D. A. T. 1976. *Determination of Food Carbohydrates.* Applied Science Publishers LTD, Essex England. pp. 178.

Souza, J. G. and J. V. Silva. 1987. Protein and carbohydrate changes in cotton leaves linked with age and development. *Trop. Agric. 64*: 46–48.

Steingrover, E., R. Osterhuis and F. Wieringa. 1982. Effect of light treatment and nutrition on nitrate accumulation in spinach (*Spinacea oleracea* L.). *Z. Pflanzenphysiol. 107*: 97–102.

Teyker, R. H., K. A. Dallmeir, G. R. St. Aubin and R. J. Lambert. 1991. Seedling nitrate reductase activity and nitrate partitioning in maize (*Zea mays* L.) strains divergently selected for post-anthesis leaf-lamina nitrate reductase activity. *J. Exp. Bot. 42*: 97–102.

Thayer, G. W., W. J. Kenworthy and M. S. Fonseca. 1984. *The Ecology of Eelgrass Meadows of the Atlantic Coast: A Community Profile.* United States Department of the Interior, Fish and Wildlife Report # FWS/OBS-84/02, Washington, DC. pp. 147.

Tomasko, D. A., C. J. Dawes, M. D. Fortes, D. B. Largo and M. N. R. Alava. 1993. Observations on a multi-species seagrass meadow offshore of Negros Oriental, Republic of the Philippines. *Bot. Mar. 36*: 303–311.

Touchette, B. W. 1999. Physiological and Developmental Responses of *Zostera marina* L. to Increases in Temperature and Water-Column Nitrate. Doctorate of Philosophy Dissertation, North Carolina State University, Raleigh, NC.

Touchette, B. W. and J. M. Burkholder. 2000. Review of nitrogen and phosphorus metabolism in seagrasses. *J. Exp. Biol. Mar. Ecol. 250*: 133–167.

Touchette, B. W. J. M. Burkholder and H. B. Glasgow Jr. 1996. The physiological responses of the seagrass, *Zostera marina* L., to increased water-column nitrate and increased temperature (abstract No. 30). *Plant Physiol. 111*: S–37.

Turpin, D. H., G. C. Vanlerberghe, A. M. Amory and R. D. Guy. 1991. The inorganic carbon requirements for nitrogen assimilation. *Can. J. Bot. 69*: 1139–1145.

Udy, J. W. and W. C. Dennison. 1997a. Growth and physiological responses of three seagrass species to elevated sediment nutrients in Moreton Bay, Australia. *J. Exp. Biol. Mar. Ecol. 217*: 253–277.

Udy, J. W. and W. C. Dennison. 1997b. Physiological responses of seagrasses used to identify anthropogenic nutrient inputs. *Mar. Freshwater Res. 48*: 605–614.

Van Handel, E. 1968. Direct microdetermination of sucrose. *Anal. Biochem. 22*: 280–283.

van Lent, F., J. M. Verschuure and M. L. J. van Veghel. 1995. Comparative study on populations of *Zostera marina* L. (eelgrass): *in situ* nitrogen enrichment and light manipulation. *J. Exp. Mar. Biol. Ecol. 185*: 55–76.

Vermaat, J. E. and C. A. Verhagen. 1996. Seasonal variation in the intertidal seagrass *Zostera noltii* Hornem.: coupling demographic and physiological patterns. *Aquatic Bot. 52*: 259–281.

Vestal, J. R. and D. C. White. 1989. Lipid analysis in microbial ecology; quantitative approaches to the study of microbial communities. *Bioscience 39*: 535–541.

Yemm, E. W. and A. J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. 57*: 508–514.

Zhen, R. G., S. J. Smith and A. J. Miller. 1992. A comparison of nitrate-selective microelectrodes made with different nitrate sensors and the measurement of intracellular nitrate activities in cells of excised barley roots. *J. Exp. Bot. 43*: 131–138.

Zimmerman, R. C., R. D. Smith and R. S. Alberte. 1987. Is growth of eelgrass nitrogen limited? A numerical simulation of the effects of light on the growth dynamics of *Zostera marina. Mar. Ecol. Prog. Ser. 41*: 167–176.

Zimmerman, R. C., R. D. Smith and R. S. Alberte. 1989. Thermal acclimation and whole-plant carbon balance in *Zostera marina* L. (eelgrass). *J. Exp. Mar. Biol. Ecol. 130*: 93–109.