

A Field Study of Nitrogen Storage and Nitrate Reductase Activity in the Estuarine Macroalgae *Enteromorpha lingulata* (Chlorophyceae) and *Gelidium pusillum* (Rhodophyceae)

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ABSTRACT: The purpose of this field study was to determine the relationship between environmental conditions, particularly high nitrate (NO_3^-), low salinity events, and both nitrogen (N) storage (NO_3^- , ammonium [NH_4^+], free amino acids [FAA], protein, and total N) and nitrate reductase (NR) activity in the macroalgae *Enteromorpha lingulata* and *Gelidium pusillum* in the lower Mobile Bay estuary (Alabama, USA). The environmental conditions at the collection site varied over the growing season with the most notable changes due to late winter and spring runoff entering the estuary (1–30 psu, 0.3–25.8 μM NO_3^- , 0.9–12.5 μM NH_4^+ , 3–28°C, 61–2,375 $\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$). Principal component analysis reduced the six environmental variables measured to three principal components. Stepwise, multiple regression analysis was then used to examine the relationship between the principal components and the internal NO_3^- , NH_4^+ , and FAA pools and NR activity. The results indicate that changes in inorganic N availability and salinity rather than changes in irradiance determine patterns of N storage and NO_3^- reduction. Both *E. lingulata* and *G. pusillum* are capable of taking up and storing NO_3^- when it becomes available. Greater NO_3^- availability produced larger NH_4^+ and FAA pools along with higher rates of NR activity in *E. lingulata*, but not *G. pusillum*, suggesting that *E. lingulata* is able to metabolize NO_3^- more rapidly during high NO_3^- , low salinity events. Differences in the susceptibility of *E. lingulata* and *G. pusillum* to NH_4^+ inhibition and salinity stress combined with their different growth strategies help to explain the seasonal trends in total N. Total N in *E. lingulata* ranged from 2.57% to 6.39% dw, while the slower growing *G. pusillum* showed no significant variation in total N content (3.8–4.1% dw). These results led to the conclusion that *E. lingulata* responds more quickly than *G. pusillum* to high NO_3^- , low salinity events and that these events have a larger effect on the overall N content of *E. lingulata*.

Introduction

Although estuaries may not always have diverse macroalgal assemblages, macroalgae are common, if not abundant, in estuaries worldwide (Owens and Stewart 1983; Lee and Olsen 1985; Lavery and McComb 1991; Sfriso et al. 1992). Even if macroalgae are not present year-round, macroalgae can affect how an estuary functions (Soulsby et al. 1982; Fong et al. 1993). Macroalgae can make substantial contributions to total primary production and are the most abundant primary producers in some estuaries (Peckol and Rivers 1996; Sfriso and Marcomini 1997; Valiela et al. 1997). In estuaries where nutrient levels are enriched, extensive macroalgal blooms can occur with their subsequent decomposition leading to anoxia (Fletcher 1996; Valiela et al. 1997).

One nutrient that can be limiting to estuarine macroalgae and other estuarine primary producers is nitrogen (N; Wheeler and Björnsäter 1992; Fong et al. 1993; Pedersen and Borum 1996). When N is

available, it is often in the form of inorganic N, either nitrate (NO_3^-) or ammonium (NH_4^+). In estuaries where inorganic N availability varies, N may be limiting only during a portion of the growing season (Wheeler and Björnsäter 1992; Fong et al. 1993; Pedersen 1995) or in a portion of the estuary (Kamer et al. 2004). Even when it is not limiting growth, the availability of inorganic N can influence the total N of estuarine macroalgae as well as the size of the different intracellular, N storage pools (Wheeler and Björnsäter 1992; Naldi and Wheeler 1999).

Many studies have focused on how estuarine macroalgae respond to changes in salinity (Kamer and Fong 2000, 2001), temperature (Rivers and Peckol 1995), inorganic N availability (Riccardi and Solidoro 1996), and irradiance (Henley 1992). These studies have often investigated how growth rates or total N change under different treatments in the laboratory (Floreto et al. 1994; Fong et al. 1996) or over the growing season in the field (Kamer et al. 2001). Only a few studies have explored how inorganic N storage or NO_3^- reduction in estuarine macroalgae varies with envi-

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ronmental conditions in the field (Thompson and Valiela 1999; Naldi and Viaroli 2002). This study is unique in that it explores how internal N pools and NO_3^- reduction respond to several environmental factors over an entire year in an estuary where high NO_3^- , low salinity events are common.

The Mobile Bay estuary, Alabama, USA, is a shallow, subtropical estuary where several species of macroalgae including *Enteromorpha lingulata*, *Ulva* sp., *Gelidium pusillum*, and *Cladophora* sp. can be found. To compare the response by two species with different growth strategies to changing environmental conditions, we chose to focus on *E. lingulata*, a fast-growing, ephemeral, green alga, and *G. pusillum*, a slow-growing, red alga. High NO_3^- , low salinity events are known to occur in the Mobile Bay estuary following later winter and spring rains. Annual fluctuations in the estuary for salinity, NO_3^- availability, NH_4^+ availability, and water temperature are 0–32 psu, 0–18 μM , 0–14 μM , and 10–30°C, respectively (Pennock et al. 1999).

The purpose of this field study was to determine whether both inorganic nitrogen and free amino acid (FAA) pools and the rate of NO_3^- reduction in *E. lingulata* and *G. pusillum* change in response to these changing environmental conditions, particularly during high NO_3^- , low salinity events. This study also sought to determine the relationship between the macroalgal response and protein content and total N.

Materials and Methods

On the east end of Dauphin Island, Alabama (30°15.015'N, 88°04.759'W) in the lower Mobile Bay estuary, *E. lingulata* and *G. pusillum* can be found growing on rocks and oyster shells. To determine the relationship between the N content of these two species and their environment, the size of several internal N pools and nitrate reductase (NR) activity were measured along with several environmental variables over one year. Measurements were taken daily (3 h after sunrise) for 8 to 10 d periods spaced throughout the year. Algal stands were sampled in October (October 26–November 4, 2000), January (January 19–27, 2001), March (March 14–22, 2001), April (April 25–May 4, 2001), and July (July 19–28, 2001).

Algal tissue was haphazardly collected from the middle of an algal stand and rinsed to remove invertebrates and sediment. Fresh weight (fw) was measured after tissue samples had been pressed firmly between paper towels. Dry weight was determined after drying for 24 h at 80°C and averaged 12.0% of fw (95% CI = 2.7%, n = 113) for *E. lingulata* and 25.5% of fw (95% CI = 2.3%, n = 27) for *G. pusillum*.

NR activity was assayed in the field using an in situ NR activity assay (Lartigue and Sherman 2002). Briefly, 0.1 to 0.3 g fw of tissue was incubated for 1 h in the dark in artificial seawater (20 psu) containing 30 mM KNO_3 and either 2.25% n-propanol (*E. lingulata*) or 3.0% n-propanol (*G. pusillum*). The production of nitrite (NO_2^-) was then quantified and NR activity expressed as $\mu\text{mol NO}_2^- \text{ g dw}^{-1} \text{ h}^{-1}$. The assay tubes were incubated at air temperature when the algae had been exposed at the time of collection or at water temperature when the algae had been submerged. Eight measurements were taken each day. While the NR activity assay was incubating, the remaining algal tissue was transported back to the Dauphin Island Sea Lab (c. 5 min).

Tissue NO_3^- , NO_2^- , and NH_4^+ and FAA were extracted from 0.4 g fw of algae homogenized using a OMNI 2000 homogenizer (OMNI International, Warrenton, Virginia) in 45 ml of deionized water. Five measurements were taken each day. The homogenate was boiled for 10 min, extracted for 24 h at 4°C, and filtered through a glass fiber filter (GF/C) before storage at –80°C. The filtrate was later analyzed for NO_3^- , NO_2^- , and NH_4^+ concentration on a Sans^{plus}Systems autoanalyzer (SKALAR, Norcross, Georgia; SKALAR 1996). The concentration of FAA was determined on a Perkin Elmer Luminescence Spectrometer (LS50B; Shelton, Connecticut) according to the fluorometric method outlined in Parsons et al. (1984) with glycine as the standard (SigmaUltra > 99%, Sigma, St. Louis, Missouri). To express the amount of FAA in $\mu\text{mol N}$, 1 μmol of glycine was considered equal to 1 μmol of FAA and 1 μmol of glycine-N (glycine has only one N atom) was considered equal to 1 μmol of FAA-N.

On one day during each monthly collection, protein content (7 samples) and total carbon (C) and N (5 samples) were measured. For the protein analysis, 0.04 to 0.06 g of tissue was ground into a fine powder using liquid nitrogen and a mortar and pestle. This powder was then suspended in 5 ml of 1% sodium dodecyl sulfate and extracted overnight at 4°C. The extracts were then centrifuged ($\times 1,170 \text{ g}$) for 20 min and the supernatant analyzed for protein content using the bicinchoninic acid (BCA) assay with bovine serum albumin (BSA, Fraction V Powder > 96%, Sigma, St. Louis, Missouri) as the standard (Smith et al. 1985). In order to express the amount of protein in μmol of N, we considered 1 g of BSA equal to 1 g of protein and used the total N ($14.40 \pm 0.05\%$ dw [standard error], n = 5) of BSA determined on a Carlo Erba CNS analyzer (NA 1500 Series 2; Saddle Brook, New Jersey) to convert the amount of BSA into an amount of N. Total C and N in *E. lingulata* and *G.*

TABLE 1. Environmental conditions at the *Enteromorpha lingulata* collection site. Values are mean (\pm SE) and asterisks indicate the level of significant difference among months (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The letters next to each mean indicate months that were found to be significantly different ($p < 0.05$) by post-hoc, pairwise comparisons. Negative depth values indicate the distance between exposed *E. lingulata* and the surface of the water.

Variable	October	January	March	April
Temperature ($^{\circ}$ C) ***	23.50 (0.28)a	9.41 (1.54)b	15.54 (0.82)c	20.97 (0.47)d
Depth (cm) ***	-0.76 (1.38)a	-18.00 (6.45)b	8.42 (5.11)a, c	12.60 (2.51)c
Irradiance (μ mol m^{-2} s^{-1})**	1715.31 (62.53)a	1772.09 (328.18)a	816.05 (284.11)a, b	787.25 (96.18)b
Salinity (psu) ***	28.56 (0.34)a	16.56 (1.83)b	3.51 (0.65)c	12.00 (1.38)b
Water column NO_3^- (μ M) ***	1.14 (0.11)a	4.50 (1.99)a	13.92 (2.24)b	0.79 (0.14)a
Water column NH_4^+ (μ M) ***	4.39 (0.55)a, b	3.33 (0.85)a	6.09 (0.92)b	2.38 (0.22)a

pusillum were determined using a Carlo Erba CNS analyzer after the algal tissue was dried at 80° C for at least 24 h and ground into a fine powder using a mortar and pestle. Drying the algae at 80° C may have led to the volatilization of some N compounds, particularly inorganic compounds. As a result, total N, the only measure where the algae was dried prior to determination, and by extension % N may be underestimated.

Prior to collecting the algae, temperature, water depth, irradiance, salinity, and water column NO_3^- , NO_2^- , and NH_4^+ concentrations were measured. Temperature was measured at the same water depth as the algal stands. Water depth was measured as the vertical distance between the center of the algal stand and the surface of the water. When the algae were submerged, this distance was recorded as a positive value and when the algae were exposed as a negative value. To test the relationship between irradiance at the time of algal collection and short-term N storage (internal NO_3^- , NH_4^+ , and FAA pools) and metabolism (NR activity), a measure of irradiance was needed that captured the irradiance history immediately preceding algal collection. Over the 30 min preceding algal collection, the mean irradiance for six consecutive 5 min periods was recorded and the average of those means was considered the irradiance for that day. Irradiance was measured at the same water depth as the center of the algal stand using a LI-193SA spherical underwater quantum sensor attached to a LI-1000 data logger when the algae were submerged and a LI-192SA spherical quantum sensor when the algae were exposed to air (LICOR, Lincoln, Nebraska). Salinity was measured and water samples were collected within 1 m of the algal stands at the same water depth as the stand when stands were submerged and from right below the water's surface when stands were exposed. These water samples were filtered through a glass fiber filter (GF/C), frozen at -80° C, and later analyzed for NO_3^- , NO_2^- , and NH_4^+ concentration on the Sans^{plus}Systems autoanalyzer.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS Base 11.5 for Windows (SPSS Inc., Chicago, Illinois) with the level of significance set at an α of 0.05. Outliers were identified using the interquartile range \times 1.5 rule and box plots (Moore and McCabe 1993). Normality was assessed using the Shapiro-Wilk test and normal quantile plots (Winer et al. 1991). The homogeneity of error variance was tested using Levene's test of equality of error variance. When either the data or the transformed data was normal with equal error variance, one-way analysis of variance performed in conjunction with a Tukey's HSD post-hoc test was used to identify any significant differences among months for the internal N pools, NR activity, and environmental variables for a given species (Zar 1999). If the data could not be transformed to meet normality and equal variance assumptions, a Kruskal-Wallis test combined with a Games-Howell post-hoc test was used (Zar 1999). For comparisons involving tissue NO_3^- , tissue NH_4^+ , FAA, or NR activity the daily means were considered replicates. In the case of protein content, % N, and C:N ratios, which were measured on only 1 d in each monthly collection, the measurements made on that 1 d were considered replicates. Principal component analysis was used to reduce the dimensionality of the environmental data (Sokal and Rohlf 1995). Stepwise, multiple regression analysis was then used to examine the relationship between principal components with eigenvalues >1 and the daily means for tissue NO_3^- , tissue NH_4^+ , FAA, or NR activity.

Results

The environmental conditions near stands of *E. lingulata* showed considerable variation over the year (Table 1). In October, temperatures were highest with algal stands above or at the water's surface. The low water depth led to high irradiance. Salinity remained high and NH_4^+ concentration in the water column was nearly four times NO_3^- concentration. In January, temperatures reached

their lowest and low early morning tides led to *E. lingulata* stands frequently being exposed. The low water depth led to high irradiance. The average salinity dropped to 16.6 ± 1.8 psu and displayed greater day to day variability than in the October with more NO_3^- than NH_4^+ in the water column. Both temperature and water depth increased in March. *E. lingulata* tended to be submerged and irradiance was lower. In March, salinity was the lowest of the year and water column NO_3^- and NH_4^+ concentrations peaked for the year with twice as much NO_3^- as NH_4^+ . In April, temperatures and water depth increased again. Irradiance remained similar to that observed in March. Salinity rose and the concentration of NH_4^+ in the water column became three times greater than the concentration of NO_3^- . Throughout the year, water column NO_2^- concentration was less than $0.5 \mu\text{M}$ and frequently less than $0.3 \mu\text{M}$.

E. lingulata was found growing at the field site during the October, January, March, and April sampling periods. During these months, tissue NO_3^- was variable ranging from $239.58 \pm 17.00 \mu\text{mol N g dw}^{-1}$ (mean \pm SE) for one day in January to less than $15 \mu\text{mol N g dw}^{-1}$ at least once during each month. Tissue NO_3^- was not significantly different between months ($p > 0.05$; Fig. 1). Tissue NO_2^- was consistently less than $0.25 \mu\text{mol N g dw}^{-1}$ and not a significant N pool. Compared to tissue NO_3^- , the internal NH_4^+ pool was smaller, frequently below $8 \mu\text{mol N g dw}^{-1}$. Unlike tissue NO_3^- , tissue NH_4^+ was more constant within a given month and significantly different between months ($p < 0.001$) with the internal NH_4^+ pool largest in March. The FAA pool, which reached its peak of $155.75 \pm 10.42 \mu\text{mol N g dw}^{-1}$ in March, was not always larger than the inorganic N pool. The size of the FAA pool was not significantly different between months ($p > 0.05$). The inorganic N and FAA pools combined were less than 10% of the total N. Roughly 20-fold more N was stored as protein compared to inorganic N and FAA with significantly more protein in January and March compared to April ($p < 0.01$). The amount of N detected in the protein pool was actually greater than the total N for the months of October and January. There was a roughly 2-fold difference in % N from October ($2.57 \pm 0.05\%$ dw) to March ($6.39 \pm 0.06\%$ dw) and each month was significantly different from the others ($p < 0.01$). C:N ratios reflected this monthly change in % N. Overall, N storage and % N were greatest in March. NR activity was significantly

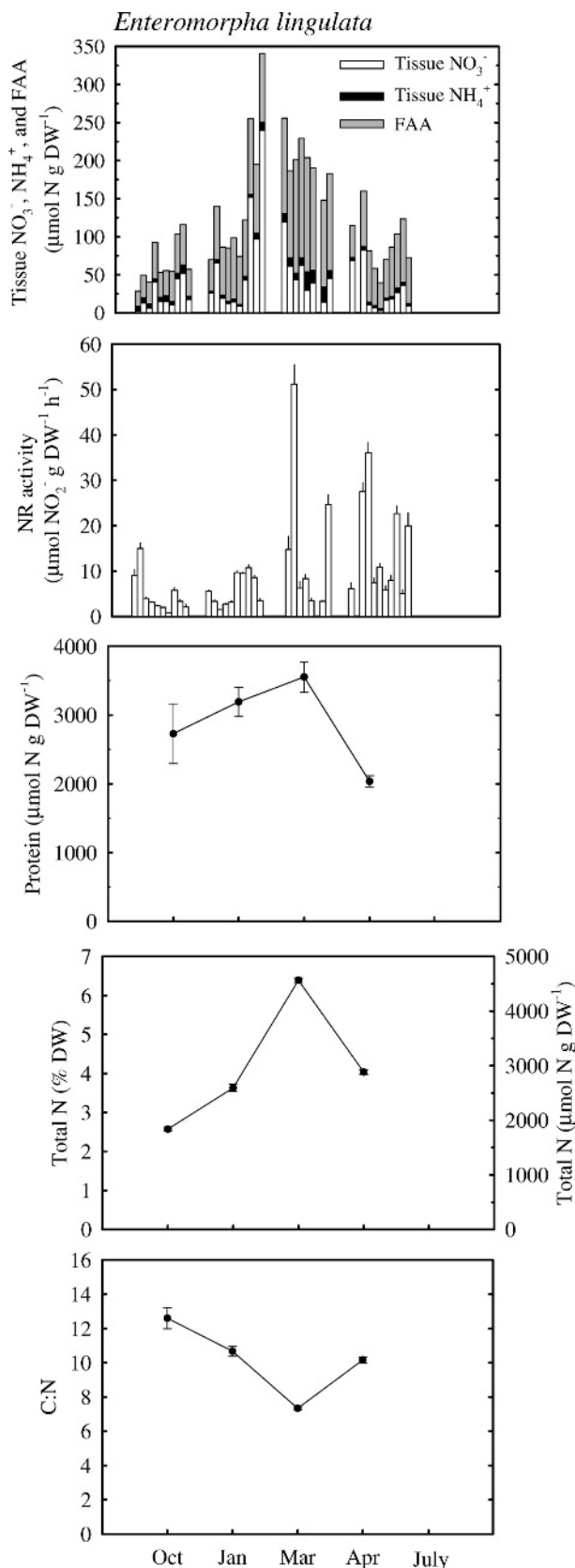


Fig. 1. Tissue NO_3^- , NH_4^+ , and FAA ($n = 8$); NR activity ($n = 8$); protein ($n = 7$); total N ($n = 5$); and C:N ratio ($n = 5$) in *Enteromorpha lingulata* over the growing season.

TABLE 2. Environmental conditions at the *Gelidium pusillum* collection site. Values are mean (\pm SE) and asterisks indicate the level of significant difference among months (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The letters next to each mean indicate months that were found to be significantly different ($p < 0.05$) by post-hoc, pairwise comparisons. Negative depth values indicate the distance between exposed *G. pusillum* and the surface of the water.

Variable	October	January	March	April	July
Temperature ($^{\circ}$ C) ***	23.50 (0.28)a	10.40 (0.33)b	15.01 (0.71)c	21.35 (0.47)d	28.37 (0.49)e
Depth (cm) ***	8.24 (1.38)a	18.60 (2.26)b	-9.35 (6.07)a	19.60 (2.51)b	41.53 (2.71)c
Irradiance (μ mol m^{-2} s^{-1})	1122.49 (114.08)	943.92 (160.28)	1024.33 (295.31)	531.44 (77.58)	571.63 (98.64)
Salinity (psu) ***	28.56 (0.34)a	14.27 (0.82)b	3.19 (0.50)c	14.30 (1.72)b	22.39 (0.74)d
Water column NO_3^- (μ M) ***	1.26 (0.12)a	6.43 (0.51)b	12.42 (0.28)c	1.46 (0.15)a, d	2.07 (0.24)d
Water column NH_4^+ (μ M) ***	4.39 (0.55)a, d	2.34 (0.25)b, c	5.51 (0.51)a	2.67 (0.39)c, d	4.94 (0.55)a

different between months ($p < 0.01$) with significantly higher values in March and April than in October. NR activity showed considerable variability within each month (15-fold in March) and while NR activity was often higher when tissue NO_3^- was high, such as at the end of January, it was not always, as seen in the beginning of October. There was not a consistent pattern between NR activity and tissue NH_4^+ either.

From October to April, temperature, salinity, and water column NO_3^- and NH_4^+ concentrations were similar at the sites where *E. lingulata* and *G. pusillum* were collected (Tables 1 and 2). Since *G. pusillum* often occurs lower in the intertidal zone than *E. lingulata*, water depth was frequently greater at the *G. pusillum* collection site and as a consequence, irradiance was lower. It is important to note that because *G. pusillum* stands are permanent, while *E. lingulata* stands are more ephemeral, migrating up and down the tidal range, there are times of the year, such as March, when *E. lingulata* stands may be deeper than *G. pusillum* stands. In July, when *E. lingulata* was not found at our field site, temperatures were the highest of the year and *G. pusillum* stands were submerged under roughly 40 cm of moderately saline water (22 psu) that contained twice as much NH_4^+ as NO_3^- .

G. pusillum was found growing at the field site throughout the year. Within a given month, the internal NO_3^- and NH_4^+ pools in *G. pusillum* displayed less day to day variability when compared to *E. lingulata* (Figs. 1 and 2). In *G. pusillum*, tissue NO_3^- was 10-fold higher in March (78μ mol g dw^{-1}) than in January and July and over twice the October and April values ($p < 0.001$; Fig. 2). Tissue NO_2^- was consistently less than 0.12μ mol N g dw^{-1} and not a significant N pool. During the year, tissue NH_4^+ spanned a smaller range than tissue NO_3^- , but like tissue NO_3^- was highest in March ($p < 0.001$). Unlike tissue NO_3^- and NH_4^+ , which were highest in March, the FAA pool was largest in January and October ($p < 0.001$). The combined inorganic N and FAA pools in *G. pusillum* still accounted for less than 10% of the total N, much like in *E. lingulata*. The size of the

protein pool was greatest in October and January ($p < 0.001$) and roughly 4-fold larger than the combined inorganic N and FAA pools. Unlike tissue inorganic N, FAA, and protein, there was no significant difference in % N (4% dw) from month to month ($p > 0.05$). C:N ratios changed little with month and, although a significant difference was found among the months ($p < 0.05$), a Games-Howell pairwise, post-hoc comparison could not identify a significant difference between any two months. NR activity was significantly different between months ($p < 0.001$) with NR activity highest in October and April and lowest in January. There was not a consistent pattern between NR activity and tissue NO_3^- or tissue NH_4^+ .

The three principal components (PC1, PC2, and PC3) extracted from the environmental variables for *E. lingulata* explained 37.86%, 32.63%, and 16.23% of the variation in the environmental data, respectively (cumulative 86.72%). The first principal component (PC1) was negatively correlated with irradiance and positively correlated with water depth and captured the variation in the environmental variables due to wind and tidal driven changes in water depth (Table 3). The second principal component (PC2) was negatively correlated with water column NO_3^- concentration and positively correlated with salinity and captured the variation in the environmental variables due to NO_3^- -rich, freshwater entering the estuary. Water column NH_4^+ concentration was the only variable with a substantial loading on the third principal component (PC3), which reflects the relative independence of water column NH_4^+ concentration from the other environmental variables.

Stepwise, multiple regression analysis indicated significant relationships in *E. lingulata* between tissue NO_3^- and PC2, tissue NH_4^+ and PC2 and PC3, the FAA pool and PC2, and NR activity and PC1, PC2, and PC3 (Table 4). Of the regression models, the tissue NH_4^+ and FAA models were the only models that explained greater than 50% of the variation in the dependent variable.

The three principal components (PC1, PC2, and PC3) extracted from the environmental variables

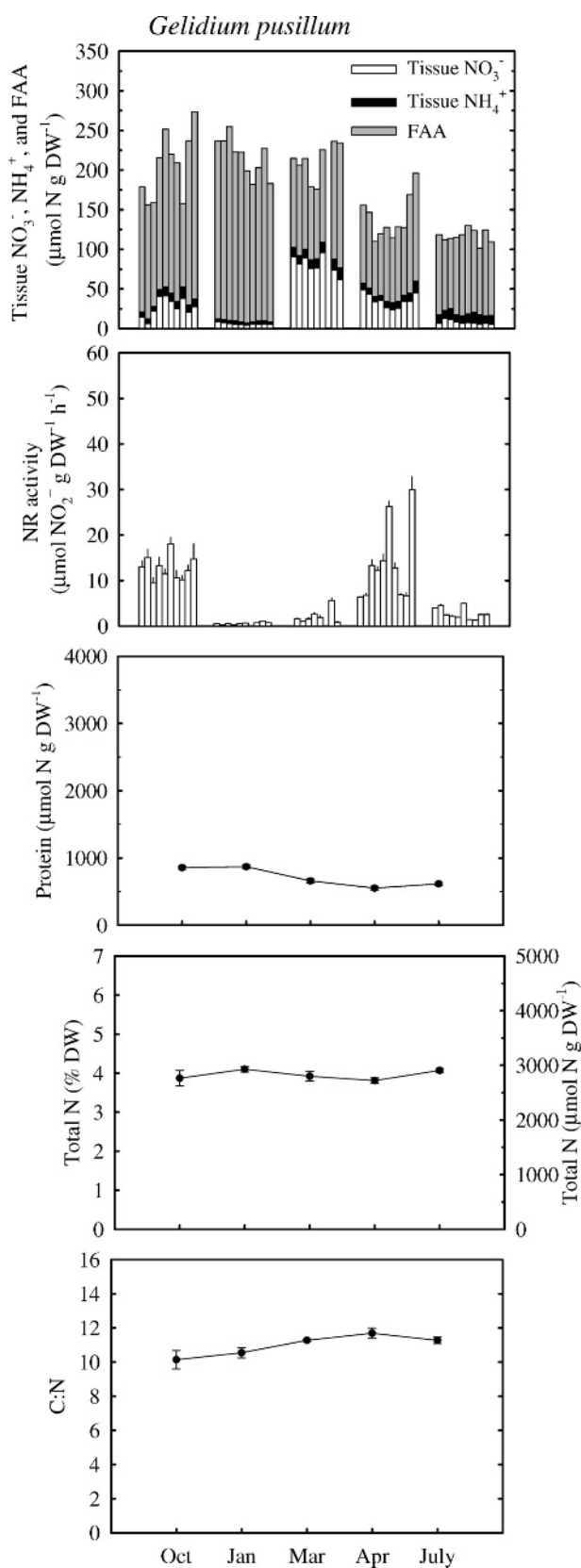


TABLE 3. Loadings for the environmental variables on the first three principal components (PC1, PC2, and PC3) extracted from the *Enteromorpha lingulata* data set. Values in bold are loadings >0.70 or <-0.70 .

Variable	PC1	PC2	PC3
Irradiance	-0.80	0.34	0.26
Water column NO_3^-	0.03	-0.81	0.31
Water column NH_4^+	-0.14	-0.14	0.93
Temperature	0.62	0.66	0.21
Salinity	-0.34	0.89	0.00
Depth	0.97	-0.03	-0.07

TABLE 4. Results of the stepwise, multiple regression analysis of tissue NO_3^- , tissue NH_4^+ , FAA, or NR activity against principal components (PC1, PC2, and PC3) for *Enteromorpha lingulata*.

Dependent Variable	Model Coefficients				adj-R ²	p
	PC1	PC2	PC3	Constant		
Tissue NO_3^-	-0.52	-0.20	0.32	1.11	0.23	<0.001
Tissue NH_4^+	-0.26	0.32	-0.20	6.36	0.64	<0.001
FAA	-0.46	0.32	-0.28	-0.23	0.73	<0.001
NR activity	0.35	-0.30	-0.28	-0.23	0.27	<0.01

for *G. pusillum* explained 45.45%, 24.10%, and 17.17% of the variation in the environmental data, respectively (cumulative 86.72%). PC1 was negatively correlated with water column NO_3^- concentration and positively correlated with temperature and salinity (Table 5). Much like PC2 for *E. lingulata*, PC1 for *G. pusillum* captured variation in the environmental variables due to NO_3^- -rich, freshwater entering the estuary along with seasonal changes in temperature. PC2 for *G. pusillum* was positively correlated with irradiance and negatively correlated with water depth reflecting the variation in the environmental variables due to wind and tidal driven changes in water depth. PC2 for *G. pusillum* was analogous to PC1 for *E. lingulata*. PC3, similar to PC3 for *E. lingulata*, was positively correlated with water column NH_4^+ concentration.

Stepwise, multiple regression analysis indicated significant relationships in *G. pusillum* between tissue NO_3^- and PC1 and PC3, tissue NH_4^+ and PC3, the size of the FAA pool and PC2, and NR activity and PC1 (Table 6). None of the regression models were able to explain more than 50% of the variation in the dependent variables.

Discussion

Based on the model coefficients and the loadings of the environmental variables onto the principal components, the regression models suggest that the

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Fig. 2. Tissue NO_3^- , NH_4^+ , and FAA ($n = 8$); NR activity ($n = 8$); protein ($n = 7$); total N ($n = 5$); and C:N ratio ($n = 5$) in *Gelidium pusillum* over the growing season.

TABLE 5. Loadings for the environmental variables on the first three principal components (PC1, PC2, and PC3) extracted from the *Gelidium pusillum* data set. Values in bold are loadings >0.70 or <-0.70 .

Variable	PC1	PC2	PC3
Irradiance	0.10	0.92	0.06
Water column NO_3^-	-0.91	0.15	0.19
Water column NH_4^+	-0.01	0.14	0.98
Temperature	0.76	-0.33	0.47
Salinity	0.91	0.08	0.08
Depth	0.47	-0.70	-0.09

appearance of NO_3^- -rich, freshwater in the estuary (decrease in PC2) led to increases in the internal NO_3^- , NH_4^+ , and FAA pools and an increase in NR activity in *E. lingulata*. Changes in light availability and water depth (PC1) appear to have had little affect on the storage of N as NO_3^- , NH_4^+ , and FAA in this species, but increasing light availability and a decline in water depth (decrease in PC1) actually led to a decrease in NR activity. For *E. lingulata* increases in water column NH_4^+ concentration (increase in PC3) translated into a larger internal NH_4^+ pool and the inhibition of NR activity.

The appearance of NO_3^- -rich, freshwater in the estuary (decrease in PC1) also led to an increase in the internal NO_3^- pool in *G. pusillum*. Unlike *E. lingulata*, NR activity decreased in *G. pusillum* during these high NO_3^- , low salinity, low temperature events. Yet, NR activity in *G. pusillum* was not affected by changing water depth or irradiance (PC2), unlike the size of the FAA pool which tended to increase in *G. pusillum* as water level dropped and irradiance increased (increase in PC2). In *G. pusillum*, much like in *E. lingulata*, increases in water column NH_4^+ availability (increase in PC3) produced increases in tissue NH_4^+ . Unlike in *E. lingulata*, *G. pusillum* displayed no evidence of NH_4^+ inhibition of NR activity and tissue NO_3^- actually increased when water column NH_4^+ concentration increased.

While both *E. lingulata* and *G. pusillum* responded to changes in inorganic N availability, there appear to be important differences in their susceptibility to NH_4^+ inhibition as well as differences in their salinity tolerance as suggested by their response to high NO_3^- , low salinity events. These differences combined with their different N demands and

growth strategies may explain the seasonal trends observed in N storage and NR activity in the two species.

Both *E. lingulata* and *G. pusillum* were capable of taking up and storing NO_3^- when it became available in the estuary. The results of this study suggest that *E. lingulata* was able to metabolize NO_3^- more rapidly during high NO_3^- , low salinity events. This difference may be due to a greater capacity to metabolize NO_3^- in *E. lingulata* stemming from its higher N demand. Foliose chlorophytes tend to have fast growth rates and *E. lingulata* is likely not an exception (Björnsäter and Wheeler 1990; Pihl et al. 1996). This finding from the field supports earlier laboratory work demonstrating that *E. lingulata* from the Mobile Bay estuary had the capacity to rapidly take up and reduce short-term NO_3^- pulses (Lartigue and Sherman 2005).

In addition to differences in N demand and overall metabolic rates, differences in salinity tolerance between *E. lingulata* and *G. pusillum* may explain their different responses to high NO_3^- , low salinity events. There is substantial evidence in the literature demonstrating that *Enteromorpha* spp. is euryhaline. *Enteromorpha intestinalis* has been repeatedly shown to tolerate a wide range of salinities (Reed and Russell 1979; Edwards et al. 1987), although there is a limit to this tolerance and prolonged exposure to 0 psu can result in a loss of pigmentation and a decline in biomass (Edwards et al. 1988; Kamer and Fong 2000). If salinity fluctuates or inorganic N availability is high, the effects of low salinity are mitigated (Kamer and Fong 2000, 2001). In the case of *Gelidium* spp., there are reports in the literature suggesting that some species of *Gelidium* are adversely affected by a decline in salinity (Oligier and Santelices 1981; Macler 1988). Oligier and Santelices (1981) found that the growth rate in *G. pusillum* decreased from 1.3% to 0.3% when salinity declined from 35 to 15 psu, suggesting salinity stress occurs at salinities as high as 15 psu. This result may explain the response of *G. pusillum* to high NO_3^- , low salinity events in this study where salinity was often below 15 psu.

The inhibition of NO_3^- uptake and NR activity by NH_4^+ has been well documented in marine algae (Lopez-Figueroa and Ruediger 1991; Riccardi and Solidoro 1996; Lomas and Glibert 1999). Some species of algae are more susceptible to NH_4^+ inhibition than others and the effect of NH_4^+ on NO_3^- uptake and reduction can range from total inhibition to simultaneous uptake and assimilation of both N sources (McCarthy and Eppley 1972; Dortch and Conway 1984; Thomas and Harrison 1987; Lomas and Glibert 1999). With regard to NR activity, our findings suggest that *E. lingulata* is more susceptible to NH_4^+ inhibition than *G. pusillum*. The

TABLE 6. Results of the stepwise, multiple regression analysis of tissue NO_3^- , tissue NH_4^+ , FAA, or NR activity against principal components (PC1, PC2, and PC3) for *Gelidium pusillum*.

Dependent Variable	Model Coefficients				adj-R ²	p
	PC1	PC2	PC3	Constant		
Tissue NO_3^-	-0.31		0.30	1.55	0.15	<0.01
Tissue NH_4^+			0.61	2.49	0.46	<0.001
FAA		0.19		7.81	0.27	<0.001
NR activity	0.33			-0.07	0.31	<0.001

increase in tissue NO_3^- in *G. pusillum* in response to increasing NH_4^+ availability suggests that *G. pusillum* may be able to simultaneously take up NO_3^- and NH_4^+ . A capacity to take up and assimilate both forms of inorganic N simultaneously could partly explain why *G. pusillum*, but not *E. lingulata*, persisted during the summer when N availability was lower.

Our results suggest that changes in inorganic N availability and salinity rather than changes in irradiance and water depth determine patterns of N storage and NO_3^- reduction in *E. lingulata* and *G. pusillum* in the Mobile Bay estuary. The absence of a positive relationship between irradiance and N storage and NO_3^- reduction in *E. lingulata* is not surprising given that the irradiance measured in this study near *E. lingulata* stands was well above the saturating irradiance for *Enteromorpha* spp. (e.g., *E. intestinalis*: $55.8 \mu\text{mol m}^{-2} \text{s}^{-1}$, Arnold and Murray 1980; *E. flexuosa*: $195.6 \mu\text{mol m}^{-2} \text{s}^{-1}$, Beach et al. 1995). The lower irradiances measured near *G. pusillum* stands in this study were also frequently above the saturating irradiances reported for *Gelidium* spp. (e.g., *G. sesquipedale*: $77\text{--}154 \mu\text{mol m}^{-2} \text{s}^{-1}$, *G. latifolium*: $217\text{--}312 \mu\text{mol m}^{-2} \text{s}^{-1}$, and *G. pulchellum*: $43\text{--}223 \mu\text{mol m}^{-2} \text{s}^{-1}$, Rico and Fredriksen 1996; *G. sesquipedale*: $76 \mu\text{mol m}^{-2} \text{s}^{-1}$, Mercado et al. 1998). The positive response to increasing irradiance in the FAA pool in *G. pusillum* indicates that the energy needed to assimilate NO_3^- and NH_4^+ might not always be immediately available in this species and could at times limit productivity.

The seasonal trend in % N and C:N ratios in *E. lingulata* suggests that prior to dying off in the summer *E. lingulata* may have been approaching N limitation. Björnsäter and Wheeler (1990) determined a critical N of 2.5% dw in *E. intestinalis*, which was similar to the October total N ($2.57 \pm 0.05\%$ dw, mean \pm SE) measured in this study. In general, total N in *E. lingulata* in this study (2.57–6.39% dw) was similar to that observed by others in *Enteromorpha* spp. in the field (1.61–4.22% dw, Kamer et al. 2001; 0.4–3.25% dw, Owens and Stewart 1983; 2.00–5.10% dw, Wheeler and Björnsäter 1992). The slower growing *G. pusillum* responded to a similar nutrient regime with little to no variation in total N or C:N ratios. Work by Vergara et al. (1993) found a critical N of roughly 1.5% dw in *G. sesquipedale* with a corresponding C:N of c. 24. It is unlikely with a total N of 4% dw and C:N ratio of 10 that *G. pusillum* in the Mobile Bay estuary was N limited during the year. Carter and Anderson (1986) reported a similar narrow range for total N (2.3–3.5% dw) in *Gelidium pristoides* from the coast of South Africa, but Fredriksen and Ruess (1989) working with cultured *G. latifolium* did

show that total N could vary 1–5% dw with N availability.

In the two instances when protein content exceeded total N in this study, the cause may have been underestimation of total N due to the volatilization of some N compounds during drying. A total N less than the protein content could also stem from an overestimation of the size of the protein pool due to differences in the amino acid composition of the BSA standard and macroalgal proteins. All colorimetric methods for determining protein concentration, including the BCA assay, are sensitive to protein-to-protein variation (Smith et al. 1985; Sapan et al. 1999).

Other factors besides N limitation likely play a role in the absence of *E. lingulata* from the Mobile Bay estuary in the summer. *E. lingulata* may not be able to tolerate high, summer temperatures, especially when exposed for long periods of time. The simple, tubular blades of *E. lingulata* are susceptible to damage and removal by herbivores and wave action. With the exception of late April, when inorganic N availability was low and herbivore abundance was high, a fast growth rate allowed *E. lingulata* stands to recover quickly following storm events. Conditions of low inorganic N availability combined with high herbivore abundance exist from late April through the summer and may prevent *E. lingulata* stands from persisting year-round.

Because of its fast growth rate and quick response to the environmental conditions, *E. lingulata* was more likely to reflect present nutrient conditions. Fong et al. (1994, 1998) came to a similar conclusion regarding total N in *E. intestinalis* and the nutrient history of the alga. Fong et al. (1994) concluded that when N was not limiting there would be a relationship between tissue nutrients and water column nutrients. Fong et al. (1998) then evaluated the use of *E. intestinalis* as an indicator of N enrichment and found that calculating the percentage change in N was a reliable predictor of differences in N availability from month to month. *G. pusillum* grew slowly as branched tufts that showed less evidence of herbivore damage and were resistant to removal by wave action. As a result, *G. pusillum* was more likely to reflect both past and present nutrient conditions.

In many subtropical and temperate estuaries, including the Mobile Bay estuary, late winter and spring runoff produce frequent high NO_3^- , low salinity events. Our findings indicated that at least a portion of this NO_3^- was likely being retained within the estuaries by *E. lingulata* and *G. pusillum* and, in the case of *E. lingulata* was rapidly being assimilated. Improving the predictive power of the NR activity regression model could result in quantitative estimates of macroalgal NO_3^- reduc-

tion in estuaries like the Mobile Bay estuary. Having demonstrated the relationship between inorganic N availability and N storage in the field, the next step is to develop a model that relates inorganic N uptakes rates to the environmental conditions in order to quantify the drawdown of inorganic N by the macroalgal component.

Only one site in the Mobile Bay estuary was sampled in this study and extension of our findings to others sites particularly in other estuaries requires further study. A comparison of our 1-yr data set with a 6-yr data set (1989–1995) for the Mobile Bay estuary collected by Pennock et al. (1999) suggests that the trends in salinity, inorganic N availability, and temperature during 2000–2001 were similar to the historical annual trends. In both data sets, late winter and spring runoff produced declines in salinity below 10 psu and increased the availability of inorganic N, particularly NO_3^- . As a result, the seasonal trends in N storage and NO_3^- reduction observed in this study in *E. lingulata* and *G. pusillum* should be representative of annual trends and are likely to be repeated at our study site in future years.

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