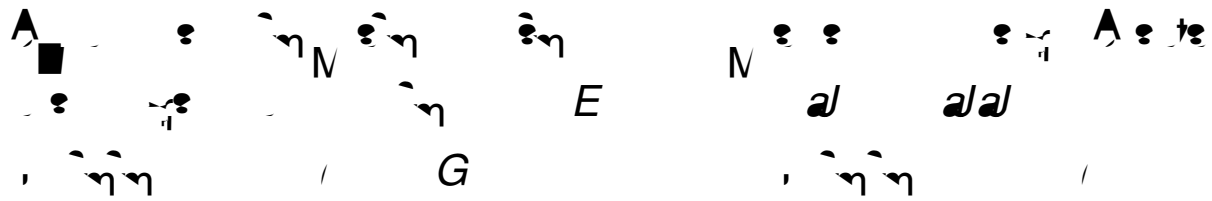


Journal of Experimental Marine Biology and Ecology



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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 $[\text{NH}_4^+]$, 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 variedwEMC /StyleSpaneThy, b154, t4a

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

TABLE 1. Environmental conditions at the *Enteromorpha lingulata* collection site. Values are mean (G

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^{2-} 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^{2-} and NH_4^+ concentrations peaked for the year with twice as much NO_3^{2-} 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^{2-} . Throughout the year, water column NO_2^{2-} concentration was less than 0.5 mM and frequently less than 0.3 mM.

E. lingulata was found growing at the field site during the October, January, March, and April sampling periods. During these months, tissue NO_3^{2-} was variable ranging from 239.58 ± 17.00 mmol N g dw²¹ (mean \pm SE) for one day in January to less than 15 mmol N g dw²¹ at least once during each month. Tissue NO_3^{2-} was not significantly different between months ($p > 0.05$; Fig. 1). Tissue NO_2^{2-} was consistently less than 0.25 mmol N g dw²¹ and not a significant N pool. Compared to tissue NO_3^{2-} , the internal NH_4^+ pool was smaller, frequently below 8 mmol N g dw²¹. Unlike tissue NO_3^{2-} , 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 ± 10.42 mmol N g dw²¹ 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

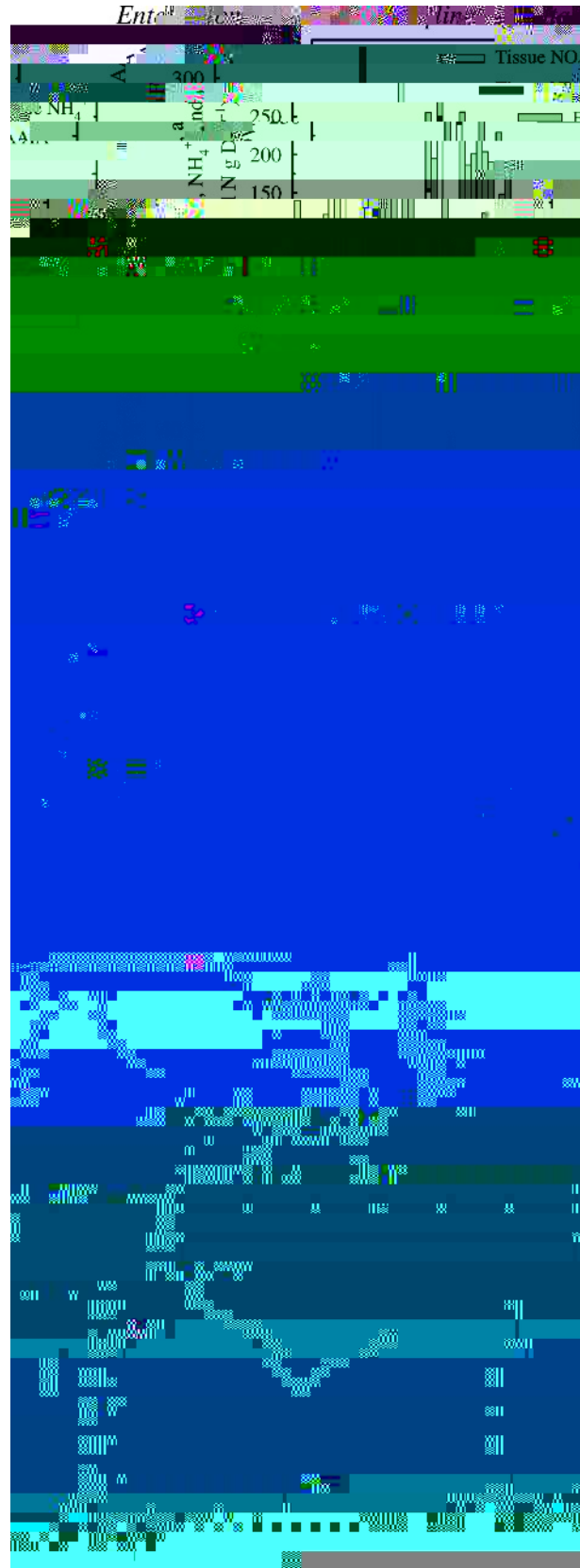


Fig. 1. Tissue NO_3^{2-} , NH_4^+ , and FAA (n = 8); NR activity (n = 8); protein (n = 7); total N (n = 5); and C:N ratio (n = 5) in *Enteromorpha lingulata* over the growing season.

TABLE 2. Environmental conditions at the *Gelidium pusillum* collection site. Values are mean (GSE) 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 (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 | 29.35 (6.07)a | 19.60 (2.51)b | 41.53 (2.71)c |
| Irradiance (mmol 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 ²⁻ (mM) *** | 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 ⁺ (mM) *** | 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 NO₃²⁻ 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 NH₄⁺ either.

From October to April, temperature, salinity, and water column NO₃²⁻ and NH₄⁺ 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 NH₄⁺ as NO₃²⁻.

G. pusillum was found growing at the field site throughout the year. Within a given month, the internal NO₃²⁻ and NH₄⁺ pools in *G. pusillum* displayed less day to day variability when compared to *E. lingulata* (Figs. 1 and 2). In *G. pusillum*, tissue NO₃²⁻ was 10-fold higher in March (78 mmol g dw⁻¹) than in January and July and over twice the October and April values (p , 0.001; Fig. 2). Tissue NO₂²⁻ was consistently less than 0.12 mmol N g dw⁻¹ and not a significant N pool. During the year, tissue NH₄⁺ spanned a smaller range than tissue NO₃²⁻, but like tissue NO₃²⁻ was highest in March (p , 0.001). Unlike tissue NO₃²⁻ and NH₄⁺, 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 NO₃²⁻ or tissue NH₄⁺.

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 NO₃²⁻ concentration and positively correlated with salinity and captured the variation in the environmental variables due to NO₃²⁻-rich, freshwater entering the estuary. Water

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^- | 20.91 | 0.15 | 0.19 |
| Water column NH_4^+ | 20.01 | 0.14 | 0.98 |
| Temperature | 0.76 | 20.33 | 0.47 |
| Salinity | 0.91 | 0.08 | 0.08 |
| Depth | 0.47 | 20.70 | 20.09 |

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

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th269]TJ-12.526670.9tualedcrs136 (8lin374(in)]TJT*[s-7.3-7.6 (-12.5323e2patissu)-7.4 -62 632TJ/Ti)-7.8 (8144.1

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

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

