Rates of inorganic nitrogen uptake by the estuarine green macroalgae *Enteromorpha intestinalis* and *Ulva expansa*

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ABSTRACT - Rates of nitrogen (N) uptake by *Enteromorpha intestinalis* and *Ulva expansa* were investigated in two separate experiments. To measure uptake rates over a range of conditions, we varied initial water column NO_3 concentrations (low, medium, and high) and initial algal tissue nutrient status (enriched versus depleted). Uptake rates were determined by measuring the disappearance of NO_3 from solution over time (1, 2, 4, 8, 12, and 24 h). *E. intestinalis* and *U. expansa* exhibited a high affinity for N. In the low water column concentration treatments, *E. intestinalis* and *U. expansa* removed all measurable NO_3 from the water within 8 h and 12 h, respectively. Nutrient-depleted algae consistently removed more NO_3 than enriched algae over each sampling interval. For *E. intestinalis,* maximum rates of NO_3 uptake increased with increasing initial water column nutrient concentrations, indicating a relationship between uptake and external substrate concentration. The same was true for *U. expansa* in the low and medium water column nutrient treatments. Maximum rates of NO_3 uptake exceeded 200 µmoles g dry wt^{-1} h^{-1} by *E. intestinalis* and 125 µmoles g dry wt^{-1} h^{-1} by *U. expansa*. Nutrient uptake rates were highly variable over 24 h, indicating surge, internally controlled, and externally controlled phases of nutrient uptake. Uptake by *E. intestinalis* varied from the maximum rate measured to a negative rate over the first 2 h, indicating a release of N from algal tissues. Similarly, *U. expansa* NO_3 uptake rates were greatest in the first hour, decreased to 0 in the second hour, and then returned to normal. Depleted algae showed greater increases in tissue N concentration in response to water column nutrient supplies than enriched algae did, and these increases were concentration dependent. Generally, macroalgae did not grow measurably in these 24-h experiments, indicating a temporal decoupling between nutrient uptake and growth.

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Critical factors in the process of nutrient uptake by macroalgae are: (1) nutrient concentration in the water column, (2) algal nutrient status, and (3) the various phases of nutrient uptake.

INTRODUCTION

Large blooms of opportunistic green macroalgae such as *Enteromorpha* and *Ulva* spp. occur in estuaries throughout the world (Sfriso *et al.* 1987 and 1992, Hernández *et al.* 1997, Raffaelli *et al.* 1999, Eyre and Ferguson 2002), often in response to increased nutrient loads from developed watersheds (Valiela *et al.* 1992, Nixon 1995, Paerl 1999). These algae are highly successful in estuarine environments where nutrient supply may be transient (Litaker *et al.* 1987), perhaps in part due to their high nutrient uptake rates and capacity to store nutrients (Fujita 1985). While these algae are natural components of estuarine systems and play integral roles in estuarine processes (Pregnall and Rudy 1985, Kwak and Zedler 1997), blooms are of ecological concern because they can reduce the habitat quality of an estuary. They can deplete the water column and sediments of oxygen (Sfriso *et al.* 1987, Valiela *et al.* 1992), leading to changes in species composition and shifts in community structure (Raffaelli *et al.* 1991, Bolam *et al.* 2000).

Macroalgal nutrient uptake rates vary with a suite of factors including, but not limited to, initial algal tissue nutrient status (Hanisak 1983). Generally, nitrogen (N) uptake rates increase as tissue N concentration decreases, reflecting N starvation and increased N demand. Fujita (1985) enriched and starved species of *Enteromorpha* and *Ulva* with N prior to using them in uptake experiments; algae that had been starved had consistently higher nutrient

uptake rates than algae that had been enriched. Similar results have been obtained by O'Brien and Wheeler (1987) for *Enteromorpha prolifera*, Duke *et al.* (1989) for *Ulva curvata*, Pedersen (1994) for *Ulva lactuca*, and McGlathery *et al.* (1996) for *Chaetomorpha linum.*

Nutrient uptake rates also vary with external substrate concentration (Hanisak 1983). Harlin (1978) measured increasing rates of NO_3 uptake by *Enteromorpha* spp. with increasing water column NO_3 concentration. Fujita (1985) found that uptake rates of dissolved inorganic nitrogen (DIN) by *Enteromorpha* and *Ulva* spp. were dependent on the concentration of DIN in the external medium. His results confirmed those of earlier studies using *Ulva fasciata* (Lapointe and Tenore 1981), *U. lactuca* (Parker 1981), and *U. curvata* (Rosenberg and Ramus 1984).

Similar to estuaries throughout the world, southern California estuaries are characterized by large seasonal blooms of *Enteromorpha* and *Ulva* spp. (Peters *et al.* 1985, Rudnicki 1986, Kamer *et al.* 2001). N may be the most limiting nutrient to the growth of macroalgae in these systems (Kamer unpub. data); therefore, the uptake dynamics of this nutrient are critical to understanding the role N plays in macroalgal bloom dynamics. The objective of this study was to measure the rates of inorganic N uptake by *Enteromorpha intestinalis* and *Ulva expansa* under initial substrate concentrations and algal tissue nutrient concentrations representative of levels measured in southern California estuaries (Fong and Zedler 2000, Kamer *et al.* 2001).

METHODS

Overview of Experimental Design

Two independent laboratory experiments were conducted to test the hypothesis that nutrient uptake rates in the green macroalgae *E. intestinalis* and *U. expansa* vary depending on the initial algal tissue nutrient status and the initial water column nutrient concentration. We measured the uptake of N by *E. intestinalis* and repeated the experiment with *U. expansa*. Each experiment had two fully crossed factors: initial algal tissue nutrient status (nutrient-enriched versus depleted) and initial water column nutrient concentration (low, medium, and high) (Table 1). Both initial tissue nutrient levels and initial water column nutrient concentrations were within the range of values measured in southern California estuaries (Fong and Zedler 2000, Kamer *et al.* 2001).

Experimental Set Up

Seven to ten days prior to the initiation of each experiment, algae were collected from Upper Newport Bay, California. Algae were kept outdoors in two shallow pans filled with aerated seawater in a temperature-controlled water bath (20 ± 2°C). Pans were covered with window screening to reduce incident light. For each experiment, the algae were pre-conditioned to achieve enriched and depleted tissue N status. Water in one pan was enriched every 3-4 d with NO_3 in order to increase the tissue nutrient concentration of the algae. The second pan contained only low nutrient seawater with no nutrient enrichment (<3.57 µM NO_3) in order to reduce tissue nutrient concentration (Fong *et al.* 1994).

Experimental units were designed to create re-circulating unidirectional water flow with consistent velocities (8.62 ± 0.58 cm/s). The flow rates were comparable to rates measured in local estuaries (Kamer unpub. data). Flow was incorporated into the experimental design in order to reduce the effect of boundary layers and avoid the limitation of nutrient uptake that can occur in static systems.

Replicate experimental units consisted of 12 L round plastic containers with a 4 L round plastic jar in the center to produce a circular water flow path between the walls of each container (Figure 1). Holes drilled into the inner jar allowed the passive flow of water from the outer container to the inner jar, while window screening kept algal tissue isolated in the outer flow path. Inside the inner jar was an aquarium pump that transported water via plastic irrigation tubing from the inner jar to the outer container. Small holes in a regular array were punched into the last 6 cm of tubing, creating equal flow in all vertical layers of the water flow path.

Low, medium, and high NO_3 concentration experimental solutions were created by adding N in the form of NO_3 to seawater. Experimental units were filled with 8 L of the appropriate solution, and each solution was sampled in triplicate to determine initial water column nutrient concentrations (Table 1).

The N enriched and depleted algae were placed in individual nylon mesh bags, spun in a salad spinner for one minute, and wet weighed. In the first experiment, 20 grams of *E. intestinalis* were placed in each experimental unit; for the second experiment, 10 grams of *U. expansa* were used. The amount of biomass in the *U. expansa* experiment was reduced because of the high uptake rates measured in the *E. intestinalis* experiment. We wanted to ensure that nutrient levels would not drop below analytical

Table 1. Initial tissue nitrogen concentrations and initial water column NO_3 concentrations in two separate experiments measuring rates of NO_3 uptake by *Enteromorpha intestinalis* and *Ulva expansa*. Values are means ± SE.

	Initial Tissue N Status (% dry wt)		Initial Water NO_3 concentrations (µM)		
Algae	Depleted	Enriched	Low	Medium	High
Enteromorpha intestinalis	1.43 ± 0.06	2.18 ± 0.06	65.24 ± 1.25	243.10 ± 8.72	523.33 ± 3.46
Ulva expansa	1.35 ± 0.02	2.17 ± 0.01	71.25 ± 0.94	274.28 ± 0.51	521.07 ± 3.80

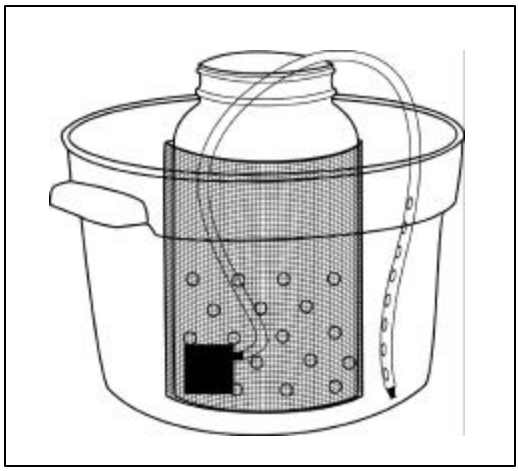

Figure 1. Diagram of experimental units showing the outer container and inner plastic jar with holes to allow passive water flow from the outer flow path to the interior of the jar through the window screening. The inner jar contained an aquarium pump, and plastic irrigation tubing was attached to the outflow of the pump. The tubing transported water from the pump to the outer flow path through an array of small holes punched in the last 6 cm of tubing.

detection limits. To begin each experiment, algae were placed in the outer container in the water flow path and isolated from the inner chamber containing the pump. Five initial sub-samples of each enriched and depleted algae were taken to determine initial tissue N status (Table 1). Sub-samples were rinsed briefly in freshwater to remove external salts, dried in a forced air oven at 60°C to a constant weight, ground in mortar and pestle, and analyzed for tissue N and P concentrations.

Experimental units were placed outdoors in a temperature-controlled water bath (20 ± 2°C) and arranged in a randomized matrix. There was four-fold replication for a total of 24 units.

At 1, 2, 4, 8, 12, and 24 h, 100 mL of water samples were taken from each unit. The total volume removed from each unit was <10% of the initial volume. The uptake rates of inorganic N were determined for each species of macroalgae by measuring the disappearance of inorganic N from solution over time. Samples were filtered with glass fiber filters (Whatman GF/C), frozen, and analyzed for NO_3, NH_4, and TKN (all forms of dissolved N except NO_3 and NO_2). The NO_3 was reduced to NO_2 via cadmium reduction; NO_2 was measured spectrophotometrically after diazotation (Switala 1999, Wendt 1999). The NH_4 was heated with solutions of salicylate and hypochlorite and determined spectrophotometrically (Switala 1999, Wendt 1999). The TKN was determined by the wet oxidation of nitrogen using sulfuric acid and a digestion catalyst. The procedure converts organic nitrogen to NH_4, which is subsequently determined (Carlson 1978). These automated methods have detection limits of 3.57 µM for N.

After the 24-h water samples were taken, algae were removed from the units, rinsed briefly in freshwater to remove external salts, dried in a forced air oven at 60°C to a constant weight, and weighed. Samples were ground in mortar and pestle and analyzed for tissue N concentration. The N was determined using an induction furnace and a thermal conductivity detector (Dumas 1981), and is reported as both the percent of dry weight and the percent of change from the initial measurement.

Statistical Analyses

All data from the experiments were tested for normality and homogeneity of variance. No transformations were necessary. For each experiment, among-treatment differences in final dry weight, final tissue N percent of dry weight, and tissue N percent of change from initial measurement were analyzed

using two-factor ANOVA (initial water column nutrient concentration x initial algal tissue nutrient status). Following a significant ANOVA, multiple comparisons were used to determine differences among individual treatments (Fisher's Protected Least Significant Differences test [PLSD]). Unless otherwise stated, no significant interactions occurred. Differences among nutrient uptake rates calculated for each sampling interval (µmoles/g dry wt/h) were analyzed using three-factor ANOVA (initial water column nutrient concentration x initial algal nutrient status x sampling interval). Regression analysis was used to quantify the relationship between water column nutrients and time for each of the six experimental treatments. Regression lines were chosen based on the best fit. All analyses were performed using either Statview 4.5 for Macintosh or SuperAnova v.1.11.

RESULTS

Nitrogen uptake by enriched and depleted *E. intestinalis*

Water column NO_3 decreased significantly over time in units with both enriched and depleted algae for all three initial nutrient concentration treatments (Figure 2). Nutrient depletion in the water was greatest in treatments when initial water column NO_3 concentration was highest and initial algae were depleted of tissue N. For all three initial water column nutrient treatments, the rate of nutrient depletion in the water was consistently higher for the depleted algae than for the enriched algae.

In the low-concentration treatment, water column NO_3 concentrations were reduced by 100% of measurable N in less than 8 h for both the depleted and enriched *E. intestinalis* (Figure 2a).

In the medium-concentration treatment, water column NO_3 concentrations decreased by 137 µM (SE=2.65) when enriched algae were present and by 208 µM (SE=6.40) when depleted algae were present (Figure 2b). This resulted in a 56% (SE=1.09) reduction in water column NO_3 for the enriched algae and an 85% (SE=2.63) reduction for the depleted algae in 24 h.

In the high-concentration treatment, water column NO_3 concentrations decreased by 153 µM (SE=7.55) when enriched algae were present (Figure 2c). This resulted in a 29% (SE=1.44) reduction in water column NO_3 over 24 h. Nutrient concentration was further reduced when depleted algae were present. Water column NO_3 concentrations decreased by 208 µM (SE=6.79), a 39% (SE=1.30) reduction in 24 h. Although the percent of the initial N that was removed was greater in the lowest water column concentration treatments, more N was always removed in the higher water column nutrient treatments. There was no pattern of water column NH_4 or TKN with either experimental factor.

There were significant differences in the final dry weight of *E. intestinalis* due to initial water column nutrient concentration (ANOVA, p=0.0032), but no differences due to initial algal nutrient status (ANOVA, p=0.8966). Growth in the low-concentration treatment was greater than medium (PLSD, p=0.0203) and high (PLSD, p=0.0013) concentration treatments, although differences were small (Figure 3a).

There was a significant effect of initial algal nutrient status on final *E. intestinalis* tissue N concentration (ANOVA, p=0.0063). Mean tissue N of enriched algae was significantly higher than depleted algae, probably reflecting initial differences. There was no effect of initial water column nutrient concentration on final tissue N (ANOVA, p=0.667), although there was a trend of increasing tissue N with increasing initial water column concentration for algae that were depleted in tissue N prior to the beginning of the experiment (Figure 3b).

The percent of change in the tissue N of *E. intestinalis* was significantly affected by initial tissue nutrient status N (ANOVA, p=0.0001), but not initial water column nutrient concentration. Algae with lower initial tissue N increased 57-79% during the experiment, while algae with higher initial tissue N increased only ~25% across all N concentration treatments (Figure 3c). There was a trend of increasing tissue N with increasing initial water column concentration for algae that were depleted in tissue N prior to the beginning of the experiment.

E. intestinalis NO_3 uptake rates were significantly affected by initial algal nutrient status (ANOVA, p=0.0001), initial water column nutrient concentration (ANOVA, p=0.0001), and sampling interval (ANOVA, p=0.0001). In the low-concentration treatment, *E. intestinalis* began taking up NO_3 immediately (Figure 4a). In the first hour, enriched algae took up 54.84 µmoles g dry wt^{-1} h^{-1} (SE=2.67) and depleted algae took up 71.65 µmoles g dry wt^{-1} h^{-1} (SE=6.28). The uptake rate increased over time for the first 4 h for both the depleted and enriched algae, with rates for depleted algae always

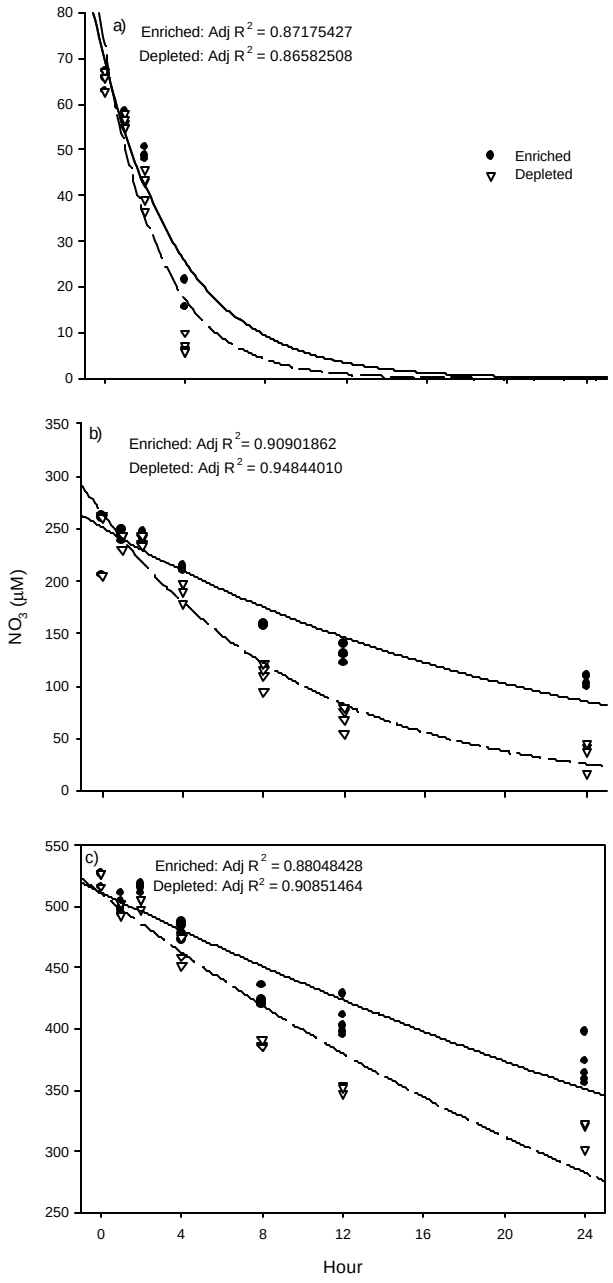

Figure 2. Decrease in water column NO_3 over time in the *Enteromorpha intestinalis* nitrogen uptake experiment for enriched and depleted algae for low (a), medium (b), and high (c) initial water column nutrient concentrations.

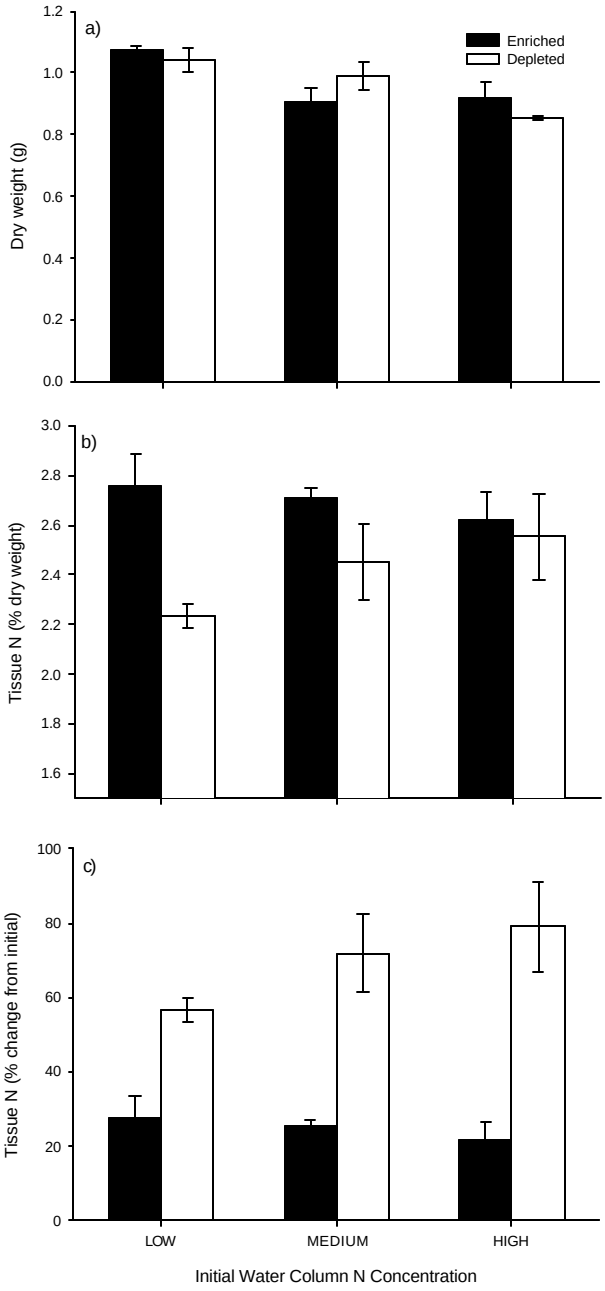

Figure 3. Final dry weight (a), tissue N concentration (b), and tissue N percent change from initial (c) of enriched and depleted *Enteromorpha intestinalis* incubated with low, medium, and high initial water column nutrient concentrations in the nitrogen uptake experiment. Bars represent ± 1 SE.

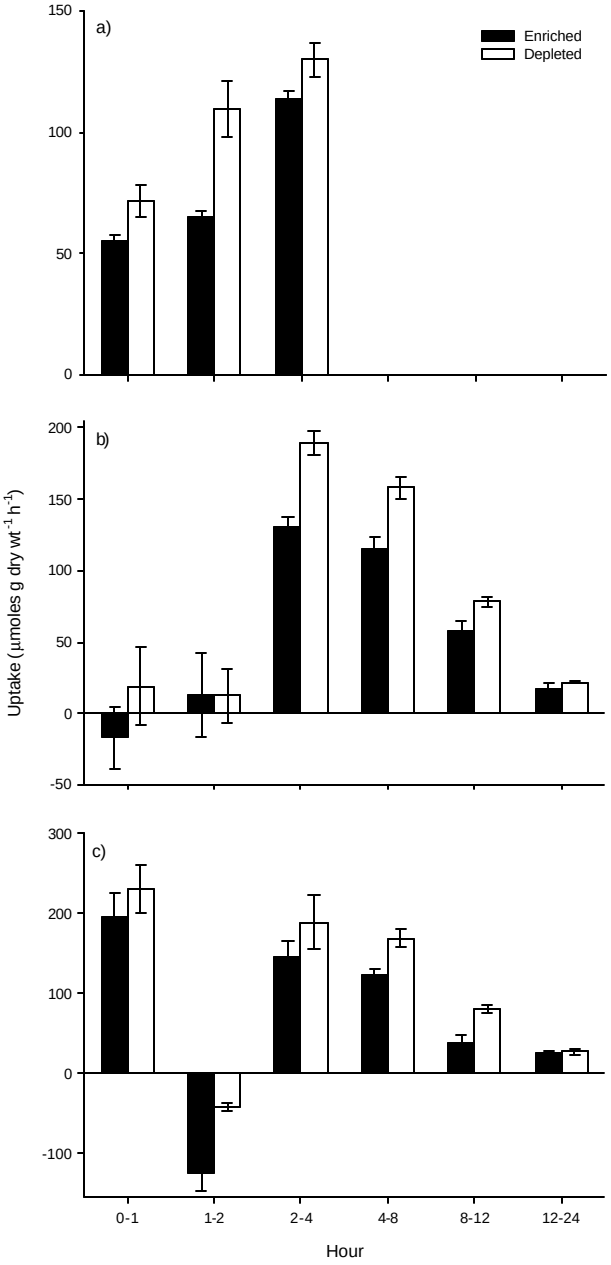

Figure 4. *Enteromorpha intestinalis* NO_3 uptake rates for enriched and depleted algae in low (a), medium (b), and high (c) initial water column nutrient concentrations in the nitrogen uptake experiment. Bars represent ± 1 SE.

higher than enriched algae. Water column NO_3 dropped below the detection limit of 3.57 µM between the 4 and 8 h sampling times for both the depleted and enriched algae, suggesting that algal tissues were not N saturated in this treatment and making it impossible to calculate uptake rates beyond the 4-h mark.

Uptake in the medium-concentration treatment was low and extremely variable (Figure 4b) until the 2-4 h sampling interval, when uptake was 131.26 µmoles g dry wt^{-1} h^{-1} (SE=5.85) for enriched algae and 190.04 µmoles g dry wt^{-1} h^{-1} (SE=8.41) for depleted algae. Uptake rates were the maximum during this interval and decreased during each sampling interval thereafter for both the enriched and depleted algae.

In the high-concentration treatment, both the depleted and enriched algae took up NO_3 over the first time interval (0-1 h) (Figure 4c). Uptake in this interval was over 200 µmoles g dry wt^{-1} h^{-1} for both the depleted and enriched algae; this was higher than any uptake rates measured in any other nutrient concentration treatment. In the 1-2 h sampling interval, NO_3 accumulated in the water column, indicating flux out of algal tissue. However, over the 2-4 h interval, uptake of NO_3 resumed and was 145.17 µmoles g dry wt^{-1} h^{-1} (SE=20.78) for enriched algae and 188.67 µmoles g dry wt^{-1} h^{-1} (SE=32.99) for the depleted algae. After 4 h, NO_3 uptake rates steadily decreased for both the depleted and enriched algae. Uptake by the depleted algae was higher than by the enriched, but the differences were not as pronounced as the medium-concentration treatment. There were no differences in uptake rates between enriched and depleted algae by the end of the experiment. As in the medium-concentration treatment, reduction in uptake over time suggests that tissues in both initially enriched and depleted algae were becoming saturated with nitrogen.

Nitrogen uptake by enriched and depleted *U. expansa*

Water column NO_3 decreased significantly over time for all three initial nutrient concentration treatments (Figure 5). Nutrient depletion in the water was greatest in treatments where initial water column NO_3 concentration was highest and initial algae were depleted of tissue N. In all three initial nitrogen concentration treatments, the rate of nutrient depletion in the water was consistently higher for the depleted algae than for the enriched algae.

In the low-concentration treatment, water column NO_3 concentrations were reduced by 100% of measurable N by the 8-h mark for the depleted *U. expansa* and in less than 12 h for all but one experimental unit containing enriched algae (Figure 5a). This pattern of N removal by *U. expansa* was similar to that of *E. intestinalis.*

In the medium-concentration treatment, water column NO_3 concentrations decreased by 110.54 µM

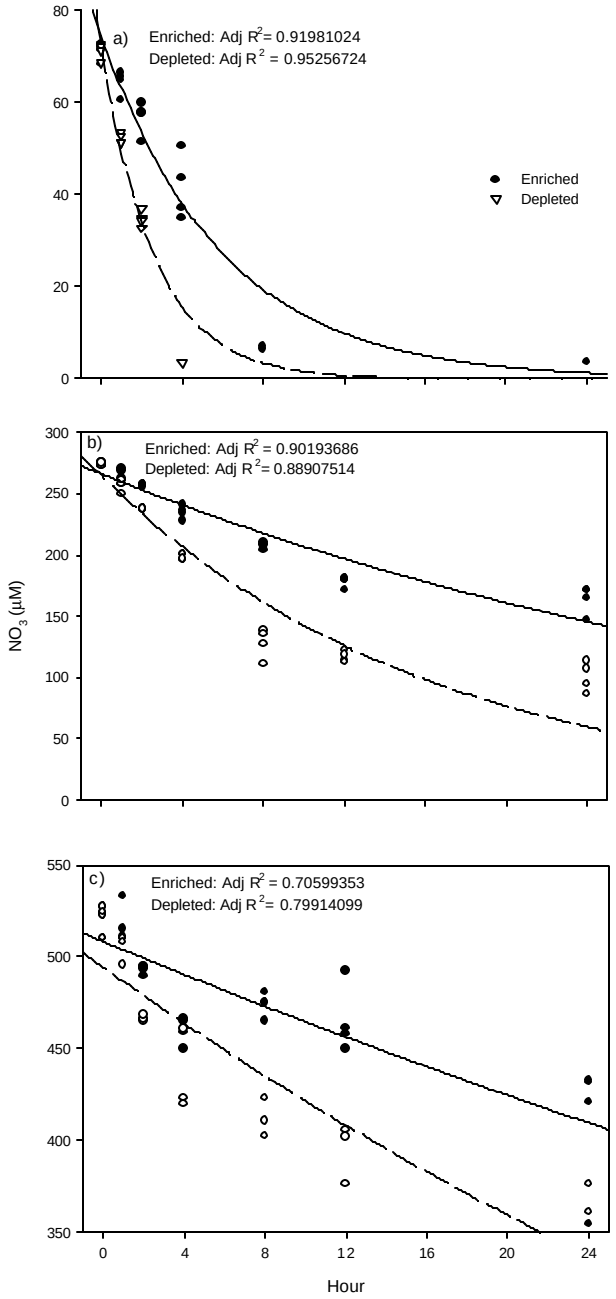

Figure 5. Decrease in water column NO_3 over time in the *Ulva expansa* nitrogen uptake experiment for enriched and depleted algae for low (a), medium (b), and high (c) initial water column nutrient concentrations.

(SE=5.74) when enriched algae were present and by 173.75 µM (SE=6.08) when depleted algae were present (Figure 5b). This resulted in a 40% (SE=2.09) reduction in water column NO_3 for the enriched algae and a 63% (SE=2.22) reduction for the depleted algae in 24 h. Overall, reductions of N in the water due to *U. expansa* were lower than for *E. intestinalis*.

In the high-concentration treatment, water column NO_3 concentrations decreased by 110.71 µM (SE=6.08) when enriched algae were present (Figure 5c). This resulted in a 21% (SE=3.58) reduction in water column NO_3 over 24 h. NO_3 concentration was further reduced when depleted algae were present. Water column NO_3 concentrations decreased by 158.69 µM (SE=7.85), a 30% reduction (SE=1.51%) in 24 h. Removal of N by *U. expansa* was also lower than that by *E. intestinalis* in these treatments. There was no pattern of water column NH_4 or TKN with either experimental factor.

U. expansa final dry weight was not significantly affected by either initial water column nutrient treatment (ANOVA, p=0.1042) or initial algal nutrient status (ANOVA, p=0.1434). Final dry weight of the algae was between 1.0-1.5 g, or ~10% of the initial wet weight (Figure 6a). The initial wet weight of E. intestinalis was twice the initial weight of *U. expansa*, but final dry weights of both species were similar due to the greater water retention capacity of *E. intestinalis*.

There was a significant effect of initial algal nutrient status on *U. expansa* final tissue N concentration (ANOVA, p=0.0001). Mean tissue N of the enriched algae was significantly higher than the depleted algae (Figure 6b). There was an interaction between the initial algal nutrient status and initial water column nutrient concentration (p=0.0074). Tissue N of the depleted algae increased as water column N increased; these changes were not seen for the enriched algae. There was no effect of initial water column nutrient treatment on *U. expansa* tissue N concentration (ANOVA, p=0.1204).

Change in tissue N of *U. expansa* was significantly affected by initial tissue nutrient status (ANOVA, p=0.0001). The percent of change from the initial N measurement in depleted *U. expansa* was greater than in enriched algae (Figure 6c). Depleted algae increased from 38-68% while enriched algae increased < 20%. There was also an effect of water column nutrient concentration (ANOVA, p=0.0366) with a significant difference between the low and the medium-concentration treatments (PLSD, p=0.0332). There were larger changes in tissue N in depleted versus enriched algae, and these changes were concentration dependent.

Uptake rate of NO_3 by *U. expansa* was significantly affected by initial algal nutrient status (ANOVA, p=0.0019), initial water column nutrient concentration (ANOVA, p=0.0001), and sampling interval (ANOVA, p=0.0001). Across all water

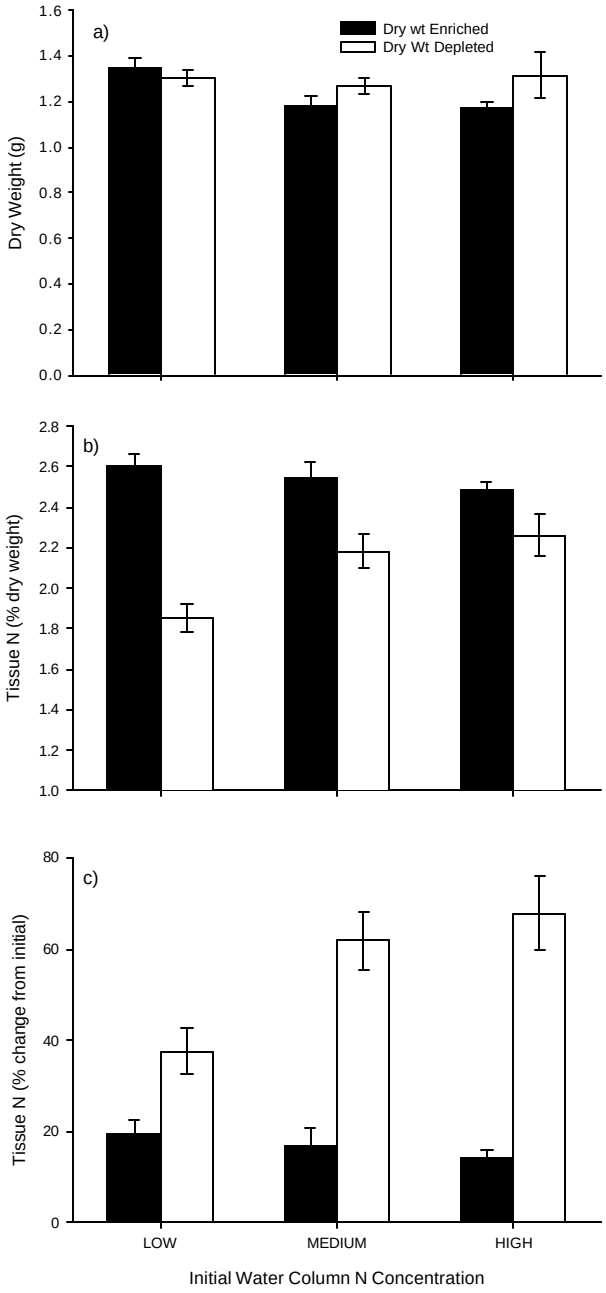

Figure 6. Final dry weight (a), tissue N concentration (b), and tissue N percent of change from initial (c) of enriched and depleted *Ulva expansa* incubated with low, medium, and high initial water column nutrient concentrations in the nitrogen uptake experiment. Bars represent ± 1 SE.

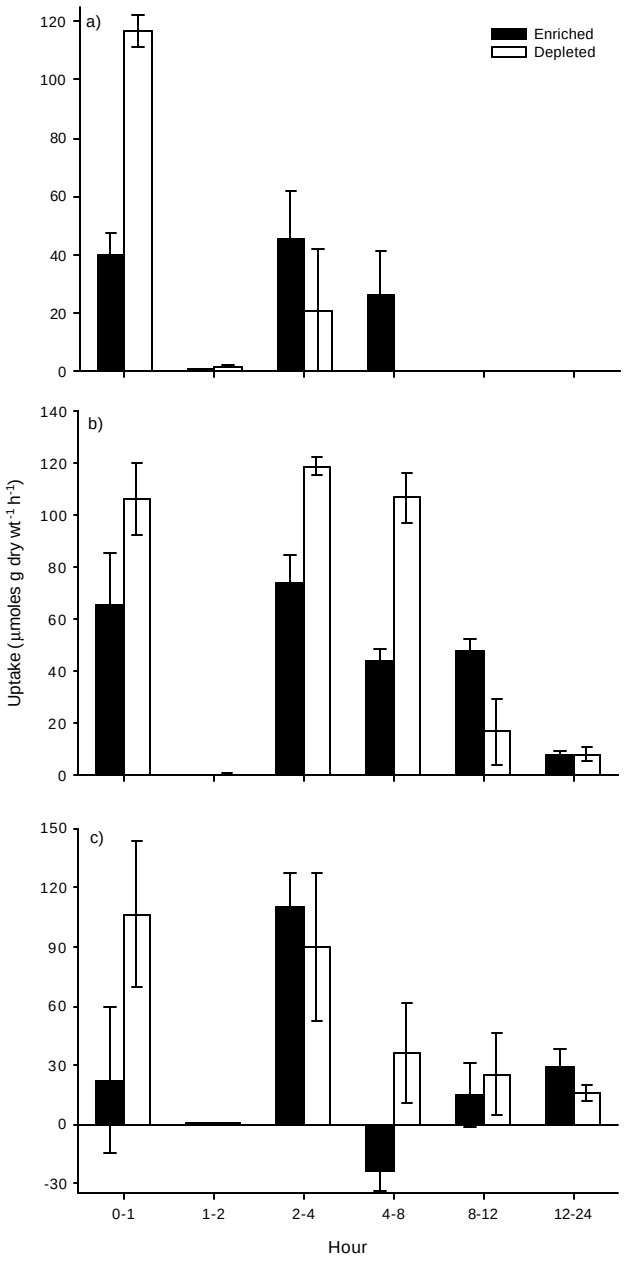

Figure 7. *Ulva expansa* NO_3 uptake rates for enriched and depleted algae in low (a), medium (b), and high (c) initial water column nutrient concentrations in the nitrogen uptake experiment. Bars represent ± 1 SE.

column nutrient treatments, uptake rates were significantly higher for the depleted than the enriched algae. A similar uptake pattern was observed in all three initial water column nutrient concentrations. Algae took up NO_3 over the first hour, but no measurable uptake occurred over the 1-2 h interval. Uptake resumed during the 2-4 h interval (Figure 7).

In the low-concentration treatment, the uptake rate of *U. expansa* was between 30-130 µmoles g dry wt^{-1} h^{-1} in the first hour, depending on algal nutrient status (Figure 7a). In the 1-2 h interval, the rate dropped to zero, then increased again in the 2-4 h interval. Water column NO_3 dropped below the detection limit during the 4-8 h interval for the depleted algae and during the 8-12 h interval for the enriched algae, suggesting that, like *E. intestinalis*, *U. expansa* algal tissue was not N saturated in this treatment. Uptake rates for *U. expansa* and *E. intestinalis* were similar in the low-concentration treatment.

In contrast to *E. intestinalis*, the highest mean uptake rates for U. expansa were measured in the medium-concentration treatment (Figure 7b). After the 2-4 h sampling interval, uptake rates for both the depleted and enriched algae decreased significantly over time. The uptake rates of the depleted algae were greater than the enriched algae up until the 8-12 h interval.

The uptake rate in the high nutrient concentration had a similar pattern to the medium-concentration treatment, with highest uptake occurring during the initial time interval, no uptake in the second interval, and uptake rates of both enriched and depleted algae decreasing over time after the 2-4 h sampling interval (Figure 7c). However, there were no differences between the enriched and depleted algae after the 0-1 h sampling interval. As with *E. intestinalis*, reduction in uptake over time suggests that tissues of both initially enriched and depleted algae were becoming saturated with N. In the high-concentration treatments, uptake by *U. expansa* was much lower than uptake by *E. intestinalis*.

DISCUSSION

Nitrogen status, measured as internal concentration of N, was very important in determining the nitrogen uptake rate of both *U. expansa* and *E. intestinalis* in these experiments. Uptake rates were always lower for enriched algae compared to nutrient-depleted tissue. This relationship has been incorporated into conceptual models of macroalgal nutrient uptake and storage (Hanisak 1983), but is rarely implicitly included in standard nutrient uptake experiments (but see Fujita 1985 and O'Brien and Wheeler 1987). The effect of algal tissue nutrient status on uptake rates suggests that knowledge of the nutrient history of the natural algal population is critical in accurately predicting what uptake rates will be in the field. Prediction of nutrient uptake rates cannot be based on nutrient supply or concentration alone.

Nitrogen uptake rates measured in these experiments were high relative to published uptake rates for other species of algae but comparable to rates published for *Enteromorpha* and *Ulva* spp. Rates measured in this experiment were at least an order of magnitude greater than the NO_3 V_{max} calculated for *Codium fragile* (2.8-10.9 µmoles g dry wt^{-1} h^{-1}), *Fucus spiralis* (1.4-2.5 µmoles g dry wt^{-1} h^{-1}), *Gracilaria tikvahiae* (9.7 µmoles g dry wt^{-1} h^{-1}), *Hypnea muciformis* (28.5 µmoles g dry wt^{-1} h^{-1}), *Laminaria longicruris* (7.0-9.6 µmoles g dry wt^{-1} h^{-1}), *Macrocystis pyrifera* (22.4-30.5 µmoles g dry wt^{-1} h^{-1}), and *Neogardhiella baileyi* (11.7 µmoles g dry wt^{-1} h^{-1}) (as cited in Hanisak 1983). Harlin (1978) calculated a V_{max} of 129.4 µmoles NO_3 g dry wt^{-1} h^{-1} for *Enteromorpha* spp. O'Brien and Wheeler (1987) measured NO_3 uptake by *E. prolifera* at >100 µmoles g dry wt^{-1} h^{-1}, similar to the uptake rates measured in our experiments over some sampling intervals. Fujita (1985) measured NH_4 uptake rates up to ~360 µmoles g dry wt^{-1} h^{-1} for *Ulva lactuca* and ~900 µmoles g dry wt^{-1} h^{-1} for *Enteromorpha* spp. during transiently enhanced uptake.

Our results demonstrated that both *E. intestinalis* and *U. expansa* efficiently removed N from the water at both the lowest and highest concentrations used in these experiments. Within the low-concentration treatments, uptake did not decrease as water column nutrient concentrations decreased over time. For both *E. intestinalis* and *U. expansa* in the low N treatments, uptake rates dropped to zero because all of the available N had been removed from the water column by the algae. However, for *E. intestinalis*, nitrogen uptake increased with water column nutrient concentrations throughout the range tested (low, medium, and high), indicating a relationship between nutrient uptake and availability. Many studies have documented that nutrient uptake rates are dependent on the concentration of that nutrient in the water, such

that uptake rates increase with increasing concentration (e.g., Harlin 1978, Rosenberg and Ramus 1984, Fujita 1985).

Among the sampling intervals of our experiments, N uptake rates varied greatly. Uptake of N by *E. intestinalis* went from the maximum rate to actual release of N within 2 h in the highest N concentration while *U. expansa* uptake went from maximum values to zero uptake in all N treatments. Thus, uptake rates were either extremely high or extremely low in any given interval. Variability in uptake among time intervals may be a result of the biochemistry of nutrient uptake. Pedersen (1994) separates uptake of NH_4 by *U. lactuca* into three phases. The first, surge uptake, is transiently enhanced nutrient uptake by nutrient-limited algae that may last only minutes to hours (Pedersen 1994). The second, internally controlled uptake, is determined by the rate-limiting step of assimilating N into organic compounds (as in Fujita *et al.* 1988), and the third, externally controlled uptake, occurs at decreasing substrate concentrations and is regulated by the rate of transport of nutrients across the alga's surface (Pedersen 1994). In our experiments, we saw indication of surge uptake during the first time interval for *U. expansa* at low, medium, and high initial water column NO_3 concentrations and for *E. intestinalis* at high NO_3 concentrations. During the 1-2 h interval, when *U. expansa* did not take up N and *E. intestinalis* appears actually to have released N, uptake may have been internally controlled, with the algae spending all available energy on assimilating inorganic N. Uptake resumed following these pauses, indicating that internal inorganic N pools had been sufficiently reduced to signal the algae to begin taking up external supplies of N again.

During the later time intervals over which uptake rates in the medium and high N treatments decreased, uptake may have been controlled by diminishing water column nutrient supplies, as suggested by Pedersen (1994). Even though considerable amounts of NO_3 remained in the water column after 24 h in the high N treatments, the algae may have recognized these levels as diminished relative to initial concentrations. An alternative explanation for decreasing uptake rates in the medium and high N treatments could be saturation of algal tissue N storage capacity. While higher maximum tissue N content has been measured in both *E. intestinalis* and *U. expansa* collected in the field (Wheeler and Björnsäter 1992, Hernández *et al.* 1997, Kamer *et al.* 2001), it is possible that once rapid uptake resulted in high internal concentrations of inorganic nutrients, the rate limiting step was no longer uptake but assimilation into organic compounds (Fujita *et al.* 1988).

In general, macroalgae did not grow measurably in these 24-h experiments. The exception was *E. intestinalis* in the N addition experiment, which grew in the lowest concentration treatment. This supports earlier findings that demonstrate nutrient uptake and growth are often temporally uncoupled (Duke *et al.* 1989). Both uptake and growth are energy-dependent processes. One explanation for temporal uncoupling of these two processes may be that opportunistic, "nutrient-specialist " types of algae allocate all available energy to uptake following pulses of high nutrient concentration to maximize short-term uptake. Once water column nutrients are depleted, energy is then available for growth. Only in these low nutrient treatments were nutrients depleted enough for growth to be significant.

These experiments identified three factors that are critical in modeling nutrient uptake by macroalgae: nutrient concentration in the water column, algal nutrient status, and the various phases of nutrient uptake. Nutrient uptake rates measured in short-term experiments (scale of hours) may only be representative of a portion of the range of rates that algae may be capable of over a longer time scale. In order to predict uptake of nutrients by macroalgae in the field, it is necessary to know the nutrient history of the algae and to measure uptake rates over time scales that encompass the different phases of nutrient uptake.

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