Nutrient limitation of the macroalga *Enteromorpha intestinalis*, across a range of water column nutrients and initial tissue nutrient status

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ABSTRACT - A laboratory experiment was conducted to quantify nutrient (nitrogen [N] and phosphorus [P]) limitation of macroalgae collected along a gradient in water column nutrients in Upper Newport Bay (UNB) estuary, a relatively nutrient-rich system in southern California. *Enteromorpha intestinalis* and water were collected for use in the experiment from five sites ranging from the lower end of the estuary to the head. Portions of the water from each site were amended with nutrients. Algae from each site were assigned to one of four experimental treatments: control (C), nitrogen enrichment (+N), phosphorus enrichment (+P), and nitrogen and phosphorus enrichment (+N+P). Experimental units consisted of glass jars containing 800 mL of experimental solution and 8 g (wet weight) of *E. intestinalis*; replication was five-fold. Each week for three weeks, water column nutrient concentrations were measured in each experimental unit; at the end of the experiment, *E. intestinalis* biomass and tissue N and P concentrations were measured. Initial algal tissue N and P concentrations and molar N:P ratios, as well as water column NO_3 and TKN, increased along a spatial gradient from the lower end of the estuary toward the head. After three weeks, *E. intestinalis* collected from sites throughout UNB, spanning the range of background water column NO_3 and PO_4 concentrations, was nutrient limited. Biomass of *E. intestinalis* from three of five sites increased with N enrichment alone and increased further when P was added in combination with N. This indicated that N was the most limiting nutrient and that P was the next most limiting nutrient after N. Growth of *E. intestinalis* from the site closest to the head of the estuary was moderate relative to the other sites and may have been limited by a factor other than nutrients.

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INTRODUCTION

As nutrient loading to coastal waters increases (Nixon 1995), a common problem in estuaries throughout the world is the development of macroalgal blooms (e.g., Sfriso *et al.* 1987 and 1992, Schramm and Nienhuis 1996, Raffaelli *et al.* 1999). While macroalgae are a natural component of estuaries, excessive production of macroalgae may result in blooms that reduce the habitat quality of an estuary by depleting oxygen levels (Sfriso *et al.* 1987, Valiela *et al.* 1992), leading to fish and invertebrate mortality (Raffaelli *et al.* 1991) and ultimately to changes in community structure (Bolam *et al.* 2000).

The growth of primary producers, such as macroalgae, is controlled in part by the availability of the resources that the primary producers require. Resources that can limit the growth of macroalgae include, but are not limited to, nutrients, salinity, light, and temperature (Schramm 1999). Nitrogen (N) and phosphorus (P) are the two most common nutrients that limit macroalgae growth (Howarth 1988, Smith *et al.* 1999).

Macroalgal production in temperate marine and estuarine systems is usually limited by N (Ryther and Dunstan 1971, Hanisak 1983, Howarth 1988). Increases in N supply to a system may release macroalgae from limitation and result in increased growth and biomass accumulation. As biomass increases, P may become the next most limiting resource (McComb *et al.* 1981, Taylor *et al.* 1995). Additionally, when algae are released from either N or P limitation by the input of more nutrients to the system, and the biomass increases, the demand for nutrients increases as well. Consequently, N or P can

again become limiting to macroalgal biomass. Given these cycles, at any point in time, macroalgal growth in an estuary may be limited by either N or P, or not limited by either of these nutrients, depending on the immediate levels of algal biomass and nutrient concentrations.

The occurrence of nutrient limitation of macroalgae in an estuary may vary spatially. Water column nutrient levels are generally higher near the head of a system and decrease toward the mouth (Rizzo and Christian 1996, Hernández *et al.* 1997, Nedwell *et al.* 2002). However, due to differential rates of nutrient processing, the relative abundance of N and P available to macroalgae may change along a spatial gradient within an estuary, and changes in nutrient limitation may occur along such a gradient.

Determining the conditions under which either N or P limits macroalgal biomass is paramount to reducing macroalgal blooms. Reduction in the supply of one or both of these nutrients to the point where the supply becomes limiting should result in a decrease in macroalgal biomass within the system. Therefore, this information can be used to most effectively control macroalgal abundance and restore estuarine systems to a more pristine state.

In southern California estuaries, the macroalga *Enteromorpha intestinalis* is a dominant bloom-forming species (Peters *et al.* 1985, Rudnicki 1986, Kamer *et al.* 2001). This green alga is highly successful, in part due to its high nutrient uptake rates and capacity to store nutrients (Fujita 1985), and its tolerance to salinity fluctuation (Kamer and Fong 2000). In several systems, such as Upper Newport Bay (UNB), high annual nutrient loads (Schiff and Kamer 2000), combined with relatively warm temperatures and high light levels in summer, have resulted in the excessive growth of *Enteromorpha intestinalis* (Kamer *et al.* 2001).

UNB is an excellent system in which to investigate the relative importance of N and P limitation across a nutrient gradient. UNB receives nutrient-laden runoff from its urbanized watershed via San Diego Creek, a significant nutrient and freshwater source to the system (California Regional Water Quality Control Board 1997). Measurements of water column nutrient concentrations have shown consistently higher levels near the head of the estuary (158-800 µM NO_3, 4.3-16.7 µM total P) relative to down-estuary areas (5-90 µM NO_3, 1.8-11.5 µM total P) (Boyle 2002). Kamer (unpub. data) also found a gradient in water column NO_3 in UNB of 414 µM near the head, 101 µM at a mid-estuary site, and 49 µM at the most seaward site.

The objective of this study was to determine whether nutrients (N or P) limit macroalgal biomass along a nutrient gradient in UNB. We hypothesized that *Enteromorpha intestinalis* from down-estuary sites would be nutrient limited and that the occurrence of limitation may decrease with increasing proximity to the head of UNB.

METHODS

A laboratory experiment was conducted to determine whether N or P limits the growth of *Enteromorpha intestinalis* from UNB. Algae and water were collected from five sites in UNB, ranging from the lower end of the estuary (Site 1) to the head (Site 5) where San Diego Creek enters UNB (Figure 1). Portions of the water from each site were amended with nutrients. Algae from each site were assigned to one of four experimental treatments: control (C), nitrogen enrichment (+N), phosphorus enrichment (+P), and nitrogen and phosphorus enrichment (+N+P). The end result was a three-factor experimental design: site x N enrichment x P enrichment.

On June 11, 2001, *Enteromorpha intestinalis* and water for use in the experiment were collected from UNB (Figure 1) and transported back to the laboratory within 5 h. At each site, *E. intestinalis* was hand collected and water was collected from mid-water depth using a battery-operated pump.

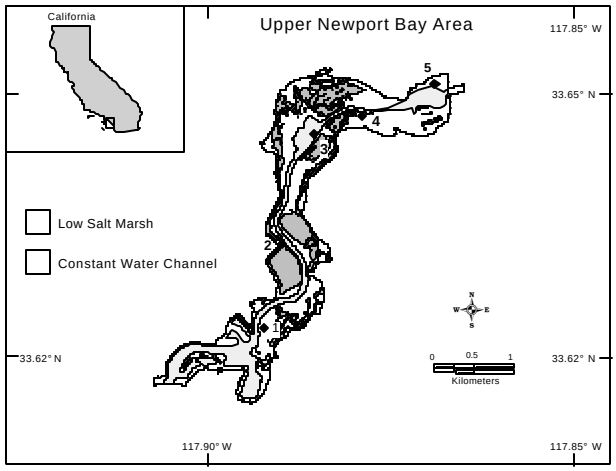

Figure 1. Map of Upper Newport Bay estuary, California, with 5 sites from which *Enteromorpha intestinalis* and water were collected for determination of nutrient limitation.

Tissue N and P of the collected *E. intestinalis* increased along a spatial gradient from the lower end of the estuary toward the head (Table 1). Tissue N:P molar ratios ranged from 16.75 to 26.40 (Table 1) and increased from Site 1 to Site 4. NO_3 in the collected water was lower from the more seaward sites (Sites 1 and 2) compared to the sites further up-estuary, and the highest NO_3 levels were found in water from Site 4 (Table 2). NH_4 and PO_4 were variable among sites. Total Kjeldahl Nitrogen (TKN, which is all forms of N except NO_3 and NO_2) was similar in water from Sites 1 through 3 and higher in water from Sites 4 and 5 (Table 2). Water column nutrient concentrations were considerably lower than values measured in other studies (Boyle 2002, Kamer unpub. data) due to the collection of water during a flood tide.

In the laboratory, *Enteromorpha intestinalis* from each site was cleaned of debris and other organisms and placed in shallow pans filled with aerated water from the corresponding site. Pans were kept outdoors overnight in a temperature-controlled water bath (20°C) and covered with window screening to reduce incident light. The collected water was placed in the dark in a 6°C cold room, where it was kept throughout the experiment.

On June 12, 2001, a portion of the water from each site was divided into four aliquots to create the experimental solutions (C, +N, +P, +N+P). The control solutions were ambient water from each site with no additions. NO_3 and PO_4 were added to the other solutions to increase concentrations over initial background levels by 400 µM N and 40 µM P. These concentrations are within the range of water column N and P concentrations measured in UNB (Boyle 2002, Kamer unpub. data).

Enteromorpha intestinalis from each site were placed in nylon mesh bags and spun in a salad spinner for 1 minute to remove excess water. Algae were wet weighed and 8.0 ± 0.1 g sub-samples were added

Table 1. Mean (± SE) initial *Enteromorpha intestinalis* tissue nitrogen and phosphorus concentrations and molar N:P ratios from each of the 5 sites in UNB. *n*=5. Superscripts denote mean values that are significantly different from each other (p<0.05, Fisher's LSD following significant 1-factor ANOVA).

Site	Tissue Nutrient Content (as percent of dry weight)		Molar N:P
	N	P	
1	1.18 (0.03)[a]	0.156 (0.002)[a]	16.75 (0.39)[a]
2	1.47 (0.06)[b]	0.164 (0.007)[a]	20.09 (1.37)[b]
3	2.21 (0.03)[c]	0.230 (0.003)[b]	21.26 (0.26)[b]
4	2.81 (0.13)[d]	0.238 (0.009)[b]	26.40 (1.98)[c]
5	2.62 (0.08)[d]	0.320 (0.009)[c]	18.16 (0.40)[a,b]

Table 2. Mean (± SE) background water column nutrient concentrations at the time of collection from each of the 5 sites in UNB. *n*=3. Superscripts denote mean values that are significantly different from each other (p<0.05, Fisher's LSD following significant 1-factor ANOVA). Among-site differences in PO_4 were not analyzed as some values were below detection limits.

Site	Water Column Nutrients (µM)			
	NO_3	NH_4	PO_4	TKN
1	12.9 (0.4)[a]	6.2 (1.2)[a]	<1.61	92.9 (8.4)[a]
2	28.3 (1.2)[b]	25.7 (1.2)[b]	2.9 (0.2)	95.2 (38.3)[a]
3	36.2 (0.6)[c]	12.6(1.4)[c]	<1.61	100 (14.9)[a]
4	55.2 (2.1)[d]	17.4 (2.7)[c]	2.5 (0.3)	238.1 (41.7)[b]
5	42.4 (0.6)[e]	<3.57	<1.61	302.4 (56.9)[b]

to glass experimental units (1.5 L total volume), each containing 800 mL of the appropriate solution. Five additional sub-samples of algae were processed for tissue N and P analysis.

Experimental units were placed in a randomized array outdoors in a temperature-controlled water bath (20 ± 2°C) and covered with window screening to reduce incident light. Replication was five-fold with the exception of the Site 3 Control and Site 3 +N treatments, which only had three replicates each due to not having collected enough algae. There was a total of 96 units. Salinity was monitored every 2 days with a hand-held refractometer, and de-ionized water was added to compensate for evaporation. Salinity was maintained within 2 ppt of initial levels measured for each site at the time of collection.

The experiment was performed for 3 weeks. At the end of each week, algae were removed from the experimental units, a 125 mL water sample was taken from each unit, and the remaining water was discarded. Water samples were filtered (Whatman GF/C) and frozen for subsequent NO_3, NH_4, TKN, and PO_4 analysis.

The procedure of creating experimental solutions using water collected from each site on June 11, 2001, was repeated at the beginning of Weeks 2 and 3 of the experiment. Each time, three sub-samples of each solution were filtered (Whatman GF/C) and frozen for subsequent analysis of NO_3, NH_4, TKN, and PO_4. The mean values of each solution for each site across all weeks are presented in Table 3. Using data from all three weeks, the mean NO_3 and TKN of experimental solutions from each site were compared using three-factor analysis of variance (ANOVA) (site x N enrichment x P enrichment). NO_3 and TKN were significantly affected by site and N enrichment ($p<0.020$ for both factors). Many NH_4 and PO_4 values were below detection limits of 3.57 and 1.61 µM, respectively, preventing statistical analyses.

Experimental units were refilled with 800 mL of the appropriate solution and the algae were placed back in their respective units. The physical array of the units in the water bath was re-randomized each week.

Table 3. Mean (± SE) concentrations across all weeks of NO_3, PO_4, NH_4, and TKN in experimental solutions (Control, +N, +P, +N+P) from each of the 5 sites in UNB. *n*=9 unless otherwise noted (*n* less than 9 occurred when samples were below detection limits of 3.57 µM for N and 1.61 µM for P). Mean values are given in µM.

Site		NO_3	PO_4	NH_4	TKN
1	Control	14.1 (0.7)	<1.61	5.6 (0.8) n=5	54.0 (10.1)
37 psu	+N	429.8 (5.4)	<1.61	6.7 (1.0) n=3	51.0 (9.6)
	+P	15.8 (0.5)	41.0 (0.6)	6.3(0.9) n=4	41.3 (5.6)
	+N+P	435.8 (5.2)	46.1 (0.4)	5.7 (0.6) n=4	123.0 (46.2)
2	Control	29.9 (0.8)	2.4 (0.3) n=5	13.5 (3.6) n=8	55.6 (15.0)
35 psu	+N	445.7 (6.4)	<1.61	9.4 (2.9)	77.0 (13.6)
	+P	31.9 (1.2)	46.5 (0.4)	13.4 (3.1)	77.0 (15.3)
	+N+P	454.2 (5.2)	45.5 (0.7)	10.9 (3.3)	66.7 (11.4)
3	Control	39.9 (1.1)	<1.61	8.8 (1.4) n=8	61.1 (10.8)
33 psu	+N	453.0 (3.9)	<1.61	10.6 (1.6)	108.7 (12.0)
	+P	39.1 (1.2)	46.2 (0.8)	9.8 (1.4)	92.1 (22.9)
	+N+P	453.8 (4.5)	45.5 (1.0)	10.3 (1.7)	107.1 (9.8)
4	Control	54.8 (1.3)	2.7 (0.1)	17.5 (1.7)	133.3 (32.6)
31 psu	+N	461.8 (5.4)	3.7 (0.9) n=6	15.3 (1.5)	148.4 (27.3)
	+P	59.1 (0.9)	46.6 (0.9)	14.1 (1.1)	95.2 (16.4)
	+N+P	467.5 (5.0)	44.0 (0.7)	15.5 (2.1)	166.7 (43.2)
5	Control	34.7 (2.0)	2.4 (0.1) n=3	8.3 (1.1) n=6	123.0 (47.8)
33 psu	+N	455.2 (10.2)	6.9 (4.5) n=3	8.3 (0.6) n=6	103.2 (20.9)
	+P	34.7 (2.7)	44.3 (0.6)	9.3 (0.5) n=6	77.0 (19.2)
	+N+P	458.9 (14.3)	42.7 (0.3)	7.8 (1.1) n=8	115.1 (25.5)

At the end of the experiment, the algae were removed from each unit and wet weighed after being spun in nylon mesh bags in a salad spinner for 1 minute. Each sample was rinsed briefly in fresh water to remove external salts, dried in a forced air oven at 60°C to a constant weight, and ground with mortar and pestle for subsequent tissue N and P analysis. The N and P content of algae are reported as both concentration (percent of dry weight) and percent of change from initial measurement.

Laboratory analyses: Water column NO_3 was reduced to NO_2 via cadmium reduction; NO_2 was measured spectrophotometrically after diazotation (Switala 1999, Wendt 1999). Water column NH_4 was heated with solutions of salicylate and hypochlorite and determined spectrophotometrically (Switala 1999, Wendt 1999). Water column TKN was determined by the wet oxidation of nitrogen using sulfuric acid and digestion catalyst. The procedure converts organic nitrogen to NH_4, which is subsequently determined (Carlson 1978). Water column PO_4 was determined spectrophotometrically following reaction with ammonium molybdate and antimony potassium under acidic conditions (APHA 1998). These methods have detection limits of 3.57 µM for all forms of N and 1.61 µM for P.

Algal tissue N was determined using an induction furnace and a thermal conductivity detector (Dumas 1981). Algal tissue P was determined by atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) following a nitric acid/hydrogen peroxide microwave digestion (Meyer and Keliher 1992).

Statistical analyses: All data were tested for normality and homogeneity of variance. Among-treatment differences in biomass and tissue nutrient concentrations were analyzed using three-factor ANOVA (site x N enrichment x P enrichment). Significant interactions did not occur unless otherwise noted. Following a significant ANOVA, multiple comparisons were used to determine differences among individual treatments (Fisher's Least Significant Difference [LSD] test). Water column NO_3, NH_4, and PO_4 values from the end of each week were often below detection limits (3.57 µM for N, 1.61 µM for P) and were not analyzed statistically; statistical analyses of the remaining values were not conducted. Water column TKN data from the end of each week were analyzed using four-factor ANOVA (site x N enrichment x P enrichment x time).

RESULTS

Biomass of *Enteromorpha intestinalis* collected from UNB generally increased from Site 1 to Site 4 (Figure 2). Algae collected from Site 5 did not follow the trend of increased biomass with increasing proximity to San Diego Creek. Biomass was significantly affected by site and N enrichment (ANOVA $p=0.0001$ for both factors) but not by P enrichment (ANOVA $p=0.6268$). Compared to controls, biomass of algae from Sites 1, 2, and 4 increased in the N enrichment alone treatments (Fisher's LSD $p<0.050$ for C versus +N at each site), indicating that N was the most limiting nutrient. There was no significant increase when N alone was added compared to controls in the biomass of algae collected from Site 3 (Fisher's LSD $p=0.054$), although variability in the control treatment was high, or Site 5 (Fisher's LSD $p=1.000$), resulting in an interaction between site and N enrichment (ANOVA $p=0.0024$).

For *Enteromorpha intestinalis* collected from Sites 1, 2 and 4, biomass increased further when P was added in combination with N (Fisher's LSD $p<0.005$ for +N versus +N+P at each site), indicating that P was the next most limiting nutrient after N. This resulted in an interaction between N and P enrichment (ANOVA $p=0.0039$). There were no

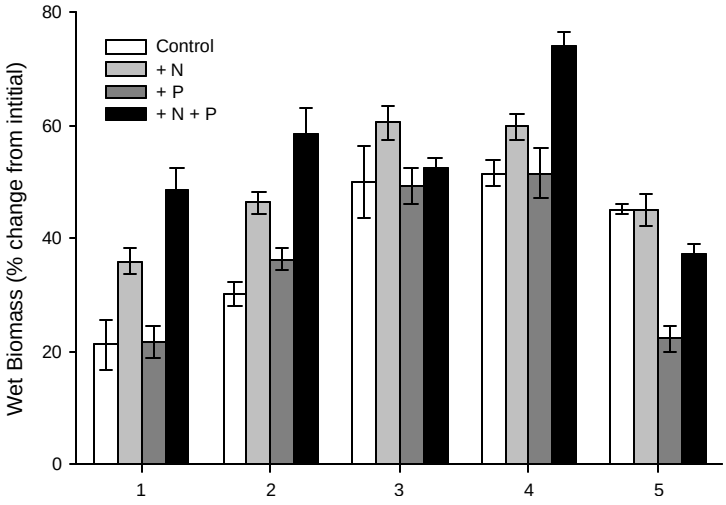

Figure 2. *Enteromorpha intestinalis* biomass (as percent change from initial measurement) grown with ambient (Control), nitrogen enrichment (+N), phosphorus enrichment (+P), or nitrogen and phosphorus enrichment (+N+P) solutions. Bars represent ± 1 SE.

differences between controls and +P treatments in biomass of algae collected from Sites 1 through 4 (Fisher's LSD p>0.100 for C versus +P at each site); therefore, there is no indication of P as the primary limiting nutrient. Biomass of *E. intestinalis* from Site 5 declined when P was added, resulting in an interaction between site and P enrichment (ANOVA p=0.0001).

Enteromorpha intestinalis final tissue N concentrations increased with site (ANOVA p=0.0001) in all treatments (Figure 3a). Tissue N was also significantly affected by N enrichment (ANOVA p=0.0001) but not by P enrichment (ANOVA p=0.2878). For all sites, tissue N concentration was greatest when N was added regardless of P addition. There was an interaction between site and N enrichment (p=0.0020), probably due to a small difference in magnitude of the increase in tissue N concentration between the +N and +N+P treatments from Site 4.

Enteromorpha intestinalis tissue N percent change from initial was significantly affected by site and N enrichment (ANOVA p=0.0001 for both factors) but not by P enrichment (p=0.2448). Final tissue N concentrations were lower than initial values for Control and +P only treatments from all sites (Figure 3b). Tissue N in the +N and +N+P treatments increased compared to initial values from Sites 1 and 2, decreased compared to initial values from Sites 3 and 4, and was similar to initial values from Site 5.

Enteromorpha intestinalis final tissue P concentration was significantly affected by site and P enrichment (ANOVA p=0.0001 for both factors) but not by N enrichment (ANOVA p=0.4439). Tissue P concentrations in every experimental treatment were greatest from Site 5 (Figure 4a), likely causing an interaction between site and P enrichment (p=0.0038). For all sites, tissue P concentration was greatest when P was added regardless of N addition.

Enteromorpha intestinalis tissue P percent change from initial was significantly affected by site and P enrichment (ANOVA p=0.0001 for both factors) but not by N enrichment (ANOVA p=0.6588). Final tissue P concentrations were lower than initial values for Control and +N only treatments from all sites (Figure 4b). Tissue P increased compared to initial values in +P and +N+P treatments from all sites, although these increases varied in magnitude with site due to differences in initial tissue P values and caused an interaction between site and P enrichment (ANOVA p=0.0001).

Water column NH_4 was low throughout the experiment. At the end of Week 1, water column NH_4 was at or below the detection limit (BDL) of 3.57 µM in all 96 experimental units. At the end of Week 2, NH_4 was BDL in 94 units and <6 µM in 2 units. At the end of Week 3, NH_4 was BDL in 61 units. In the remaining 35 units, NH_4 was 9.06 ± 0.80 µM (mean ± SE).

Water column NO_3 supply was greatly reduced in all units each week of the experiment. At the end of Week 1, water column NO_3 was at or BDL of 3.57 µM in 55 of the 96 units. In the remaining 41 units, NO_3 was 5.51 ± 0.19 µM. At the end of Week 2, NO_3 was at or BDL in 94 units and 4.29 µM in 2 units. At the end of Week 3, NO_3 was at or BDL in 44 units. In the remaining 52 units, NO_3 was 6.20 ± 0.23 µM.

Water column PO_4 was also low in many units at the end of each week of the experiment. At the end

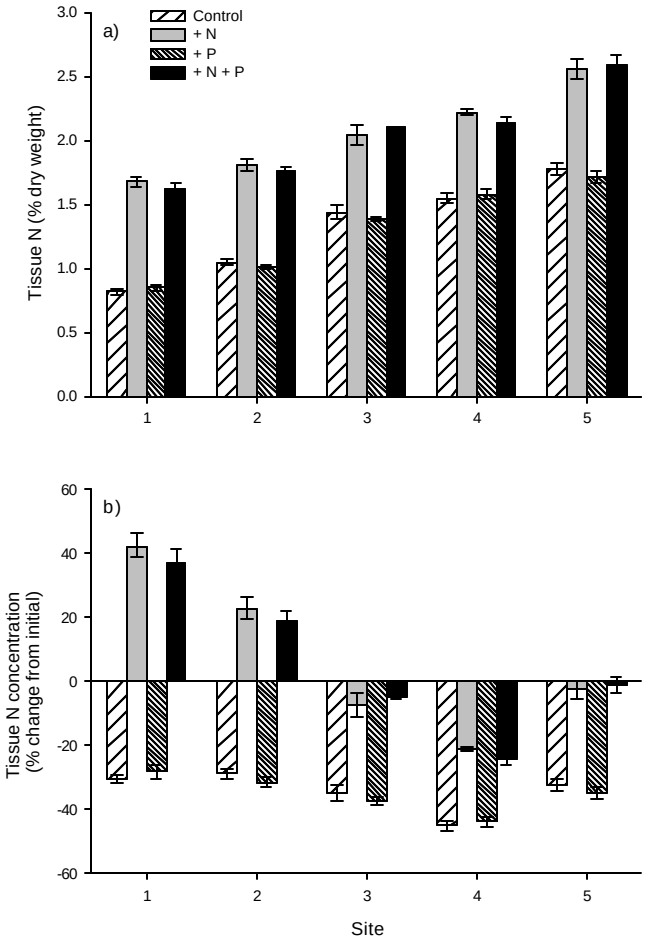

Figure 3. *Enteromorpha intestinalis* tissue nitrogen concentration as percent dry weight (a) and percent change from initial value (b) after 3 weeks in ambient (Control), nitrogen enrichment (+N), phosphorus enrichment (+P), or nitrogen and phosphorus enrichment (+N+P) solutions. Bars represent ± 1 SE.

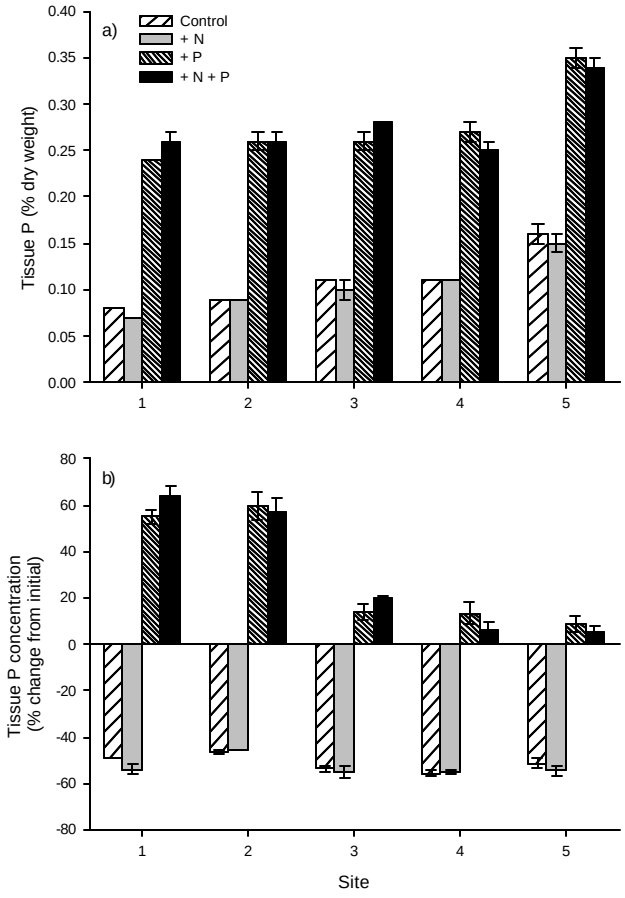

Figure 4. *Enteromorpha intestinalis* tissue phosphorus concentration as percent of dry weight (a) and percent change from initial value (b) after 3 weeks in ambient (Control), nitrogen enrichment (+N), phosphorus enrichment (+P), or nitrogen and phosphorus enrichment (+N+P) solutions. Bars represent ± 1 SE.

of Week 1, water column PO_4 was BDL of 1.61 µM in 75 of 96 experimental units and was 4.58 ± 0.25 µM in the other 21 units. At the end of Weeks 2 and 3, PO_4 was at or BDL in 55 units; at the end of Week 2, PO_4 was 4.31 ± 0.23 µM; and at the end of Week 3, PO_4 was 4.67 ± 0.24 µM in the remaining units.

Water column TKN at the end of each week was significantly affected by site (p=0.0004, ANOVA) and time (p=0.0001, ANOVA) but not by N enrichment (p=0.8506, ANOVA) or P enrichment (p=0.7949, ANOVA). There was interaction between time and site (p=0.0008), probably due to variation in the magnitude of the differences in water column TKN between sites at the end of each week (Table 4). TKN decreased over the course of the experiment.

DISCUSSION

Enteromorpha intestinalis collected from sites throughout UNB was nutrient limited. Algae from down-estuary sites were limited by N, as expected, based on low background water column NO_3 concentrations. However, *E. intestinalis* from Site 4, which had the highest background water column NO_3 concentration, was also limited by N. Furthermore, water from Site 4 had among the highest background water column TKN levels, and algae were still N limited in spite of this potential source of N. Therefore, in our experiment, *E. intestinalis* was N limited across a range of available water column N.

Once N was supplied in sufficient quantity, P limitation occurred in algae collected from sites with both the lowest and highest background water column PO_4 concentrations. Background PO_4 levels were variable among sites and did not exhibit the same spatial gradient at NO_3 and TKN. However, PO_4 addition stimulated growth of algae collected from sites with PO_4 levels below the detection limits of 1.61 and up to 2.9 µM, and these sites were both down-estuary and closer to the head.

The lack of statistical difference in biomass of *Enteromorpha intestinalis* from Control and +N treatments from Site 3 was likely due to high variability in the Control treatment. Furthermore, replication of the Control and +N treatments from Site 3 was only three-fold, compared to five-fold replication in all other experimental treatments (this was due to not having collected enough algae from Site 3). It is probable that with greater replication of the Control and +N treatments from Site 3, the trend toward increased biomass with N enrichment alone would have been significant.

There was no indication of either N or P limitation of *Enteromorpha intestinalis* from Site 5. Growth of *E. intestinalis* from Site 5 was moderate compared to the growth of algae from Sites 1 through 4, even though algae from Site 5 had among the highest initial tissue N and P concentrations, presumably due to the proximity of the site to the mouth of San Diego Creek, a large freshwater and nutrient source to UNB. Growth of algae from Site 5 may have been limited by other factors not investigated in this study. Due to its proximity to San Diego Creek, water collected at Site 5 may have had greater concentrations of toxics, herbicides, or other constituents that could have been responsible for the patterns observed.

Enteromorpha intestinalis was most limited by N across a range of initial tissue nutrient levels. N

Table 4. Mean (± SE) water column TKN in units from all experimental treatments for each site at the end of each week of the experiment. Values from C, +N, +P, and +N+P treatments were averaged when there were no effects of either N enrichment or P enrichment (ANOVA, p>0.050 for both factors) on water column TKN. n=20, except for Site 3 where n=16.

Site	TKN (μM)		
	Week 1	Week 2	Week 3
1	203.93 (35.74)	47.86 (2.75)	33.93 (1.71)
2	290.71 (41.77)	42.50 (2.10)	32.14 (2.04)
3	157.59 (25.22)	29.91 (1.63)	28.57 (1.30)
4	177.14 (21.99)	32.50 (1.68)	32.86 (1.82)
5	263.92 (29.24)	33.21 (2.15)	30.71 (1.47)

limitation occurred in *E. intestinalis* with initial tissue N concentrations that varied by more than two-fold. N limitation may have been expected in algae from Sites 1 and 2, which had initial tissue N concentrations <2%. However, N limitation of algae from Site 4 occurred as well. Algae from this site had initial tissue N concentrations well over 2%, the suggested critical concentration below which macroalgal maximal growth rates are limited by internal N concentration (Hanisak 1983, O'Brien and Wheeler 1987, Pedersen and Borum 1996) and above which further increases in N supply, and therefore tissue N, should not stimulate increased growth.

The initial tissue molar N:P ratios of *Enteromorpha intestinalis* that exhibited N limitation also spanned a range. Algae from Site 4 had the highest initial N:P ratio, and N limitation of the algae would not have been predicted based on this value. Generally, macroalgal tissue molar N:P ratios <16 indicate N limitation, ratios between 16 and 24 indicate that N and P are present in sufficient supply, and ratios >24 indicate P limitation (Björnsäter and Wheeler 1990, Wheeler and Björnsäter 1992). However, as pointed out by Larned (1998), algal tissue N:P ratios may reflect either nutrient requirements or nutrient-storage capacities and cannot alone be used to accurately predict whether N or P may limit macroalgal growth. As seen here, an alga with an initial tissue N:P ratio >24 was N limited.

Even in nutrient-rich estuaries such as UNB (California Regional Water Quality Control Board 1997, Kamer *et al.* 2001), nutrient limitation is an important ecological process affecting primary producers. *Enteromorpha intestinalis* from several sites in UNB was most limited by N, as is common in temperate estuaries (Hanisak 1983, Howarth 1988, Pedersen and Borum 1996). When released from N limitation via N enrichment, P was the next most limiting nutrient to these algae. P limitation of macroalgae is not as common but has been documented in several systems (Birch *et al.* 1981, O'Brien and Wheeler 1987).

Documentation of limitation by one nutrient and then another when the first is supplied in excess is relatively rare. A review of nutrient limitation work on tropical macroalgae by Larned (1998) showed that when the synergistic effects of N and P were tested, there was rarely a response by the algae. However, similar to the results of our experiment, Lapointe (1989) found that *Gracilaria tikvahiae* was most limited by P and that N became limiting when P supply increased. McComb *et al.* (1981) and Taylor *et al.* (1995) found that phytoplankton abundance was most limited by N and that P became limiting when N supply increased.

End-of-experiment patterns in *Enteromorpha intestinalis* biomass and tissue nutrients were reflections of the variation in initial tissue N and P concentrations and ambient water column N supplies from each site. Final tissue N concentrations were highest in algae from Site 5, although N in water from Site 5 was lower than N in water from Site 4. This was likely the result of relatively high initial tissue N levels but only moderate growth; less dilution of tissue N concentration due to growth occurred in algae from Site 5 compared to algae from Site 4, which grew more.

TKN concentrations in water collected for this study were ~2 to 7 times higher than NO_3 concentrations. Dissolved organic N (DON) in the TKN fraction of the water, such as amino acids and urea, may be taken up by macroalgae (Tyler *et al.* in preparation) and stimulate growth. In addition, macroalgae also release DON during growth (Tyler *et al.* 2001). Release of DON from algae would account for the increases in TKN in units from several sites at the end of Week 1 of our experiment. The observed decreases in TKN from Week 1 to Weeks 2 and 3 may have been due to uptake of DON and NH_4 by the algae, re-mineralization of organic to inorganic N in the water used over the course of the experiment, or transformation of NH_4 to NO_3/NO_2.

This experiment was conducted in only one

season. Future investigation of nutrient limitation of macroalgae should include seasonal manipulations to determine the temporal extent of nutrient limitation, as well as the incorporation of other factors, such as light and temperature, which may limit macroalgal growth during the winter months.

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