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Macroalgal Nutrient Dynamics in Upper Newport Bay

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INTRODUCTION

Newport Bay is the second largest estuarine embayment in southern California and provides critical natural habitat for terrestrial and aquatic species. The upper portion of the Bay is a state ecological reserve and serves as refuge, foraging areas, and breeding grounds for a number of threatened and endangered species. The Bay also provides significant spawning and nursery habitats for commercially and recreationally fished species. However, the Bay is subject to anthropogenic stressors. For example, high nutrient loads from the surrounding watershed have resulted in excessive growth of macroalgae (*Enteromorpha* and *Ulva* spp.) and impairment of the Bay's beneficial uses.

Macroalgae exhibit distinct seasonal patterns of biomass accumulation in Upper Newport Bay (UNB). *Enteromorpha* and *Ulva* spp. are generally sparse in the winter and early spring (December-April) when growth is likely limited by environmental factors such as light, temperature, and flow. *Enteromorpha* and *Ulva* are prolific throughout the summer and early fall months (June-October) when light and temperature conditions are optimal for maximum growth. During these months, macroalgal biomass may be limited by factors such as nutrients, space, or herbivory. While the seasonal patterns of algal blooms in UNB and the biomass of algae during these events is documented to some degree, the relative importance of the different factors that may control macroalgal biomass are not yet fully understood.

In part due to the excessive growth of macroalgae, UNB has been added to the Clean Water Act 303(d) list for nutrients, as well as other constituents. The 303(d) listing precipitated the development and adoption of a Total Maximum Daily Load (TMDL) for nitrogen (N) and phosphorus (P) in San Diego Creek, the largest tributary to UNB, in 1998. The implementation phase of the nutrient TMDL has several elements, one of which calls for the evaluation of N and P water quality objectives (WQOs).

A report titled *Comparison of Nutrient Inputs, Water Column Concentrations, and Macroalgal Biomass in Upper Newport Bay, California* (Schiff and Kamer, 2000) was the first step toward evaluating the current total inorganic nitrogen (TIN) WQOs. The goal of the report was to find published studies of estuaries in which water quality and macroalgal biomass data had been collected synoptically and to determine if the WQOs in San Diego Creek were overly conservative, not conservative enough, or if insufficient data existed to evaluate the appropriateness of the current WQOs for N and P. The report concluded that insufficient data were available to make this assessment based upon several factors. First, there was a limited amount of synoptic water quality and quantitative macroalgal abundance data from other estuaries. Second, there was no relationship between water quality and macroalgae; it was not possible to reliably predict macroalgal biomass from water column nutrient concentrations. Third, southern California estuaries were distinctly different from other estuaries in the US and abroad, which limits attempts to extrapolate data from other regions and apply them locally. Fourth, significant secondary mechanisms involved in nutrient dynamics were not fully understood and needed to be investigated in order to understand the impacts of nutrient loads on estuaries.

Since there was no relationship between water column N or P and macroalgal biomass, there was a clear need for mechanistic studies investigating the processes in Upper Newport Bay (UNB) that control macroalgal biomass. Increased understanding of the processes and mechanisms that regulate the growth and biomass accumulation of *Enteromorpha* and *Ulva* spp. is paramount to making informed management decisions for estuaries such as UNB. Studies identified as high priority by Schiff and Kamer (2000) were to: 1) investigate the contribution of nutrients from estuarine sediments to macroalgal growth and tissue nutrient content; 2) determine if N or P is the nutrient most limiting to macroalgae; 3) measure rates of N and P uptake by *Enteromorpha intestinalis* and *Ulva expansa*, the dominant, green, bloom-forming macroalgal species; and 4) investigate the effects of varia-

tion in the frequency and concentration of nutrient pulses on macroalgal growth and tissue nutrient content. These are some of the important mechanisms that may enable macroalgae to bloom under enriched conditions of southern California estuaries.

PROCESS-BASED STUDIES

Effects of Estuarine Sediments on Macroalgae

Biomass of *Enteromorpha* and *Ulva* spp. is regulated, in large part, by availability of dissolved nutrients. These algae can obtain the dissolved nutrients they need from multiple sources, such as the water column or estuarine sediments (i.e. dissolved nutrient flux from porewaters). In estuaries, water column nutrient levels are generally higher near the head of the system, where rivers flow in, and decrease toward the mouth or the opening to the ocean. Therefore, the availability of water column N to macroalgae usually decreases along a spatial gradient within an estuary from the head toward the mouth. Nutrient concentrations of estuarine sediments may vary spatially as well within an estuary. This variation is likely controlled by sediment quality (i.e., grain size, organic content), which may not vary linearly along a spatial gradient. As a result, estuarine sediments may also be a significant source of nutrients to macroalgae, particularly when water column nutrients are low. However, there is little empirical data on the extent to which dissolved nutrients fluxing from estuarine sediments are either taken up by macroalgae or are of benefit to macroalgae. Our objective was to determine the importance of water column vs. sediments as sources of nutrients to macroalgae in UNB.

We hypothesized that the importance of water column vs. sediment sources of nutrients to *Enteromorpha intestinalis* varied along a nutrient resource gradient within UNB. We tested this hypothesis by constructing experimental units using water and sediments collected from 3 sites in UNB. We measured changes in water column and sediment nutrients in three sets of experimental units for each site: sediments + water; sediments + water + *E. intestinalis* (algae); inert sand (no nutrients) + water + algae. In units containing algae, we measured growth and tissue nutrients (N and P).

The importance of the water column versus sediments as sources of nutrients to *Enteromorpha intestinalis* varied with the magnitude of the different sources. When initial water column dissolved inorganic N (DIN) and PO_4 levels were low, nutrients from estuarine sediments increased *E. intestinalis* growth and tissue nutrient concentrations. Macroalgal growth and tissue P increased with increases in initial sediment nutrient concentrations. In units from the site where initial water column DIN was high, there was no effect of estuarine sediments on algal growth or tissue N content. However, reduced salinity of water from this site may have limited macroalgal growth overall, thereby masking any effects of sediment nutrients. Water column inorganic N was consistently depleted throughout the experiment, regardless of initial water column N concentration. Thus, the water column was a primary source of nutrients to the algae when water column nutrient supply was high, and the sediments supplemented nutrient supply to the algae when water column nutrient sources were low. The sediments acted as both a source and a sink of nutrients in this experiment, depending on water column nutrient concentrations.

These data provide direct experimental evidence of the role of sediment nutrients in estuarine nutrient dynamics. Macroalgae can utilize nutrients stored in estuarine sediments, which implies that nutrient inputs and the response of increased biomass of macroalgae may be temporally uncoupled via storage in the sediments. Therefore, calculations of nutrient loads to estuaries or annual budgets for these systems should include fluxes from the sediments as well as loading from the watershed.

Nitrogen vs. Phosphorus Limitation of Macroalgae

The growth of macroalgae is controlled, in large part, by nutrients as well as salinity, light, and temperature. N and P are the two most common nutrients that limit macroalgal growth. However, nutrient limitation of macroalgae in an estuary may vary along a gradient of resource availability due to differential rates of nutrient processing (e.g., P mineralizes much more quickly than N). Therefore, we investigated N and P limitation of macroalgae along a spatial nutrient gradient in UNB.

Enteromorpha intestinalis and water were collected from 5 sites ranging from the lower end of UNB to the head near San Diego Creek. Portions of the water from each site were amended with nutrients to create 4 experimental solutions (control [C], nitrogen enrichment [+N], phosphorus enrichment [+P], and nitrogen and phosphorus enrichment [+N+P]). Algae were added to replicate experimental units, each filled with one of the experimental solutions. We measured water column nutrient concentrations in each experimental unit for three weeks and, at the end of the experiment, we measured *E. intestinalis* biomass and tissue N and P concentrations.

Biomass of *Enteromorpha intestinalis* from 3 of 5 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 increased among algae from the most seaward site toward the head of the estuary reflecting initial tissue nutrient concentrations and ambient water column N supplies. In contrast, growth of *E. intestinalis* from the site closest to the head of the estuary after additions of N and P was moderate relative to the other sites and, therefore, may have been limited by a factor other than nutrients. Final tissue N and P concentrations reflected both initial water column levels and N and P enrichment, respectively. Depletion of tissue nutrients during the experiment suggested that algae were growing on internal reserves of nutrients.

Since N was the nutrient most limiting to *Enteromorpha intestinalis*, a reduction of the supply of N to UNB should result in a decrease in macroalgal biomass within the system. Since P was also limiting to macroalgae at several sites, reductions in P supply in conjunction with reductions in N supply may also decrease macroalgae in UNB.

Uptake of Nitrogen and Phosphorus by *Enteromorpha Intestinalis* and *Ulva Expansa*

As both N and P may limit macroalgal biomass in UNB, the uptake dynamics of both nutrients may be important in the system's nutrient and macroalgal bloom dynamics. Macroalgal nutrient uptake rates vary with a suite of factors including, but not limited to, initial algal tissue nutrient status. Generally, N uptake rates increase as tissue N concentration decreases, reflecting N starvation and increased N demand. Nutrient uptake rates also vary with external substrate concentration. The objective of this study was to measure the rates of inorganic N and P uptake by *Enteromorpha intestinalis* and *Ulva expansa* under varying initial water column concentrations and algal tissue nutrient concentrations representative of levels measured in UNB.

Rates of N and P uptake by *Enteromorpha intestinalis* and *Ulva expansa* were investigated in 4 separate experiments. We varied initial water column nutrient concentrations (low, medium and high) and initial algal tissue nutrient status (enriched vs. depleted). In each experiment, uptake of either N or P by either *E. intestinalis* or *U. expansa* was measured at 1, 2, 4, 8, 12, and 24 h. Uptake rates of inorganic nitrogen and phosphorus were determined by measuring the disappearance of inorganic nutrients from solution over time.

Enteromorpha intestinalis and *Ulva expansa* exhibited a high affinity for N across all treatments, but little or no affinity for P. In the low water column concentration treatments, *E. intestinalis* and *U. expansa* removed all

measurable NO_3 from the water within 8 and 12 h, respectively. Nutrient depleted algae consistently removed more NO_3 than enriched algae over each sampling interval. 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*. These rates were up to two orders of magnitude greater than rates measured for other species, but similar to rates measured for these species by other researchers.

Maximum rates of NO_3 uptake increased with increasing initial water column nutrient concentrations, indicating a relationship between uptake and external substrate concentration. Nutrient uptake rates were highly variable over 24 h, indicating surge, internally controlled, and externally controlled phases of nutrient uptake. Over the first 2 h of the *Enteromorpha intestinalis* NO_3 uptake experiment, uptake rates went from the maximum measured to negative rates, indicating a release of N from algal tissue. Similarly, *U. expansa* NO_3 uptake rates were greatest in the first hour, decreased to zero in the second hour, and then resumed. For both species, depleted algae showed greater increases in tissue N and P concentration in response to water column nutrient supplies than enriched algae did, and the tissue N increases were concentration dependent.

Initial tissue nutrient status was very important in determining the rate of N uptake by both *Ulva expansa* and *Enteromorpha intestinalis*. Algae depleted of N before the experiments had greater demands for N and therefore higher uptake rates than enriched algae. Other critical factors in nutrient uptake by macroalgae were nutrient concentration in the water column and the various phases of nutrient uptake. Therefore, prediction of nutrient uptake rates cannot be based on nutrient supply or concentration alone. In order to accurately predict uptake of nutrients by macroalgae in the field, it is also 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. The high N uptake rates exhibited by *E. intestinalis* and *U. expansa* are characteristics that explain their success in UNB, where N availability can be spatially and temporally patchy, and demonstrate the difficulty in controlling blooms.

Effects of Frequency and Concentration of Nutrient Pulses on Macroalgae

The ways in which temporal variation in the supply of nutrients to macroalgae may affect growth and tissue nutrient status are largely unknown. Nutrient inputs to estuaries can vary over temporal scales from hours to months, and macroalgae can store nutrients and use reserves for growth when external nutrient supplies are low. This strategy likely enables them to succeed in environments such as estuaries with episodic inputs of nutrients. Our objective was to quantify how the frequency and concentration of nutrients supplied to *Enteromorpha intestinalis* and *Ulva expansa* affect growth and tissue nutrient dynamics.

Over a 28-day period, *Enteromorpha intestinalis* and *Ulva expansa* were each given equal supplies of NO_3-N (28 mg) and PO_4-P (6.2 mg) via pulses of different frequency and therefore different concentration. NO_3-N doses given to 10 g wet wt of algae in 1 L seawater were: 1 mg (once per day), 7 mg (once per week), 14 mg (once per two weeks), or 28 mg (once per month). Phosphorus was also added in a 10:1 (molar) ratio. Water column nutrient levels were monitored throughout the course of the experiment. Algal biomass and tissue N and P concentrations were measured at the end of the experiment.

Enteromorpha intestinalis and *Ulva expansa* responded to all nutrient doses used in this study. Growth in both algae increased most with daily doses, although significant growth of both algae was measured for all frequencies of doses. Therefore, algae were able to store enough nutrients from the large, one-time pulses to sustain positive increases in biomass in low nutrient environments for up to 28 d. Tissue nutrient content was also related to the frequency and concentration of the doses. Total mass of N and P in algal tissue (mg $unit^{-1}$) increased as frequency of doses increased. Overall, tissue N concentrations were greater in *U. expansa*, and tissue P concentrations were greater in *E. intestinalis*. *E. intestinalis* and *U. expansa* removed substantial portions of

the nutrient doses. Twenty-nine % to 96% of added nutrients were removed from the water by algae within 24 h.

Storage of pulsed nutrients in macroalgal tissues means that the effects of nutrient pulses on macroalgae may last longer than the nutrient pulse itself remains in the water column. Due to nutrient storage in algal tissue, effects of large nutrient pulses may be as important and persistent in natural systems as chronic, low-grade nutrient inputs. This effect may be exacerbated in natural systems due to sediment storage and release of nutrients. The frequency of nutrient inputs to coastal systems may be critical in determining macroalgal biomass, and temporal scales should be taken into account when regulating nutrient loads in order to minimize macroalgal biomass.

CONCLUSIONS

- *Sediments were an important source of nutrients to macroalgae when water column supply was low, and contributions from sediments need to be incorporated into nutrient budgets for UNB.*

  Growth and tissue nutrient concentrations (N and P) of *Enteromorpha intestinalis* were greater in the presence of estuarine sediments containing measurable nutrients compared to nutrient-free sediment controls. Moreover, macroalgal growth and tissue concentrations increased with increases in initial sediment nutrient concentrations. Similarly, Lavery and McComb (1981) documented macroalgal growth after exposure to estuarine sediments. Since sediments represent a potentially significant source of nutrients to macroalgae, further studies to quantify the flux of N and P from sediments will be required for a basic understanding of nutrient cycling and utilization in UNB.

- *N was the most limiting nutrient to macroalgal growth. P was secondarily limiting at some sites within UNB.*

  When N and P were added to experimental units with *Enteromorpha intestinalis* from UNB, biomass of algae from 3 sites increased with N only additions. Biomass increased further when P was added in combination with N, but no increases in biomass were seen with P only additions. Therefore, strategies to reduce macroalgal biomass in UNB should focus primarily on reductions in N loads to the Bay and secondarily on P loads.

- *Nitrogen uptake rates were a function of water column nutrient concentration and the recent exposure of algae to nutrients.*

  Enteromorpha intestinalis and *Ulva expansa* are very efficient algae at consuming NO_3 with uptake rates measured up to >200 µmoles g dry wt^{-1} h^{-1}. These rates are up to an order of magnitude higher than other macroalgae such as *Codium* or *Gracilaria*. Uptake rates increased with water column concentrations; higher initial water column NO_3 concentrations resulted in higher rates of NO_3 uptake. In addition, nutrient depleted algae had consistently higher rates of nutrient uptake than algae that were enriched with NO_3. Short-term temporal fluctuations in uptake were a function of algal physiology (i.e., surge uptake, internally controlled uptake, and externally controlled uptake). Each of these factors is important for accurately predicting nutrient uptake and macroalgal growth.

- *Algae efficiently stored large pulses of nutrients and used them for growth when nutrient supply was low.*

  Enteromorpha intestinalis and *Ulva expansa* grew most and had the greatest increases in tissue N and P content with daily, low concentration nutrient additions (cumulative dose of 28 mg N and 6 mg P over 28 days). As demonstrated earlier, however, these algae are also efficient scavengers of nutrients. As a result, significant algal growth was observed in all treatments, regardless of frequency of nutrient addition, including a single dose of 28 mg N and 6 mg P. Therefore, large infrequent nutrient loads should be considered in any macroalgae control plan, in addition to controlling chronic, low-grade nutrient inputs.

RECOMMENDATIONS

There are several steps that can be taken to further understand the relationship between nutrients and macroalgae so that regulators and stakeholders can set water quality limits and reduce macroalgal biomass, and its effects, in UNB. These activities can continue in parallel with other management actions targeted at reducing or controlling macroalgal biomass.

- *Improved estimates of nutrient loading from sediments are needed if accurate nutrient budgets for UNB are desired.*

  A nutrient budget for UNB will not be accurate unless it incorporates nutrient loading from sediments. Research in other estuarine systems has shown that the magnitude and direction of sediment N and P flux vary temporally and spatially. Therefore, in order to achieve a comprehensive understanding of nutrient loading to the Bay, sediment-water column nutrient exchange should be quantified at several sites in UNB over an annual cycle. In addition, sediment-water column nutrient dynamics can vary with changing physical conditions (salinity, oxygen levels, sediment grain size). Therefore, sediment-water column nutrient flux should be estimated under varying environmental conditions.

- *The mechanism and process studies conducted herein are extremely useful for creating a dynamic nutrient model that predicts algal growth in UNB.*

  Building a believable nutrient-macroalgal model is a complex, but worthwhile undertaking. The process for creating such a model requires many steps building from a basic nutrient budget, quantifying parameters that characterize the multitude of processes that occur in the Bay, to calibrating and validating the model with locally collected empirical data. The benefit of a well-calibrated and validated model is the ability to predict macroalgal biomass based on knowledge of nutrient inputs to UNB.

  Resource Management Associates, Inc. (RMA) has developed a water quality model for UNB in support of the nutrient TMDL. The RMA modeling effort has completed some of the initial steps in the model building process. RMA relied on values and rate constants from studies conducted in other geographic regions for determining the macroalgal responses to nutrients in UNB. The literature review conducted by Schiff and Kamer (2000), however, showed that UNB is dissimilar from other geographic regions. Based on the studies described herein, we now have local site-specific data on rates and processes in

UNB, which will lead to more accurate model-based predictions of water quality and macroalgae in UNB.

Now that site-specific data is available for UNB, we recommend that one of the first steps is to update and re-evaluate the RMA model to determine its ability to model this system. Additional validation data will be necessary for this re-evaluation. We recommend that additional data collection be integrated with the existing UNB Regional Monitoring Program to enhance cost-efficiency.

- *Once sufficiently validated, the dynamic model should be used to assess the most effective and efficient strategies for achieving management endpoints.*

  The power of a dynamic nutrient model is its predictive ability. The model then becomes an extremely useful management tool that regulators and stakeholders can use to run multiple implementation scenarios for reducing macroalgal biomass. In this way, UNB managers can evaluate the most effective and efficient mechanisms for achieving management endpoints of concern, including setting realistic concentration- or load-based water quality objectives.

REFERENCES

Schiff, K. and K. Kamer. 2000. Comparison of nutrient inputs, water column concentrations, and macroalgal biomass in Upper Newport Bay, California. Southern California Coastal Water Research Project, Westminister, CA.

APPENDIX A

Estuarine Sediments Enhance the Growth and Tissue Nutrient Content of the Estuarine Macroalga *Enteromorpha intestinalis*

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ABSTRACT

Large blooms of opportunistic green macroalgae such as *Enteromorpha intestinalis* are a common problem in estuaries worldwide. Macroalgae derive their nutrient requirements from both the water column and estuarine sediments, and we hypothesized that the importance of these sources to *E. intestinalis* varies along a nutrient resource gradient within an estuary. We tested this by constructing experimental units using water and sediments collected from 3 sites in Upper Newport Bay estuary, California, US. We measured changes in water column and sediment nutrients in three sets of experimental units for each site: sediments + water; sediments + water + *Enteromorpha intestinalis* (algae); inert sand + water + algae. In units containing algae, we measured growth and tissue nutrients (N and P). The importance of the water column versus sediments as sources of nutrients to *E. intestinalis* varied with the magnitude of the different sources. When initial water column DIN levels were low, estuarine sediments increased *E. intestinalis* growth and tissue nutrient concentrations. In units from sites where initial water column DIN was high, there was no effect of estuarine sediments on algal growth or tissue N content. Water column inorganic N was consistently depleted throughout the experiment. Thus, the water column was a primary source of nutrients to the algae when water column nutrient supply was high, and the sediments supplemented nutrient supply to the algae when water column nutrient sources were low. The sediments acted as both a source and a sink of nutrients in this experiment, depending on water column nutrient concentrations. These data provide direct experimental evidence that macroalgae can utilize nutrients stored in estuarine sediments. This concept is critical in understanding estuarine nutrient dynamics and in water quality management decisions designed to protect coastal waters.

INTRODUCTION

Large blooms of opportunistic green macroalgae such as *Enteromorpha* and *Ulva* spp. occur in estuaries throughout the world (Owens and Stewart 1983, Pregnall and Rudy 1985, Rudnicki 1986, Sfriso et al. 1987 and 1992, Lavery and McComb 1991, Hernández et al. 1997, Farris and Oviatt 1999, Martins et al. 1999, Pihl et al. 1999, Raffaelli et al. 1999, Kamer et al. 2001, Tyler et al. 2001) often in response to increased nutrient loads from developed watersheds (Valiela et al. 1992, Nixon 1995, Duarte 1995, Paerl 1997, Valiela et al. 1997, Paerl 1999). While these algae are natural components of estuarine systems and play integral roles in estuarine processes (Pregnall and Rudy 1985, Kwak and Zedler 1997, Boyer and Fong. in review), 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, Sfriso et al. 1992) leading to changes in species composition, shifts in community structure, and loss of ecosystem function (Raffaelli at al. 1991, Ahern et al. 1995, Thiel and Watling 1998).

Biomass of *Enteromorpha* and *Ulva* spp. is often regulated by nutrient availability (Owens and Stewart 1983, Sfriso et al. 1987, Hernández et al. 1997, Schramm 1999). These algae efficiently remove nitrogen (N) from the water column (Owens and Stewart 1983, Fujita 1985, O'Brien and Wheeler 1987, Duke et al. 1989 a and b, Fong et al. 1998, Kamer and Fong 2000, 2001). In estuaries, N levels are generally higher near the head of the system, where rivers flow in, and decrease toward the mouth or the opening to the ocean (Rizzo and Christian 1996, Hernández et al. 1997, Nedwell et al. 2002, Boyle et al. unpub. data). Therefore, the availability of water column N to macroalgae usually decreases along a spatial gradient within an estuary.

Estuarine sediments may also be a significant source of nutrients to macroalgae, particularly when water column nutrients are low. The release of nitrogen and phosphorus (P) from sediments is well established (Boynton et al.

1980, Nixon 1981, Nowicki and Nixon 1985, Gardner et al. 1991, Seitzinger et al. 1991, Cowan and Boynton 1996, Rizzo and Christian 1996, Trimmer et al. 1998, 2000, Clavero et al. 2000, Grenz et al. 2000). Estuarine nutrient budget calculations show that N and P fluxing from sediments could potentially meet a significant portion of the nutrient requirement of primary producers (Boynton et al. 1980, Blackburn and Henriksen 1983, Cowan and Boyton 1996, Trimmer et al. 1998). Birch et al. (1981) found that macroalgal tissue nutrient content varied with sediment nutrient content and inferred that nutrient exchange between the sediments and the algae occurred.

The importance of sediments as a source of nutrients to macroalgae is critical in understanding nutrient dynamics in estuaries and factors controlling algal blooms (Valiela et al. 1997). However, there is little direct evidence that nutrient fluxes from estuarine sediments are either taken up by macroalgae or are of benefit to macroalgae. Exceptions are Thybo-Christesen et al. (1993) and Bierzychudek et al. (1993), which showed decreases in water column N and P along depth gradients from the sediment surface toward floating algal mats, indicating uptake of nutrients fluxing from the sediments by the macroalgae. Lavery and McComb (1991) found increased growth of *Chaetomorpha linum* over a 3-week period in the presence of estuarine sediments compared to in their absence. Further work is needed to understand the significance of nutrient contributions from estuarine sediments to macroalgae both spatially and temporally.

Our objective was to determine the relative importance of water column versus sediment nutrients to macroalgae across a gradient of resource availability. We hypothesized that sediment sources would become more important to macroalgae when water column nutrients are low, such as may happen at the seaward end of a nutrient gradient within an estuary. For example, in Upper Newport Bay (UNB), a large southern California estuary subject to blooms of *Enteromorpha intestinalis* and *Ulva expansa* (Kamer et al. 2001), water column nutrient concentrations were consistently high 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 et al. unpub. data), while sediment nutrient concentrations varied throughout the estuary (0.034-0.166 % dry wt TKN, 0.044-0.072 % dry wt P) and did not exhibit spatial patterns (Boyle et al. unpub. data). Sediments have the potential to be a significant source of nutrients to macroalgae throughout much of the estuary, particularly where water column nutrients are lower.

MATERIALS AND METHODS

To test the relative importance of the water column vs. sediments as a source of nutrients to macroalgae, we collected sediment cores and water from 3 sites in UNB across a water column nutrient gradient (Figure 1); water column dissolved inorganic nitrogen (DIN) decreased from the head to the seaward end of the estuary (Table 1). Using sediment and water from each site, we constructed three sets of experimental units varying in complexity: sediment + water; sediment + water + *Enteromorpha intestinalis* (algae); inert sand + water + algae. The sediment + water cores served to eliminate algae as a nutrient sink, and the inert sand + water + algae cores served to eliminate sediments as a nutrient source/sink. Quantifying nutrients in each component of the experimental system at the beginning and end of the experiment allowed us to determine nutrient allocation among these compartments under different nutrient supply conditions.

The first site from which we collected sediment cores and water was at the head of the estuary (Head, Figure 1), near the mouth of San Diego Creek, a large freshwater and nutrient source to UNB. This site had the greatest water column DIN concentration, mostly in the form of NO_3, the lowest Total Kjeldhal nitrogen (TKN) and PO concentrations, and the lowest salinity (Table 1). The second site (Middle) was mid-way between the head and the seaward end of the estuary and had intermediate DIN concentration and salinity (Table 1). The third site

(Lower) was just above the transition zone between the natural estuarine habitat of UNB and the highly modified and developed Lower Bay; thus we consider this to be the lower boundary of the estuarine system (Kamer et al. 2001). This site had the lowest water column DIN concentration, TKN and PO_4 concentrations similar to the Middle site, and the highest salinity (Table 1).

At each site we took 10 individual sediment cores to a depth of 8 cm from exposed intertidal mudflats. Cores were taken in a row parallel to the water line using polycarbonate tubes 7.3 cm ID x 20 cm high. We used the edge of the vegetation as an elevational guide to ensure sampling of similar elevation among sites. Bottoms of the cores were capped and sealed in the field; tops were left open. Care was taken not to disturb the vertical stratification of the cores.

At each site, we also took 5 smaller sediment samples from areas immediately adjacent to the areas from which the cores were taken. We used open-ended 60 ml syringes (2.5 cm ID x 8 cm deep) to collect samples for initial N and P content (see below for nutrient methods) and grain size distribution (Bouyoucos 1962). Sediment nutrients (N and P) were greatest at the Middle site, which had the least sand and the highest silt content (Table 2); P, but not N, was significantly different among sites. The Lower site had the sandiest sediments, the least silt content, and the lowest sediment N and P (Table 2). All three sites had similar clay content (Table 2).

Water was collected at each site from 0.5-1 m depth using a battery operated pump. In the laboratory, water from the corresponding site was added to each polycarbonate tube so that 300 ml were overlying each sediment core. The bottom 8 cm of each tube was wrapped with duct tape to block light from entering the sediments through the sides of the tube. Only a portion of the water collected at the beginning of the experiment was used immediately; the remaining water was stored in the dark at 6°C and used over the course of the experiment when the water in each experimental unit was replaced.

To separate the contribution of nutrients from the estuarine sediment to macroalgae from the contribution from the water column, we constructed units that were identical with the exception of an 8 cm deep layer of sand in the bottom of each instead of estuarine sediment. The sand simulated the physical presence of the estuarine sediments, yet had no measurable nutrients to contribute to the macroalgae. Use of these units allowed us to compare the response of the algae in units with sediments and water to the response in units with sand and water, thereby determining the effects of the sediments on algae.

Five inert sand + water + algae experimental units per site were constructed using sand that was prepared by heating in a muffle furnace to 400°C for 10 h to remove any organic material, then washing the cooled sand in dilute acid (3% HCl in de-ionized water). The sand was then rinsed of acid and dried to a constant weight at 60°C in a forced air oven. Five sub-samples of the sand mixture were analyzed for N and P content. Sand N was below the detection limit of 0.05 % dry wt, and P was below the detection limit of 0.01 % dry wt (Table 1).

Ten days prior to the initiation of the experiment, *Enteromorpha intestinalis* was collected from the field. Algae were kept outdoors in a shallow pan filled with aerated, low nutrient seawater (<3.57 DIN, <1.61 PO_4, 35 psu) in a temperature controlled water bath (20 ± 2ºC) and covered with window screening to reduce incident light. Keeping algae under these conditions prior to the beginning of an experiment reduces internal nutrient stores and variability in initial tissue nutrient levels (Fong et al. 1994). Initial tissue N was 1.19 ± 0.02 % dry wt (n=5, mean ± SE) and initial tissue P was 0.11 ± 0.00 % dry wt (n=5).

To complete the experimental units, we added *Enteromorpha intestinalis* to 5 tubes from each site containing estuarine sediments and to all the tubes containing inert sand. *E. intestinalis* was placed in nylon mesh bags and

spun in a salad spinner for 1 minute to remove excess water. Algae were weighed and 5.0 ± 0.1 g sub-samples were added to experimental units designated as "+ algae" treatments. The units were placed in individual 1 l glass beakers containing 450 ml of water from the corresponding site. This was done to detect and contain any leaking that might occur. Water levels in two units, each in different treatments, indicated that water was leaking out, and all data from these units were excluded from analyses. Beakers containing experimental units were placed outdoors in a temperature controlled water bath (20 ± 2ºC) and covered with one layer of window screening to reduce incident light. Treatments were arranged in a randomized matrix. We had three treatments (sediments + water; sediments + water + algae; inert sand + water + algae) for each of the three sites (Head, Middle, Lower) with 5-fold replication for a total of 45 units. The experiment ran for three weeks. During this time, salinity was monitored with a hand-held refractometer and de-ionized water was added to compensate for evaporation.

At the end of each week, we sampled the water in each experimental unit for nutrient analyses. Algae were removed from the tubes, and, with a 60 cc syringe, we removed the water from each unit, except for a thin layer (5-10 mm) overlying the core. Care was taken to ensure that the core surface was not visibly disturbed. A subsample of the water removed from each unit was filtered with glass fiber filters (Whatman GF/C), frozen, and analyzed for NO_3+NO_2, NH_4, TKN (all forms of dissolved N except NO_3 and NO_2), and PO_4. NO_3 was reduced to NO_2 via cadmium reduction; NO_2 was measured spectrophotometrically after diazotation (Switala 1999, Wendt 1999). NH_4 was heated with solutions of salicylate and hypochlorite and determined spectrophotometrically (Switala 1999, Wendt 1999). 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). PO_4 was determined spectrophotometrically following reaction with ammonium molybdate and antimony potassium under acidic conditions (APHA 1998). These automated methods have detection limits of 3.57 µM for N and 1.61 µM for P.

At the end of weeks 1 and 2, we refilled each unit with 300 ml of water that was collected at the beginning of the experiment from each site. Each time this stored water was added to experimental units, triplicate samples from each site were analyzed for nutrient concentrations. We added water by pouring it slowly over a small petrie dish so that it ran down the side of the core tube and did not disturb the sediment surface. Algae were replaced in the appropriate units, the water in the surrounding beakers was replaced with a fresh 450 ml from the appropriate batch, and the units were returned to the water bath.

At the end of the experiment, *Enteromorpha intestinalis* was removed from each unit, placed in individual nylon mesh bags, spun in a salad spinner for 1 minute and wet weighed. Samples were individually rinsed briefly in freshwater to remove external salts, dried in a forced air oven at 60°C to a constant weight, and re-weighed. Samples were ground in mortar and analyzed for tissue N and P. N was determined using an induction furnace and a thermal conductivity detector (Dumas 1981). 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). N and P content of algae are reported as total mass $unit^{-1}$, which is calculated by multiplying the nutrient concentration of a sample (% dry weight) as a proportion by the dry weight of that sample:

$$\text{mg N or P unit}^{-1} = [\%\ \text{tissue N or P}/100] * \text{dry wt (g)} * 1000\ \text{mg/g}$$

Each sediment or sand core was removed from its unit at the end of the experiment and homogenized. A subsample of each core was dried in a forced air oven at 60°C to a constant weight, ground in a mortar and pestle and analyzed for N and P. N was determined by use of a dynamic flash combustion system coupled with a gas chromatographic separation system and a thermal conductivity system (Dumas 1981). P was determined by AAS and ICP-AES following a nitric acid/hydrogen peroxide microwave digestion (Meyer and Keliher 1992). Final sediment N and P are reported as % change from initials.

All data were tested for normality and homogeneity of variance. Non-normal sediment P values were transformed by adding a constant and taking the square root of the sum. Among treatment differences in *Enteromorpha intestinalis* final wet biomass and tissue N and P total mass unit^{-1} were analyzed using 2-factor ANOVA (site x core material, where core material was either estuarine sediment or inert sand). Among treatment differences in final sediment N and P content were analyzed using 2-factor ANOVA (site x algae, where algae was either present or absent). Following a significant ANOVA, multiple comparisons were used to determine differences among individual treatments (Fisher's Least Significant Difference test (LSD)). Final sand N and P values were not included as they were all below the detection limits of 0.05 % dry wt and 0.01 % dry wt respectively.

NO_3 and NH_4 values of water removed from each experimental unit at the end of each week were often below the detection limit of 3.57 µM. PO_4 values from the end of weeks 1 and 2 were often below the detection limit of 1.61 µM. Statistical analyses of the remaining values were not conducted due to low sample size. Differences in water column PO_4 at the end of the third week were analyzed using 2-factor ANOVA (site x treatment, where treatment was either estuarine sediment only, estuarine sediment + algae, or inert sand + algae). TKN data from the end of each week was analyzed using 2-factor repeated measures ANOVA (site x treatment x time). Significant ANOVA was followed by Fisher's LSD to determine differences among individual treatments. Unless otherwise stated, no significant interactions occurred between factors in ANOVA.

RESULTS

Enteromorpha intestinalis biomass was significantly affected by both site (p=0.003, ANOVA) and core material (p=0.001, ANOVA) and there was a significant interaction between the two terms (p=0.001). Algal biomass increased most in units containing estuarine sediments from the Middle site (Figure 2), followed by units containing sediments from the Lower site (p=0.001, Fisher's LSD). In units from the Middle and Lower sites, biomass was greater when estuarine sediments were present as compared to the inert sand (p=0.001 for Middle and p=0.004 for Lower, Fisher's LSD). Algal biomass was not different between sand and sediment treatments from the Head (p=0.834, Fisher's LSD), and growth in these units was low, possibly due to low salinity conditions. Algal biomass was similar among sites when incubated with sand (p=0.272, 0.390, 0.815, Fisher's LSD), increasing 12-16% from initial weight in 3 weeks.

The N contained in *Enteromorpha intestinalis* tissue (total mass unit^{-1}) at the end of the experiment was significantly affected by both site and core material (p=0.001 for both factors, ANOVA). Overall, tissue N was highest in units from the Head (Figure 3a; p=0.001 for all comparisons, Fisher's LSD) and decreased with distance down-estuary, tracking water column patterns. Tissue N was higher in units containing sediments versus sand from the Lower (p=0.001, Fisher's LSD) and Middle (p=0.007, Fisher's LSD) sites. There was no difference between sediment and sand treatments from the Head (p=0.199, Fisher's LSD).

The P contained in *Enteromorpha intestinalis* tissue (total mass unit^{-1}) at the end of the experiment was significantly affected by both site and core material (p=0.001 for both factors, ANOVA) and there was significant interaction between the terms (p=0.006). Tissue P was greater in units containing sediments from each site compared to sand (Figure 3b; p=0.001 for each comparison, Fisher's LSD). When sediments were present, tissue P was greatest in units from the Middle site followed by the Lower (p=0.002, Fisher's LSD) and then the Head (p=0.028, Fisher's LSD). Within sand treatments, tissue P did not vary with site (p=0.595, 0.630, 0.981, Fisher's LSD).

Water column N supplies were greatly reduced in all units each week of the experiment. At the end of the first week, water column NO_3 and NH_4 were below detection limit (BDL) of 3.57µM in all experimental units except the sediment + water treatments from the Head. NO_3 in these units was 37.29 ± 12.54 µM (mean ± SE), which is much less than initial values from this site. At the end of the second and third weeks, NO_3 and NH_4 were BDL in all units except for 1 or 2 units in which DIN was always <11 µM. Water column TKN was significantly affected by time (p=0.001, ANOVA) but not by site (p=0.172, ANOVA) or treatment (p=0.922, ANOVA). Mean TKN for all treatments at the end of weeks 1, 2, and 3 was 40.03 ± 1.54 µM, 26.08 ± 1.38 µM, and 30.23 ± 1.38 µM, respectively (n=43 for each week).

Water column P supplies were also reduced during each week of the experiment. Water column PO_4 was BDL (1.61 mM) in 29 of 43 of units at the end of week 1 and 31 of 43 units at the end of week 2. Mean PO_4 (± SE) at the end of the first week was 4.95 ± 0.54 µM (n=14) and 7.23 ± 1.33 µM (n=12) at the end of the second week. At the end of the third week, PO_4 was detected in 35 of 43 units. PO_4 was significantly affected by site (p=0.011, ANOVA) but not treatment (p=0.512, ANOVA). Mean PO_4 for all units with measurable values was 5.43 ± 0.48 µM from the Lower site, 6.26 ± 0.79 µM from the Middle, and 3.55 ± 0.29 µM from the Head. PO_4 was similar in units from the Lower and Middle sites (p=0.516, Fisher's LSD), and PO_4 in units from the Head was lower than in units from the Lower site (p=0.020, Fisher's LSD).

Final sediment N (% change from initial) was not affected by either site (p=0.073, ANOVA) or presence of algae (p=0.353, ANOVA). Overall, variability in sediment N was high in all treatments (Figure 4a). Final sediment P (% change from initial) varied significantly with site (p=0.001, ANOVA) but not with the presence of algae (p=0.186, ANOVA). Sediment P increased in units from the Lower site and decreased in units from the Middle (Figure 4b). In units from the Head, sediment P increased when algae were not present and there was no change from initial levels of sediment P when algae were present.

DISCUSSION

The importance of the water column versus sediments as sources of nutrients to *Enteromorpha intestinalis* varied with the magnitude of the different sources in this experiment. Estuarine sediments were more important to the growth of *E. intestinalis* when water column N was low, such as in units from the Middle and Lower sites, compared to when water column N was high, such as in units from the Head. This is evidenced by the differences in algal growth between estuarine sediment and inert sand treatments. Furthermore, the magnitude of the effect of estuarine sediments on macroalgal growth appears to be related to the nutrient content of the sediments. Overall, growth was greatest when algae were incubated with sediments from the Middle site, which had the highest initial sediment N and P content.

When water column N was high, such as in units from the Head, estuarine sediments did not significantly influence *Enteromorpha intestinalis* growth. However, overall *E. intestinalis* growth in these units was low. Salinity in Head waters was 8-10 psu. Prolonged exposure to salinity < 25 psu can significantly reduce the growth of *E. intestinalis* (Kamer and Fong 2000). Therefore, the lack of the effect of sediments on *E. intestinalis* growth may have had less to do with high water column N meeting the algae's nutrients demand than the inhibition of growth due to low salinity. However, nutrients from watersheds are usually transported to estuaries via freshwater; high nutrient levels often correlate with low salinity (Valiela et al. 1992). As such, estuarine sediments may not have significant effects on macroalgal growth when salinity is the limiting factor. Sediments may only affect macroalgal growth when salinity or other factors do not inhibit growth.

The influence of water column versus sediment nutrients on algal tissue nutrient status also varied with the magnitude of the sources. *Enteromorpha intestinalis* tissue N content increased in the presence of estuarine sediments when water column N was low, such as in the Middle and Lower site units. Greater N content in these algae presumably led to greater biomass. When water column N was high, the presence of sediments did not affect tissue N content. *E. intestinalis* tissue N levels were greatest overall in Head units, probably due to the greater supply of N available in the water column. Algae in these units derived most, if not all, of their N requirement from the water column, and the low salinity conditions did not impair the algae's ability to remove N from the water column. Tissue N content of another estuarine macroalga, *Cladophora albida*, was more closely related to water column N concentrations than sediment N content (Birch et al. 1981).

Enteromorpha intestinalis tissue P content was greatly enhanced by the presence of sediments in units from all three sites. Tissue P was greatest in units containing sediments from the Middle site, which had the highest initial sediment P value. Similarly, Birch et al. (1981) found that *Cladophora albida* tissue P was tightly linked to sediment P content.

While the sediments appeared to be a source of nutrients to macroalgae based on the enhanced growth and condition of the algae in the presence of sediments, they were also a sink. Significant quantities of NO_3 and PO_4 flux into sediments in estuarine systems (Boynton et al. 1980, Nowicki and Nixon 1985, Cowan and Boynton 1996, Rizzo and Christian 1996, Trimmer et al. 1998, 2000, Grenz et al. 2000). These nutrients can flux back out of the sediments and may again be available to primary producers (Boynton et al. 1980, Blackburn and Henriksen 1983, Thybo-Christesen et al. 1993, Bierzychudek et al. 1993, Cowan and Boyton 1996, Trimmer et al. 1998)

We did not see strong indications of loss of N and P from the sediments reflecting the increases we saw in *Enteromorpha intestinalis* growth and tissue N and P. It is possible that there was a flux of N and P from the top layers of sediment (Lavery and McComb 1991, Cowan and Boynton 1996, Pihl et al. 1999, Clavero et al. 2000, Svensson et al. 2000, Trimmer et al. 2000), and analyzing only the top layers of the cores may have provided better resolution of changes over time. Alternatively, the mass of nutrients contained within the sediments may have been much greater than the mass of N and P in the algae. While it was possible to detect changes in algal nutrient concentration, if concentration changes occurred in sediments as well they may not have been detectable.

Our data provide direct experimental evidence that algae can take advantage of nutrients stored in estuarine sediments, confirming the long-standing hypothesis that sediments can supply nutrients to primary producers. While many studies have calculated fluxed nutrients from estuarine sediments (e.g., Boynton et al. 1980, Blackburn and Henriksen 1983, Nowicki and Nixon 1985, Koop et al. 1990, Cowan and Boynton 1996), few studies have investigated whether algae are able to use these sediment-derived nutrients. Lavery and McComb (1991), Thybo-Christensen et al. (1993) and Bierzychudek et al. (1993) provided evidence that macroalgal mats can intercept nutrients fluxing from the sediments. Our study provides further experimental evidence that nutrients released from sediments can be taken up by primary producers, and furthermore that these nutrients are of ecological significance to the algae by enhancing growth rates and tissue nutrient content.

The importance of sediments as a source of nutrients to macroalgae is also critical in understanding temporal nutrient dynamics in estuaries. This experiment demonstrated that sediments can be both a source of nutrients and a sink. Sediments adsorbed nutrients, and sediment-derived nutrients were important to macroalgae when water column nutrient sources were low. This can happen either spatially along a nutrient gradient within an estuary, or temporally, when nutrient inputs to estuaries are seasonally low. In the Chesapeake Bay, water

column supplies of nitrogen were lowest in the summer, which is when demand by primary and secondary producers was greatest (Baird et al. 1995). It was concluded that spring supplies of nitrogen sustained the summer demand, probably through recycling via the sediments.

The temporal link sediments can serve between nutrient inputs and resulting algal blooms may be particularly important in systems with Mediterranean climates where the majority of the annual precipitation occurs during cool winters and the remainder of the year is relatively dry. Temporal decoupling between wet season nutrient inputs and dry season algal blooms occurs in the Peel-Harvey system (McComb and Lukatelich 1995, McComb et al. 1998) and the Palmones River estuary (Hernández et al. 1997). In UNB, loads of N and P were greatest during winter and spring, when macroalgal cover was relatively sparse, and lowest in the summer and fall, when macroalgae were most abundant (Kamer et al. 2001, Boyle et al. unpub. data). Therefore, calculations of nutrient loads to estuaries or annual budgets for these systems should include fluxes from the sediments as well as loading from the watershed.

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Table 1. Mean initial water column nutrients and salinity ranges from 3 sites in Upper Newport Bay. Means that are significantly different from each other (p<0.05, Fisher's LSD following significant 1-factor ANOVA) are indicated with superscripts. For nutrient data, n=9; means were calculated from triplicate samples from weeks 1, 2, and 3. Values in ( ) are SE.

Site	Water column nutrients (µM)					Salinity (psu)
	NO_3	NH_4	DIN	TKN	PO_4	
Head	414 (8.0)[a]	11.4 (2.3)[a]	422 (5.8)[a]	33.3 (6.6)[a]	<1.61	8
Middle	101 (3.4)[b]	21.0 (0.5)[b]	122 (3.4)[b]	61.9 (4.3)[b]	5.95 (2.37)[a]	25
Lower	49 (1.8)[c]	23.2 (3.7)[b]	72 (4.5)[c]	55.6 (6.9)[b]	3.41 (0.19)[a]	30

Table 2. Mean initial nitrogen and phosphorus content and grain size distribution of sediments from 3 sites in Upper Newport Bay and inert sand used in sand + water + algae treatments. n=5. Means that are significantly different from each other (p<0.05, Fisher's LSD following significant 1-factor ANOVA) are indicated with superscripts. Inert sand was not included in either nutrient content statistical analyses as N and P were below detection limits or grain size statistical analyses as data were invariate. Values in ( ) are SE.

Site	Nutrients as a % of dry weight			% Sediment type		
	Total N	Total P (Molar)	N:P	Sand	Silt	Clay
Head	0.066 (0.004)[a]	0.05 (0.000)[a]	2.92	61 (3)[a,b]	25 (2)[a]	14 (1)[a]
Middle	0.072 (0.002)[a]	0.058 (0.002)[b]	2.76	53 (3)[b]	32 (2)[b]	15 (1)[a]
Lower	0.062 (0.006)[a]	0.042 (0.004)[c]	3.28	69 (1)[a]	18 (1)[c]	13 (1)[a]
Inert sand	<0.05	<0.01	-	96 (0)	1 (0)	3 (0)

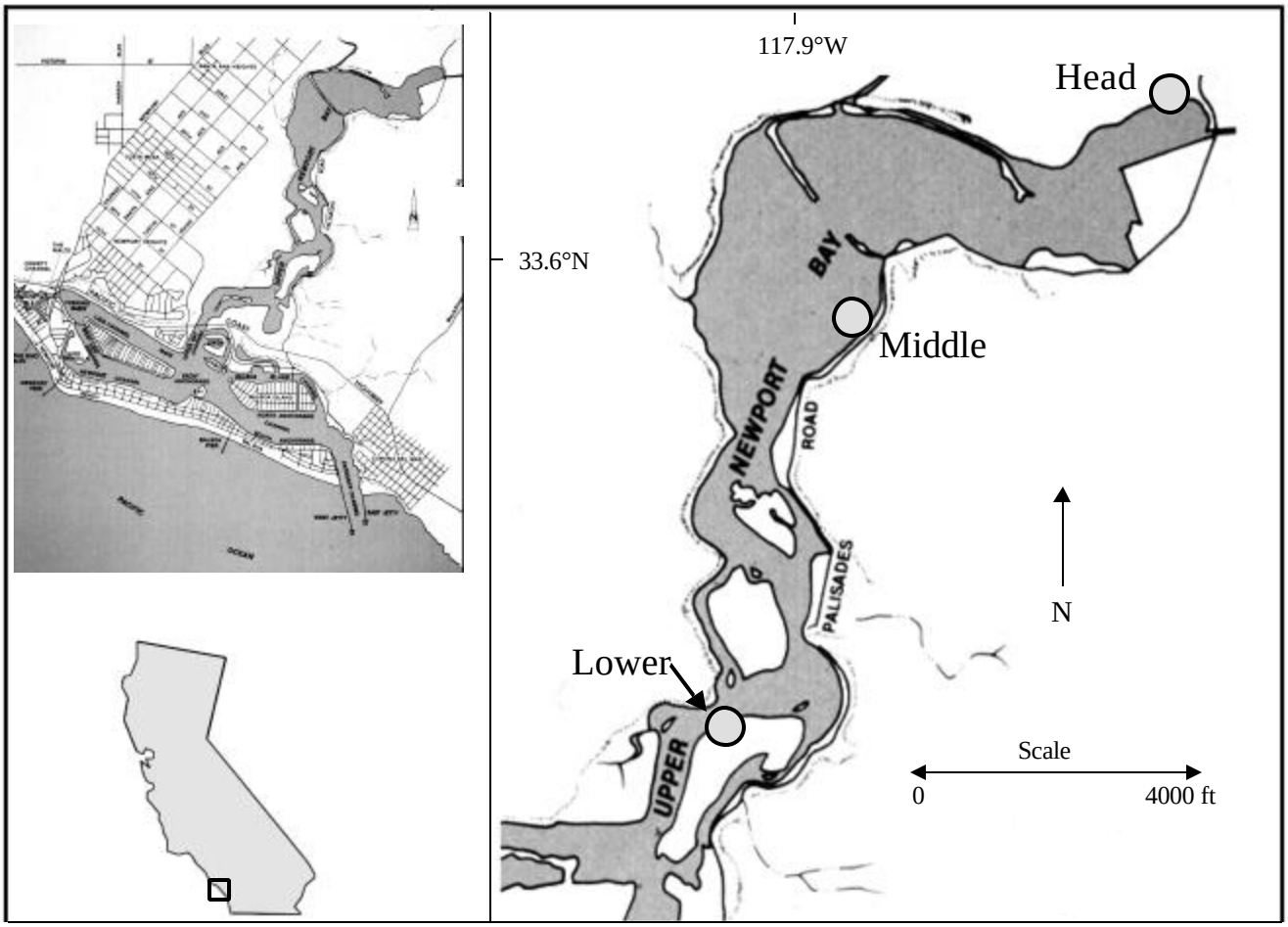

Figure 1. Map of Upper Newport Bay estuary, California, US, with 3 sites (Lower, Middle, and Head) from which water and sediments were collected to construct experimental units.

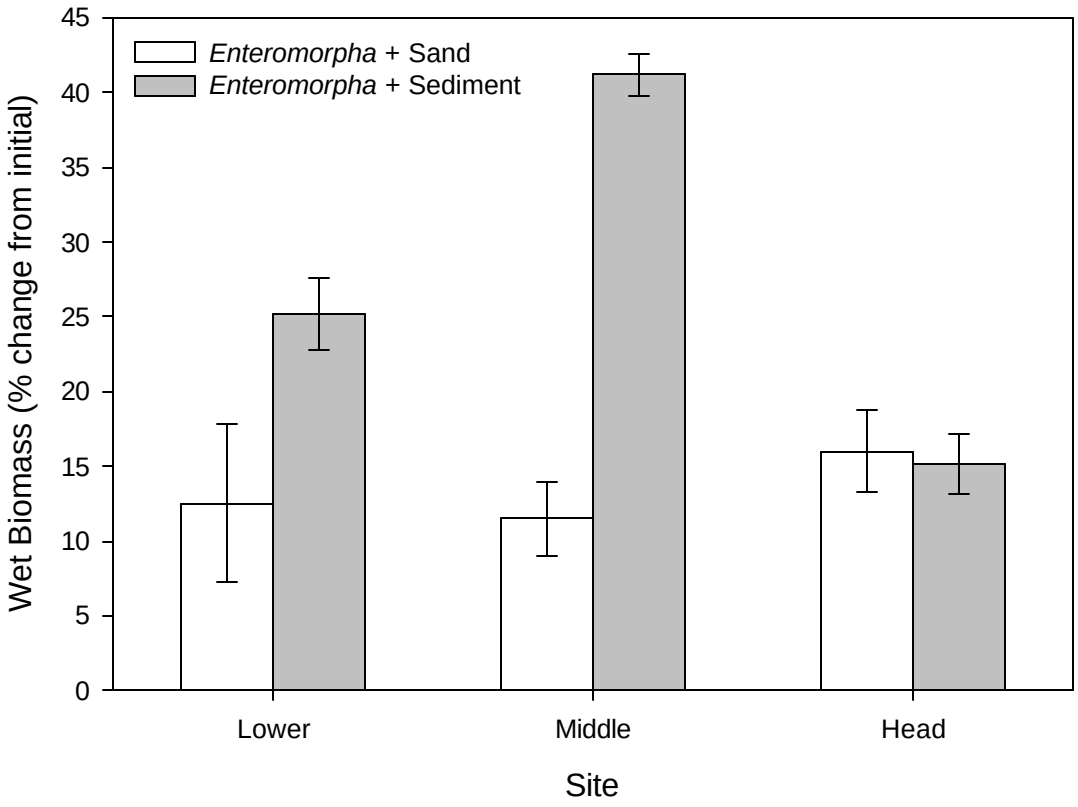

Figure 2. *Enteromorpha intestinalis* biomass (as % change from initial) grown with either inert sand or estuarine sediment from 3 sites in Upper Newport Bay.

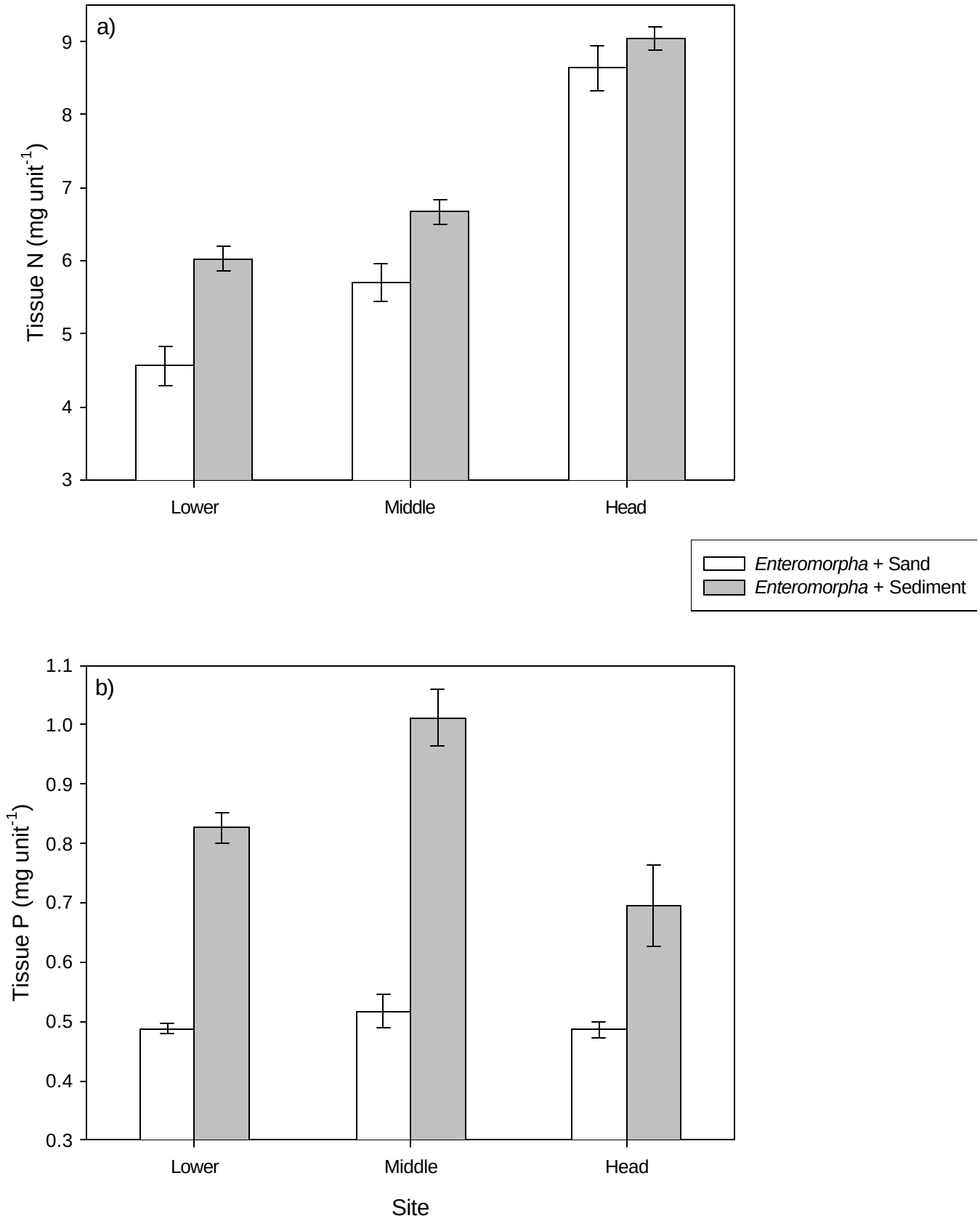

Figure 3. Mass of N (a) and P (b) in *Enteromorpha intestinalis* tissue grown with either inert sand or estuarine sediment from 3 sites in Upper Newport Bay.

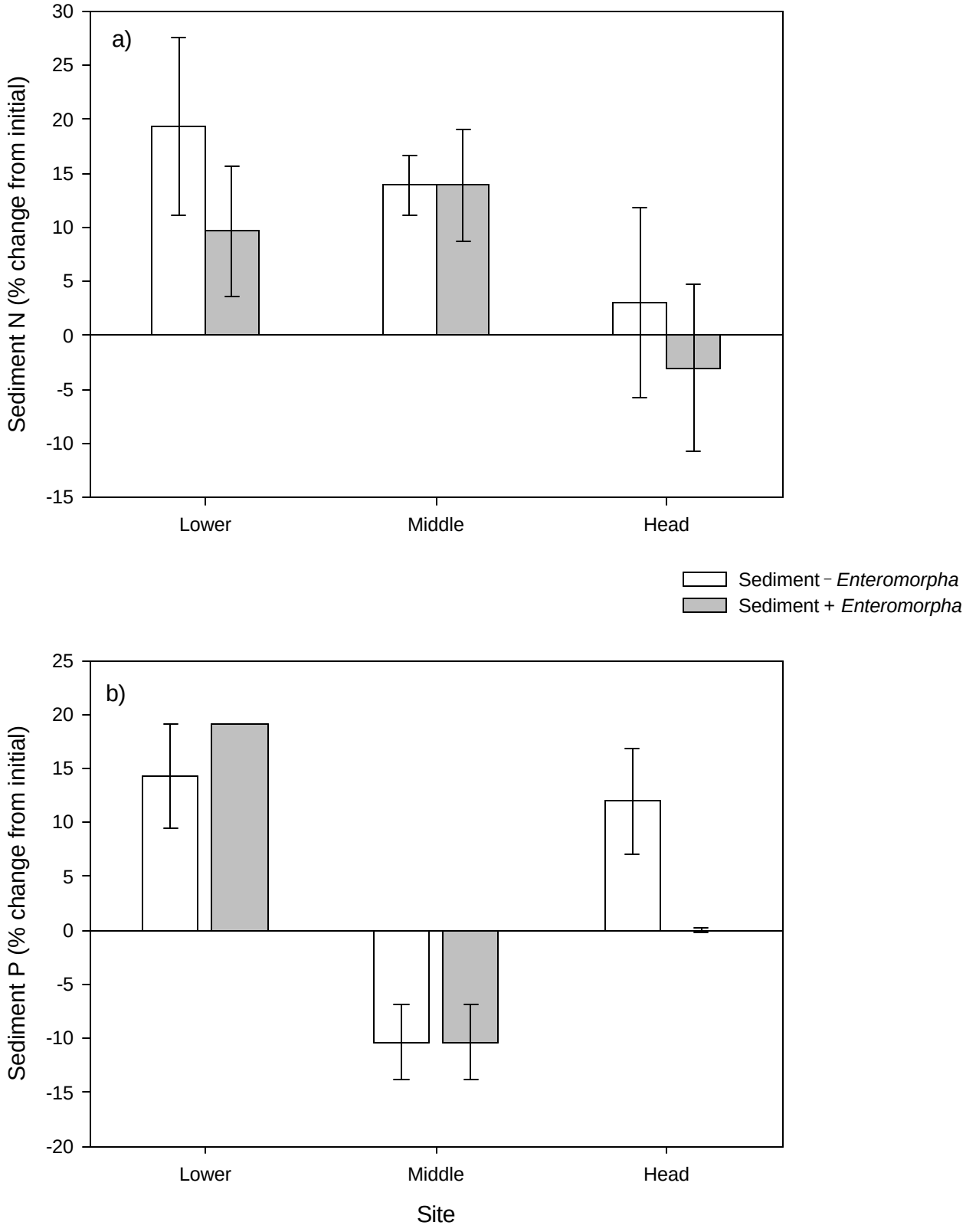

Figure 4. Sediment N (a) and P (b) (as % change from initial) from 3 sites in Upper Newport Bay incubated in the presence or absence of *Enteromorpha intestinalis*.

APPENDIX B

Nitrogen and Phosphorus Limitation of Growth of *Enteromorpha intestinalis* in Upper Newport Bay

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ABSTRACT

We investigated nutrient (nitrogen (N) and phosphorus (P)) limitation of macroalgae along a nutrient gradient in Upper Newport Bay estuary (UNB), a relatively nutrient-rich system in southern California, USA. We collected *Enteromorpha intestinalis* and water from 5 sites ranging from the lower end of the estuary to the head. Initial tissue N and P levels increased along a spatial gradient from the lower end toward the head. Water column NO_3 and TKN were lower at the more seaward sites compared to the sites further up-estuary; NH_4 and PO_4 were variable among sites. Portions of the water from each site were amended with nutrients to create 4 experimental solutions: control (C), nitrogen enrichment (+N), phosphorus enrichment (+P), and nitrogen and phosphorus enrichment (+N+P). At 3 of 5 sites, *E. intestinalis* biomass increased with N enrichment 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 among the lower 4 sites increased along a spatial gradient reflecting initial tissue nutrient concentrations and ambient water column N supplies. Final tissue N and P concentrations reflected both initial levels and N and P enrichment, respectively. Depletion of tissue nutrients during the experiment suggested that algae were growing on internal reserves of nutrients. Growth of *E. intestinalis* from the site closest to the head of the estuary was moderate relative to the other sites and appeared to be limited by a factor other than nutrients. Reduction of the supply of N to UNB should result in a decrease in macroalgal biomass within the system. Reductions in P supply may also decrease macroalgae at several sites within UNB.

INTRODUCTION

As nutrient loading to coastal waters increases (Nixon 1995), a common problem in estuaries through out the world is the development of macroalgal blooms (McComb et al. 1981, Sfriso et al. 1987, Raffaelli 1989, Sfriso et al. 1992, King and Hodgson 1995, Thornton et al. 1995, Hernández et al. 1997, Farris and Oviatt 1999, Martins 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, Young et al. 1998) leading to fish and invertebrate mortality (Raffaelli et al. 1991) and ultimately to changes in ecosystem structure (Raffaelli et al. 1989, 1999, Ahern et al. 1995, Thiel and Watling 1998) and function.

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, Holmboe et al. 1999, Smith et al. 1999, Guildford and Hecky 2000).

Macroalgal production in temperate marine and estuarine systems is usually limited by N (Ryther and Dunstan 1971, Harlin and Thorne-Miller 1981, Hanisak 1983, Howarth 1988, Thybo-Christensen et al. 1993, Pedersen and Borum 1996). 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.

When nutrient limitation of macroalgae does occur, it may vary spatially within an estuary. 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). Therefore, 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 (Fujita 1985, Cohen and Fong in prep., Kennison et al. in prep.), its 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* (AHA 1997, 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 mM NO_3, 4.3-16.7 mM total P) relative to down-estuary areas (5-90 mM NO_3, 1.8-11.5 mM total P) (Boyle et al. unpub. data). Kamer et al. (in prep.) also found a gradient in water column NO_3 in UNB of 414 mM near the head, 101 mM at a mid-estuary site, and 49 mM 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.

MATERIALS AND METHODS

We tested whether N or P limits the growth of *Enteromorpha intestinalis* from 5 sites in UNB. Sites ranged from the lower end of the estuary (Site 1) to the head (Site 5) where San Diego Creek enters UNB (Figure 1). We collected algae and water from each site and amended portions of the water from each site with nutrients. For each site there were 4 experimental treatments: control (C), nitrogen enrichment (+N), phosphorus enrichment (+P), and nitrogen and phosphorus enrichment (+N+P). The end result was a 3-factor experimental design: site x N enrichment x P enrichment.

On 11 June 2001, *Enteromorpha intestinalis* was collected from each site in UNB (Figure 1) and transported back to the laboratory within 5 h. Tissue N and P concentrations of 5 initial samples of *E. intestinalis* from each site showed that initial tissue N and P levels increased along a spatial gradient from the lower end of the estuary toward the head (Table 1). Tissue nutrient concentrations reflect the ambient nutrient conditions that an alga recently experienced (Björnsäter and Wheeler 1990) and therefore are better indicators of local nutrient regimes

than traditional water column sampling methods, which often miss episodic, transient pulses of nutrients (Fong et al. 1998) or can be dramatically affected by the tidal cycle. Mean initial *E. intestinalis* tissue N:P molar ratios ranged from 16.75 to 26.40 (Table 1) and did not show any clear spatial patterns.

Water was also collected in large carboys from each site from mid-water depth using a battery operated pump. Water column NO_3 was lower at the more seaward sites (sites 1 and 2) compared to the sites further up-estuary, and the highest initial water column NO_3 was found at 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 at sites 1-3 and higher at sites 4 and 5 (Table 2).

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 12 June 2001, a portion of the water from each site was divided into 4 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 40µM P. These concentrations are within the range of water column N and P concentrations measured in UNB (Boyle et al. in prep, Kamer et al. in prep.). This procedure of creating experimental solutions using water collected 11 June, 2001, from each site was repeated at the beginning of each week of the experiment when the water in each unit was replaced. Each week, 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 means of each solution for each site across all weeks are presented (Table 3). Using data from all three weeks, mean NO_3 and TKN of experimental solutions from each site were compared using 3-factor ANOVA (site x N enrichment x P enrichment). NO_3 was significantly affected by site and N enrichment ($p=0.001$ for both factors), and TKN was significantly affected by site ($p=0.001$) and N enrichment ($p=0.019$). Many NH_4 and PO_4 values were below detection limits of 3.57 and 1.61 mM, respectively, preventing statistical analyses.

Enteromorpha intestinalis from each site was 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 to glass experimental units (1.5 l total volume), each containing 800 ml of the appropriate solution. Units were placed in a randomized array outdoors in a temperature controlled water bath (max. temperature 22 °C during the day) and covered with window screening to reduce incident light. Replication was 5-fold except for the site 3 Control and site 3 +N treatments, which only had 3 replicates each due to not having collected enough algae. There was a total of 96 units.

The experiment ran for 3 weeks. At the end of each week, algae and water were removed from each unit and a 125 ml water sample was taken from each unit. Samples were filtered (Whatman GF/C) and frozen for subsequent NO_3, NH_4, TKN, and PO_4 analysis.

Experimental units were refilled with 800 ml of the appropriate solution and the algae were placed back in their respective units. Units were re-randomized each week. Salinity was monitored every two days with a hand-held refractometer and de-ionized water was added to compensate for evaporation.

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 freshwater 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. N and P content of algae are reported as both concentration (% dry weight) and % change from initial.

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 3-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 test [LSD]). 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 mM for P) and statistical analyses of the remaining values were not conducted. Means of these data are reported only when all replicates in a treatment had values above the detection limits. Water column TKN data from the end of each week was analyzed using 3-factor repeated measures ANOVA (site x N enrichment x P enrichment x time).

RESULTS

Enteromorpha intestinalis biomass generally increased from site 1 to site 4 (Figure 2), reflecting both initial tissue nutrient concentrations and ambient water column N supplies. 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 ($p=0.0001$ for both factors, ANOVA) but not by P enrichment ($p=0.2878$, ANOVA). Compared to controls, biomass of algae from sites 1, 2 and 4increased in the N enrichment alone treatments ($p<0.050$ for C vs. +N at each site, Fisher's LSD), indicating that N was the most limiting nutrient. There was no significant increase when N alone was added compared to controls in biomass of algae collected from site 3 ($p=0.054$, Fisher's LSD), though variability in the control treatment was high and replication of the control and +N treatments was 3-fold whereas replication of all other treatments was 5-fold. There was also no significant difference in biomass of algae from site 5 between control and +N treatments ($p=1.000$, Fisher's LSD), which, along with the response of algae from site 3, resulted in an interaction between site and N enrichment ($p=0.0024$, ANOVA).

For *Enteromorpha intestinalis* collected from sites 1, 2 and 4, biomass increased further when P was added in combination with N ($p<0.005$ for +N vs. +N+P at each site, Fisher's LSD). This indicated that P was the next most limiting nutrient after N as P addition caused further growth once the algae were released from N limitation. This resulted in an interaction between N and P enrichment ($p=0.0039$, ANOVA). There were no differences between controls and +P treatments in biomass of algae collected from sites 1-4 ($p>0.100$ for C vs. +P at each site, Fisher's LSD); therefore there was 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 ($p=0.0001$, ANOVA).

Enteromorpha intestinalis final tissue N levels increased with site ($p=0.0001$, ANOVA) in all treatments (Figure 3a). Tissue N was also significantly affected by N enrichment ($p=0.0001$, ANOVA) but not by P enrichment ($p=0.2878$, ANOVA). For all sites, tissue N concentration was greatest when N was added regardless of P addition. There was an interaction between site x 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 % change from initial was significantly affected by site and N enrichment ($p=0.0001$ for both factors, ANOVA) 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) indicating that algae were growing on internal stores of N. Tissue N in the +N and +N+P treatments increased compared to initials from sites 1 and 2, decreased compared to initials from sites 3 and 4, and was similar to initials from site 5. Though algae from site 5 experienced only moderate growth relative to the other sites, N uptake by algae was still great enough that final tissue N levels were similar to initial levels despite moderate growth.

In contrast to tissue N, there were no differences in *Enteromorpha intestinalis* final tissue P from sites 1-4 (Figure 4a) despite differences in initial P concentrations among these sites. However, tissue P concentration was greater from site 5 ($p=0.0001$, ANOVA), causing an interaction between site and P enrichment ($p=0.0038$). Tissue P was also significantly affected by P enrichment ($p=0.0001$, ANOVA) but not by N enrichment ($p=0.4439$, ANOVA). For all sites, tissue P concentration was greatest when P was added regardless of N addition.

Enteromorpha intestinalis tissue P % change from initial was significantly affected by site and P enrichment ($p=0.0001$ for both factors, ANOVA) but not by N enrichment ($p=0.6588$, ANOVA). 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 initials in +P and +N+P treatments from all sites, though these increases varied in magnitude with site due to differences in initial tissue P values and caused an interaction between site and P enrichment ($p=0.0001$, ANOVA).

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 mM 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 was 4.29 mM 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 at the end of each week of the experiment. At the end 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; PO_4 was 4.31 ± 0.23 µM at the end of week 2 and 4.67 ± 0.24 µM at the end of week 3.

Water column TKN at the end of each week was significantly affected by site (p=0.0004, ANOVA) and time (p=0.0001) but not by N enrichment (p=0.8506) or P enrichment (p=0.7949). 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 3). TKN decreased over the course of the experiment.

DISCUSSION

Even in nutrient-rich estuaries such as UNB (California Regional Water Quality Control Board 1997, Kamer et al. 2001, Boyle et al. unpub. data), nutrient limitation of primary producers can occur. Algae from several sites in UNB weremost limited by N. When released from N limitation via N enrichment, P was the next most limiting nutrient. Macroalgae are often N limited in temperate estuaries (Harlin and Thorne-Miller 1981, Hanisak 1983, Howarth 1988, Thybo-Christensen et al. 1993, Pedersen and Borum 1996). P limitation is not as common but has been documented in several systems (Birch et al. 1981, O'Brien and Wheeler 1987).

Enteromorpha intestinalis collected from sites spanning a gradient in water column nutrients was nutrient limited. Algae from down-estuary sites were limited by N as expected based on low background water column NO3 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 (Tyler et al. in prep.). 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 of 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 3-fold, compared to 5-fold replication in all other experimental treatments, 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 other sites, even though algae from site 5 had among the highest initial tissue N and P concentrations. 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 limited by N across a range of initial tissue nutrient levels. N limitation may have been expected in algae from sites 1 and 2, which had initial tissue N concentrations <2%. Roughly 2% has been suggested as the critical concentration, the threshold below which macroalgal maximal growth rates are limited by internal N concentration (Birch et al. 1981, Hanisak 1983, O'Brien and Wheeler 1987, Pedersen and Borum 1996) and above which further increases in tissue N should not stimulate increased growth. 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 (Birch et al. 1981, 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.

P limitation of *Enteromorpha intestinalis* may have been expected as well at all of our sites based on initial tissue P concentrations. A suggested threshold for critical P concentration, below which maximal growth rates are limited by internal P concentration, is 0.33% (Birch et al. 1981), and algae from each of our sites were initially below this level. Over the course of the experiment, depletion of tissue P indicated that algae were utilizing internal stores of P for growth as would be expected if initial P concentrations were below the critical concentration.

Documentation of limitation by one nutrient and then another when the first is supplied in excess is relatively uncommon. 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. However, 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. While it seems common for macroalgae to be limited by either N or P, it appears less common for them to be limited by both nutrients.

TKN concentrations in water collected for this study were ~2-7 times higher than NO_3 concentrations. Dissolved organic nitrogen (DON, a portion of the TKN) such as amino acids and urea are taken up by macroalgae (Tyler et al. in prep.) and may stimulate growth. In addition, macroalgae also release DON during growth (Tyler et al. 2001, Kamer and Fong unpub. data). 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. Week to week decreases in TKN may have been due to uptake of DON and NH_4 by the algae and transformation of the N from DON and NH_4 to NO_3 and NO_2.

In conclusion, *Enteromorpha intestinalis* in UNB was limited by N at a majority of sites during the time period in which we tested for nutrient limitation, and P limitation occurred when the algae were released from N limitation. Reduction of N inputs should result in reduction of algal biomass and associated problems such as anoxia and mortality of estuarine organisms. Further study of nutrient limitation in UNB should include a seasonal component to investigate temporal nutrient dynamics.

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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 means that are significantly different from each other (p<0.05, Fisher's LSD following significant 1-factor ANOVA).

	Tissue nutrient content (as % dry weight)		Molar N:P
Site	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 means 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.

	Water column nutrients (μM)			
Site	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]

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 five sites in UNB. *n*=9 unless otherwise noted (*n* less than 9 occurred when samples were below detection limits of 3.57 mM for N and 1.61 mM for P). Means are given in mM.

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)

Table 3. 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.

	TKN (μM)		
Site	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)

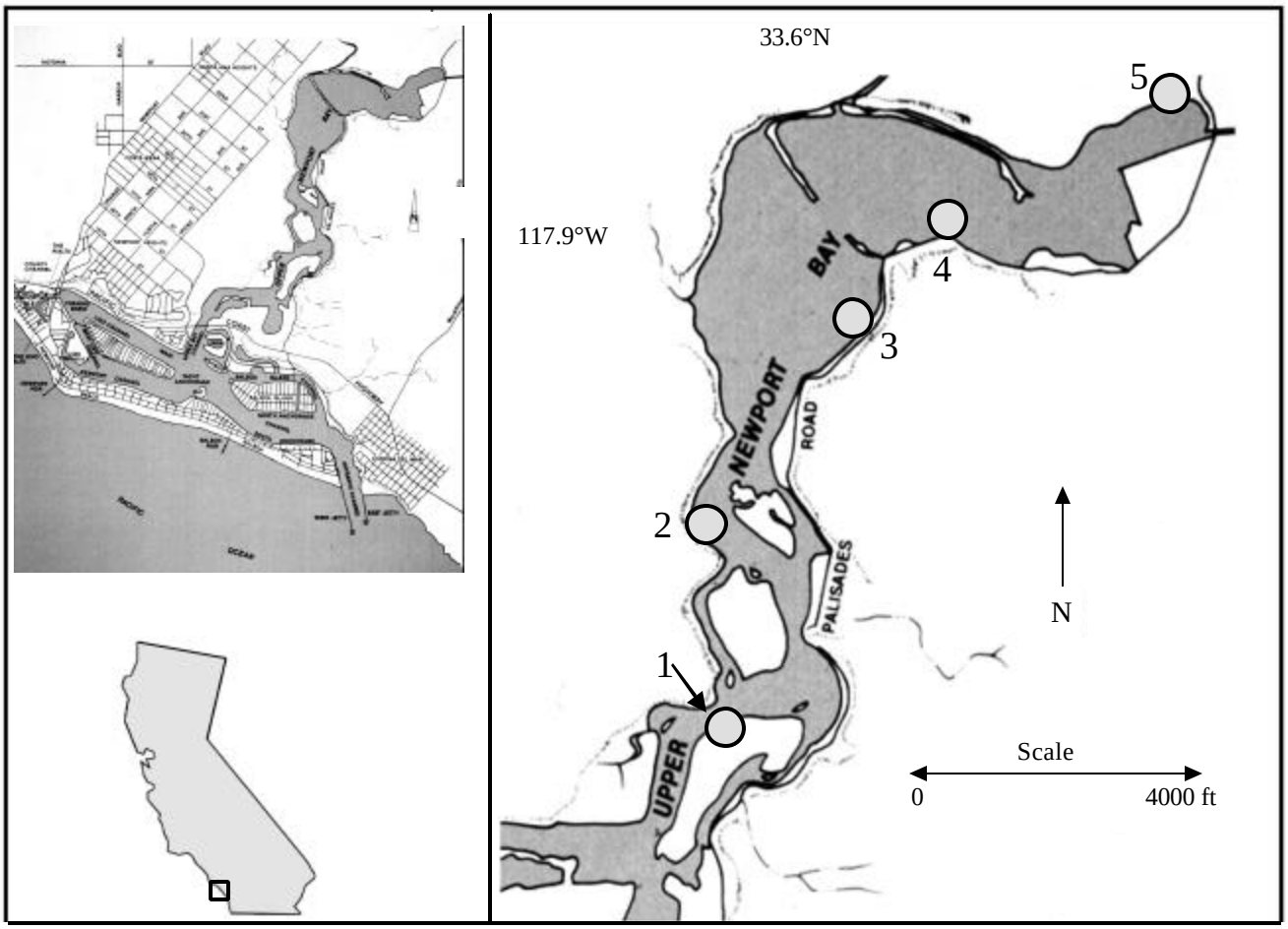

Figure 1. Map of Upper Newport Bay estuary, California, US, with 5 sites from which *Enteromorpha intestinalis* and water were collected for determination of nutrient limitation.

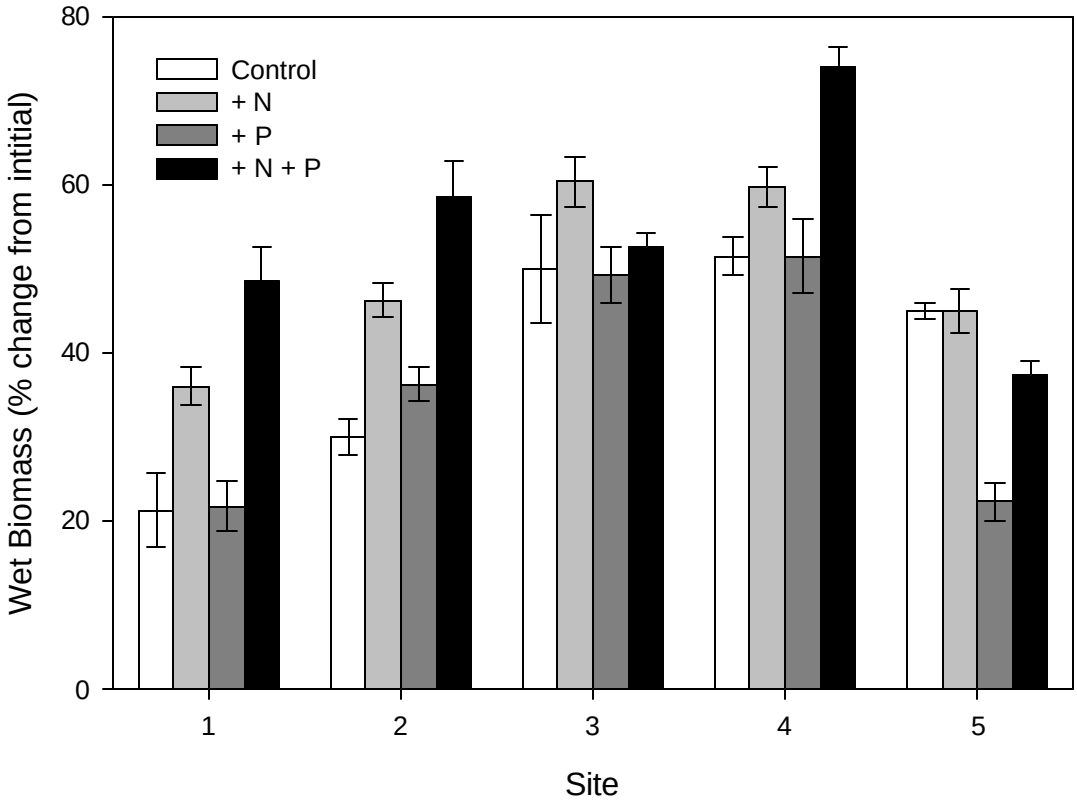

Figure 2. ***Enteromorpha intestinalis*** **biomass (as % change from initial) grown with ambient (Control), nitrogen enrichment (+N), phosphorus enrichment (+P), or nitrogen and phosphorus enrichment (+N+P) solutions.**

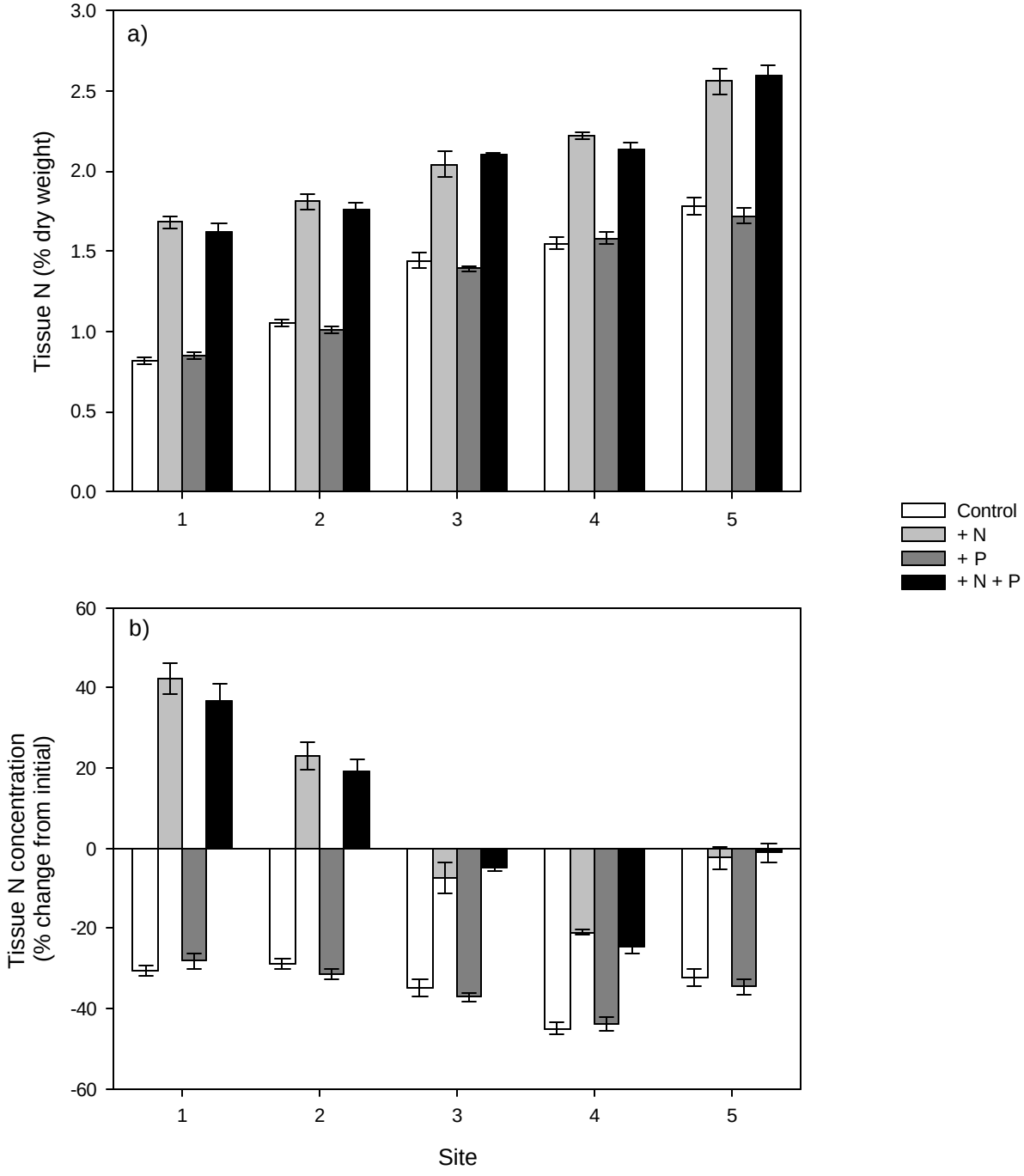

Figure 3. ***Enteromorpha intestinalis*** **tissue nitrogen concentration as % dry wt (a) and % change from initial (b) after 3 weeks in ambient (Control), nitrogen enrichment (+N), phosphorus enrichment (+P), or nitrogen and phosphorus enrichment (+N+P) solutions.**

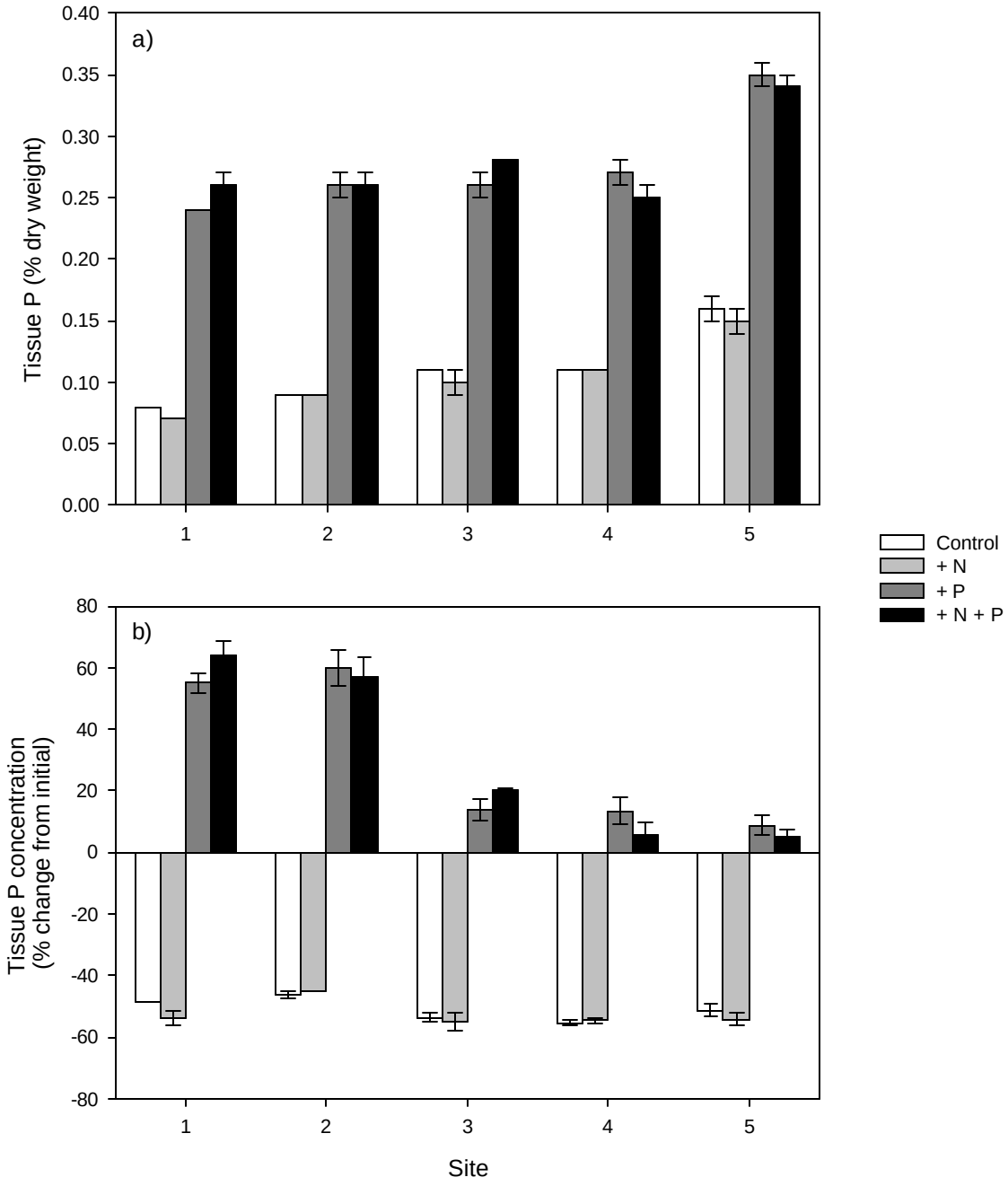

Figure 4. ***Enteromorpha intestinalis*** **tissue phosphorus concentration as % dry wt (a) and % change from initial (b) after 3 weeks in ambient (Control), nitrogen enrichment (+N), phosphorus enrichment (+P), or nitrogen and phosphorus enrichment (+N+P) solutions.**

APPENDIX C

Rates of Inorganic Nitrogen and Phosphorus Uptake by the Estuarine Green Macroalgae *Enteromorpha intestinalis* and *Ulva expansa*

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ABSTRACT

Rates of nitrogen (N) and phosphorus (P) uptake by *Enteromorpha intestinalis* and *Ulva expansa* were investigated in 4 separate experiments. In each experiment, uptake of either N or P by either *E. intestinalis* or *U. expansa* was measured at 1, 2, 4, 8, 12, and 24 h. To measure nutrient uptake rates over a range of conditions, we varied initial water column nutrient concentrations (low, medium and high) and initial algal tissue nutrient status (enriched vs. depleted) by pre-conditioning the algae. *E. intestinalis* and *U. expansa* exhibited a high affinity for N but little or no affinity for P. In the low water column concentration treatments, *E. intestinalis* and *U. expansa* removed all measurable NO_3 from the water within 8 and 12 h, respectively. Nutrient depleted algae consistently removed more NO_3 than enriched algae over each sampling interval. Rates of NO_3 uptake by *E. intestinalis* were >200 μmoles g dry wt^{-1} h^{-1} and ~125 μmoles g dry wt^{-1} h^{-1} by *U. expansa*, up to two orders of magnitude greater than rates measured for other species. Nutrient uptake rates were highly variable over 24 h, indicating surge, internally controlled, and externally controlled phases of nutrient uptake. Over the first 2 h of the *E. intestinalis* NO_3 uptake experiment, uptake rates went from the maximum measured to negative rates, indicating a release of N from algal tissue. Similarly, *U. expansa* NO_3 uptake rates were greatest in the first hour, decreased to 0 in the second hour, and then resumed. Depleted algae showed greater increases in tissue N and P concentration in response to water column nutrient supplies than enriched algae did, and the tissue N increases were concentration dependent. Critical factors in nutrient uptake by macroalgae are nutrient concentration in the water column, algal nutrient status, and 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 (Owens and Stewart 1983, Pregnall and Rudy 1985, Sfriso et al. 1987, Lavery and McComb 1991, Sfriso et al. 1992, Hernández et al. 1997, Farris and Oviatt 1999, Martins et al. 1999, Pihl et al. 1999, Raffaelli et al. 1999, Kamer et al. 2001, Tyler et al. 2001, Eyre and Ferguson 2002) often in response to increased nutrient loads from developed watersheds (Valiela et al. 1992, Nixon 1995, Duarte 1995, Paerl 1997, Valiela et al. 1997, Paerl 1999). These algae are highly successful in estuarine environments where nutrient supply may be transient (Litaker et al. 1987, Ramus and Venable 1987, Sutula et al. in review), 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, Boyer and Fong in review), 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, Sfriso et al. 1992) leading to changes in species composition, shifts in community structure, and loss of ecosystem function (Raffaelli at al. 1991, Ahern et al. 1995, Thiel and Watling 1998).

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) fed 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. O'Brien and Wheeler (1987) measured lower dissolved inorganic nitrogen (DIN) uptake rates for *Enteromorpha prolifera* with higher tissue N content than with lower tissue N content. Duke et al. (1989a) showed that NH_4 uptake in *Ulva curvata* was primarily regulated by tissue N concentration; N uptake was inversely related to tissue N. Pedersen (1994) found that uptake of N by *Ulva lactuca* increased as initial N content of the algae decreased. McGlathery et al. (1996) showed that maximum NH_4 and NO_3 uptake rates by *Chaetomorpha linum* varied inversely with algal N status. Red and brown algae

had higher rates of inorganic N uptake when they had been grown under N- poor conditions compared to N-enriched conditions (D'Elia and DeBoer 1978, Ryther et al. 1981, Rosenberg et al. 1984).

Nutrient uptake rates also vary with external substrate concentration (Hanisak 1983). Harlin (1978) measured increasing rates of NO_3 uptake with increase in water column NO_3 concentration for *Enteromorpha* spp. Fujita (1985) found that uptake rates of DIN by *Enteromorpha* and *Ulva* spp. were dependent on the concentration of DIN in the external medium. Lapointe and Tenore (1981) found NO_3 uptake rates by *Ulva fasciata* were proportional to substrate loading. Parker (1981) measured NH_4 uptake rates by *U. lactuca* that were directly proportional to NH_4 concentration. Rosenberg and Ramus (1984) found that *Ulva curvata* NH_4 and NO_3 uptake rates increased with N concentration.

Macroalgal production in temperate marine and estuarine systems is usually limited by N (Ryther and Dunstan 1971, Harlin and Thorne-Miller 1981, Hanisak 1983, Howarth 1988, Thybo-Christensen et al. 1993), which has lead to the predominance of N-based uptake studies (Harlin 1978, Lapointe and Tenore 1981, Parker 1981, Hanisak 1983, Rosenberg and Ramus 1984, Fujita 1985, Fujita et al. 1988, Duke et al. 1989 a and b, Pedersen 1994). There are few studies of phosphorus (P) uptake by macroalgae (O'Brien and Wheeler 1987), though limitation of macroalgae by P does occur (Birch et al. 1981, O'Brien and Wheeler 1987, Kamer et al. in prep.).

Enteromorpha and *Ulva* spp. commonly form blooms in southern California estuaries (Peters et al. 1985, Rudnicki 1986, Kamer et al. 2001, Kennison et al. unpub. data). There is a lack of data for southern California estuaries, in general, (Bricker et al. 1999) and on nutrient dynamics, in particular (Williams and Zedler 1992). Understanding nutrient uptake by macroalgae is critical to understanding the effects of nutrient availability on their productivity (sensu Fujita 1985). Rates of nutrient uptake for macroalgae in southern California have not been determined.

The objective of this study was to measure the rates of inorganic N and P uptake by *Enteromorpha intestinalis* and *Ulva expansa* under initial substrate concentrations and algal tissue nutrient concentrations representative of levels measured in Upper Newport Bay (UNB) (Kamer et al. 2001, Boyle et al. unpub. data), a large estuary in southern California that experiences large seasonal blooms of *E. intestinalis* and *U. expansa* (AHA 1997, Kamer et al. 2001). Of available DIN in coastal marine and estuarine waters, NO_3 is usually more abundant than NH_4 (Sharp 1983). In UNB, NO_3 concentrations are much greater than NH_4, sometimes by 10 to 20-fold (Boyle et al. unpub. data). Macroalgae in UNB showed limitation have been shown to be limited by both N and P (Kamer et al. in prep.). Therefore, the availability of N and P may regulate macroalgal biomass and the uptake dynamics of both nutrients are of critical concern in understanding the role they play in macroalgal bloom dynamics.

MATERIALS AND METHODS

Overview of Experimental Design

We conducted four independent laboratory experiments to test the hypothesis that nutrient uptake rates in the green macroalgae *Enteromorpha intestinalis* and *Ulva expansa* vary depending on initial algal tissue nutrient status and initial water column nutrient concentration. We measured the uptake of both N and P by *E. intestinalis* in separate experiments and repeated these experiments using *U. expansa*. Each experiment had 2 fully-crossed factors: initial algal tissue nutrient status (nutrient enriched vs. depleted) and initial water column nutrient concentration (low, medium, and high). Both initial tissue nutrient levels and initial water column nutrient concentrations were within the range of values measured in Upper Newport Bay and other southern California estuaries

(Kamer et al. 2001, Boyle et al. unpub. data, Kamer et al. in prep.). Each experiment ran for 24 hours. The experiments using *E. intestinalis* were conducted on 10 July for N addition and 7 August for P addition. Identical experiments for *U. expansa* were conducted on 24 July for N addition and 31 July for P addition, all in 2001.

Experimental Set Up

Seven to ten days prior to the initiation of each experiment, algae were collected from UNB. 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, water in one pan was enriched every 3 days with NO_3 (579.76 ± 64.28 µM, mean ± SE, n=6) for the N uptake experiments and PO_4 (19.57 ± 6.67 µM, n=6) for the P uptake experiments 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, < 1.61 µM PO_4) in order to reduce tissue nutrient status (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 (K. Kamer unpub. data). Flow was incorporated into the experimental design in order to reduce the effect of boundary layers and avoid limitation of nutrient uptake that can occur in static systems.

Replicate experimental units consisted of 12L round plastic containers with a 4L 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 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.

After pre-conditioning the algae to achieve enriched and depleted nutrient status (Table 1), algae were placed in individual nylon mesh bags, spun in a salad spinner for one minute and wet weighed. Five initial samples were taken to determine initial tissue N and P status. 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 concentration (Table 1). For the first experiment (*Enteromorpha intestinalis* N uptake), 20 grams of algae were placed in each experimental unit; for the rest of the experiments, 10 grams of algae were used. We reduced the amount of biomass in subsequent experiments because of the high uptake rates measured in experiment 1. We wanted to ensure that nutrient levels would not drop below analytical detection limits. To begin each experiment, algae were placed in the outer container in the water flow path and isolated from the inner chamber with the pump. Experimental units were placed outdoors in a temperature controlled water bath and arranged in a randomized matrix. There was four-fold replication for a total of 24 units.

Three water samples were taken from each nutrient solution at the beginning of each experiment to quantify initial water column nutrient concentrations (Table 1). At 1, 2, 4, 8, 12 and 24 hours, additional 100 ml water samples were taken from each unit. The total volume removed from each unit was <10% of the initial volume. We determined the uptake rates of inorganic nitrogen and phosphorus for each species of macroalgae by measuring the disappearance of inorganic nutrients from solution over time. Samples were filtered with glass fiber filters (Whatman GF/C), frozen, and analyzed for NO_3, NH_4, TKN (all forms of dissolved N except NO_3 and NO_2) and PO_4. NO_3 was reduced to NO_2 via cadmium reduction; NO_2 was measured spectrophotometrically after diazotation (Switala 1999, Wendt 1999). NH_4 was heated with solutions of salicylate and hypochlorite and determined spectrophotometrically (Switala 1999, Wendt 1999). 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). PO_4 was determined spectrophotometrically following reaction with ammonium molybdate and antimony potassium under acidic conditions (APHA 1998). These automated methods have detection limits of 3.57 µM for N and 1.61 µM for P.

After the 24 hour 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 and P concentration. N was determined using an induction furnace and a thermal conductivity detector (Dumas 1981). P was determined by atomic absorption spectometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) following a nitric acid/hydrogen peroxide microwave digestion (Meyer and Keliher 1992). N and P content of algae are reported as % dry wt and percent change from initial.

Statistical Analyses

All data from the four 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 and P (% dry weight), and tissue N and P % change from initial were analyzed using 2-factor ANOVA (initial water column nutrient concentration x initial algal 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/hr) were analyzed using 3-factor ANOVA (initial water column nutrient concentration x initial algal nutrient status x sampling interval). Regression analysis quantified the relationship between water column nutrients and time for each of the 6 experimental treatments. Regression lines were chosen based on the best fit.

RESULTS

Experiment 1: Nitrogen uptake by enriched and depleted *Enteromorpha intestinalis*

Water column NO_3 decreased significantly over time in units with both enriched and depleted algae for all three initial water column nutrient concentrations (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 concentrations, 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 hours for both the depleted and enriched *Enteromorpha 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 hours.

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 hours. 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 hours. Although the % 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, TKN, or PO_4 with either experimental factor.

There were significant differences in final dry weight of *Enteromorpha 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 *Enteromorpha intestinalis* final 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 nutrient concentration for algae that were depleted in tissue N prior to the beginning of the experiment (Figure 3b).

There was a significant effect of initial water column nutrient concentration on *Enteromorpha intestinalis* final tissue P concentration (ANOVA, p=0.0373). Tissue P from the low initial water column nutrient concentration treatment was significantly less than from the medium treatment (PLSD, p=0.0135). However, differences among means were small (Figure 3c) and may be attributed to the higher growth in the low treatment, diluting the tissue P concentration. There was no effect of initial algal nutrient status (ANOVA, p=0.4520).

Percent change in *Enteromorpha intestinalis* tissue N was significantly affected by initial algal 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 water column nutrient concentration treatments (Figure 4a). 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.

Percent change in the tissue P of *Enteromorpha intestinalis* varied significantly with both initial algal nutrient status (ANOVA, p=0.0002) and initial water column nutrient concentration (ANOVA, p=0.0377). The % change in tissue P in the enriched algae was always less than in the depleted algae (Figure 4b). In addition, for both enriched and deplete algae, the % change in tissue P was highest in the medium concentration treatment.

Enteromorpha 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 time (ANOVA, p=0.0001). In the low concentration treatment (Figure 5a), *E. intestinalis* began taking up NO_3 immediately. In the first hour, enriched algae took up 54.84 µmoles/g dry wt/hr (SE=2.67) and depleted algae took up 71.65 µmoles/g dry wt/hr (SE=6.28). The uptake rate increased over time for the first four hours for both the depleted and enriched algae with rates for depleted algae always higher than enriched. Water column NO_3 dropped below the detection limit of 3.57 µM during the 4-8 hour sampling interval, for both the depleted and enriched algae, suggesting the algal tissues were not N saturated in this treatment and making it impossible to calculate uptake rates beyond the four hour mark.

Uptake in the medium concentration treatment (Figure 5b) was low and extremely variable until the 2-4 hour sampling interval when uptake was 131.26 µmoles/g dry wt/h (SE=5.85) for enriched algae and 190.04 µmoles/ g dry wt/h (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 (Figure 5c), both the depleted and enriched algae took up NO_3 over the first time interval (0-1 hours). Uptake in this interval was over 200 µmoles/g dry wt/h for both the depleted and enriched algae; this is 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/h (SE=20.78) for enriched algae and 188.67 µmoles/g dry wt/h (SE=32.99) for the depleted algae. After 4 hours, 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 and 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 suggested that tissues in both initially enriched and depleted algae were becoming saturated with N.

Experiment 2: Nitrogen uptake by enriched and depleted *Ulva expansa*

Water column NO_3 decreased significantly over time for all three initial water column nutrient concentrations (Figure 6). 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 concentrations, 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 8 h for the depleted *Ulva expansa* and by 12 h for all but one experimental unit for enriched algae (Figure 6a). This pattern of N removal by *U. expansa* was similar to that of *Enteromorpha intestinalis.*

In the medium concentration treatment, water column NO_3 concentrations decreased by 110.54 µM (SE=5.74) when enriched algae were present and by 173.75 µM (SE=6.08) when depleted algae were present (Figure 6b). 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 hours. Overall, reductions of N in the water due to *Ulva expansa* were less than for *Enteromorpha 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 6c). This resulted in a 21% (SE=3.58) reduction in water column NO_3 over 24 hours. 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 hours. Removal of NO_3 by *Ulva expansa* was also less than that by *Enteromorpha intestinalis* in these treatments. There was no pattern of water column NH_4, TKN, or PO_4 with either experimental factor.

Ulva expansa final dry weight was not significantly affected by either initial water column nutrient concentration (ANOVA, p=0.1042) or initial algal nutrient status (ANOVA, p=0.1434). Final dry weight of the algae was between 1.0-1.5g, or ~10% of the initial wet weight (Figure 7a). The initial wet weight of *Enteromorpha 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 *Ulva expansa* final tissue N concentration (ANOVA, p=0.0001). Mean tissue N of the enriched algae was significantly higher than the depleted algae (Figure 7b). There was an interaction between 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 concentration on *U. expansa* tissue N concentration (ANOVA, p=0.1204). There was no effect of either initial water column nutrient concentration (ANOVA, p=0.9287) or initial algal nutrient status (ANOVA, p=0.081) on *U. expansa* tissue P concentration (Figure 7c).

Percent change in tissue N of *Ulva expansa* was significantly affected by initial tissue nutrient status (ANOVA, p=0.0001). Percent change from initial N in depleted *U. expansa* was greater than in enriched algae (Figure 8a). Tissue N of depleted algae increased from 38-68% while enriched algae tissue N increased <20%. There was also an effect of initial 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 vs. enriched algae, and these changes were concentration dependent.

There was a significant effect of initial algal nutrient status on percent change in *Ulva expansa* tissue P (ANOVA, p=0.0223) but no change due to initial water column nutrient concentration (ANOVA, p=0.9156). Depleted algae had greater increases in P than enriched algae in all treatments except for the low initial NO_3 water concentration (Figure 8b).

Uptake rate of NO_3 by *Ulva expansa* was significantly affected by initial algal nutrient status (ANOVA, p=0.0019), initial water column nutrient concentration (ANOVA, p=0.0001), and time (ANOVA, p=0.0001). Across all water column nutrient treatments uptake rates were significantly higher for the depleted than the enriched algae. In all three initial water column nutrient concentrations, there was a similar uptake pattern. 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 9).

In the low concentration treatment (Figure 9a), uptake rate of *Ulva expansa* was between 30-130 µmoles/g dry wt/hr in the first hour, depending on algal nutrient status. 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 detection during the 4-8 h interval for the depleted algae and during the 8-12 h interval for the enriched algae suggesting that, like *Enteromorpha 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 treatments.

In contrast to *Enteromorpha intestinalis*, the highest mean uptake rates for *Ulva expansa* were measured in the medium concentration treatment (Figure 9b). After the 2-4 hour 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 rates 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 hour sampling interval (Figure 9c). However, there were no differences between the enriched and depleted algae after the 0-1 hour sampling interval. As with *Enteromorpha 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 *Ulva expansa* was much lower than uptake by *E. intestinalis*.

Experiment 3: Phosphorus uptake by enriched and depleted *Enteromorpha intestinalis*

There was little change in water column PO_4 over time (Figure 10). Unlike NO_3, PO_4 was not reduced in the water column by *Enteromorpha intestinalis* in 24 h.

There was no significant effect of initial algal nutrient status on *Enteromorpha intestinalis* final dry weight (ANOVA, p=0.5036). There was also no effect of initial water column nutrient concentration (ANOVA, p=0.3362) (Figure 11a).

There was a significant effect of initial water column nutrient concentration on *Enteromorpha intestinalis* final tissue N concentration (ANOVA, p=0.0274), with the medium treatment having the most tissue N, but differences were small among treatments (Figure 11b). There was no difference in tissue N between the enriched and depleted algae (ANOVA, p=0.5021).

There was a significant effect of initial algal nutrient status on *Enteromorpha intestinalis* final tissue P concentration (ANOVA, p=0.0001). The enriched algae had higher tissue P than the depleted algae (Figure 11c), reflecting initial differences. There was no effect of initial water column nutrient concentration (ANOVA, p=0.5780).

There was a significant effect of initial water column nutrient concentration on *Enteromorpha intestinalis* tissue N percent change from initial (ANOVA, p=0.0267). Tissue N increased more in the medium treatment than in the low or high treatments (Figure 12a), (PLSD, p=0.0118 for the low and p=0.034 for the high). However, there were no differences in tissue N percent change from initial between the depleted and the enriched algae (ANOVA, p=0.2286).

The depleted *Enteromorpha intestinalis* had greater percent change in tissue P from initial than the enriched algae (ANOVA, p=0.0001; Figure 12b). There was no difference in percent change of tissue P due to initial water column nutrient concentration. (ANOVA, p= 0.3678).

There was a significant effect of initial water column nutrient concentration on *Enteromorpha intestinalis* PO_4 uptake rates (ANOVA, p=0.002) and a significant effect of time (ANOVA, p=0.0001). In general, PO_4 uptake was extremely variable among nutrient concentration treatments over time. In both the medium and high treatments the algae took up PO_4 initially, but there was no further uptake after the first interval (Figure 13). There were no significant differences in uptake rates between the enriched and the depleted algae (ANOVA, p=0.9976).

Experiment 4: Phosphorus uptake by enriched and depleted *Ulva expansa*

Water column PO_4 did not decrease significantly over time (Figure 14). Generally, removal by *Ulva expansa* was less than by *Enteromorpha intestinalis*.

There were no significant differences in final dry weight between the depleted and enriched algae (ANOVA, p=0.1268). There was no effect of initial water column nutrient concentration on dry weight (ANOVA, p=0.7001), (Figure 15a).

There were no differences in *Ulva expansa* final tissue N concentration (Figure 15b) due to either initial algal nutrient status (ANOVA, p=0.9512) or initial water column nutrient concentration (ANOVA, p=0.0542). There were significant effects of both initial water column nutrient concentration (ANOVA, p=0.002) and initial algal

nutrient status (ANOVA, p=0.001) on final tissue P concentration (Figure 15c). Tissue P was significantly lower in the medium concentration treatment than in the low and high concentration treatments (PLSD, p=0.0005 for low and p=0.0374 for high), and tissue P was lower in the depleted algae compared to the enriched, reflecting pre-conditioning.

There were no differences in *Ulva expansa* tissue N percent change from initial due to either initial water column nutrient concentration or initial algal nutrient status (Figure 16a). There were significant effects of both initial water column nutrient concentration (ANOVA, p=0.0016) and initial algal nutrient status (ANOVA, p=0.0001) on tissue P percent change from initial (Figure 16b). In the medium nutrient concentration treatment, tissue P percent change was less than for low nutrient concentration (PLSD, p=0.0004) and high nutrient concentration (PLSD, p=0.0341). Tissue P % change was always greater for depleted than for enriched algae.

The rates of uptake of PO_4 by *Ulva expansa* were not significantly affected by initial water column nutrient concentrations or initial algal nutrient status (Figure 17). The uptake rates were low and variable across time. Unlike *Enteromorpha intestinalis, U. expansa* did not experience initially high uptake in the medium and high concentrations.

DISCUSSION

Nitrogen status, measured as internal concentration of N, was very important in determining the rate of nitrogen uptake by both *Ulva expansa* and *Enteromorpha intestinalis* in these experiments. Uptake rates were always lower for enriched compared to nutrient depleted tissues. 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 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* 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 moles g dry wt^{-1} h^{-1} for *Enteromorpha* spp. during transiently enhanced uptake.

Our results demonstrated that both *Enteromorpha intestinalis* and *Ulva 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 *Ulva expansa* in the low N treatments, uptake rates dropped to zero because all 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 (Harlin 1978, Hanisak 1983, Lapointe and Tenore 1981, Parker 1981, Rosenberg and Ramus 1984, Fujita 1985).

Among the sampling intervals of our experiments, N uptake rates varied greatly. Uptake of N by *Enteromorpha intestinalis* went from the maximum rate to actual release of N within 2 hours in the highest N concentration while *Ulva 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 *Ulva 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 to actually 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 *Enteromorpha intestinalis* and *Ulva 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).

Enteromorpha intestinalis and *Ulva expansa* did not demonstrate the same high and sustained affinity for phosphorus that they did for nitrogen. Neither alga reduced water column PO_4 over 24 h indicating a lack of uptake of PO_4. Although *Enteromorpha intestinalis* demonstrated initial high PO_4 uptake rates in the medium and high PO_4 concentration treatments, rates were extremely low after the first hour. In addition, initial P status had no effect on uptake rate, but only affected final tissue nutrient concentration. Other research has suggested that N is the most limiting nutrient in Newport Bay (Boyle et al. in prep., Kamer et al. in prep), in other southern California estuaries (Fong et al. 1993a,b, 1994a,b, 1996) and in most temperate estuaries around the world (for a review see Nixon 1995). Thus, development of very efficient uptake and storage mechanisms for N, but not P, may be an adaptation to a long history of N-limitation.

In general, macroalgae did not grow measurably in these 24-hour experiments. The exception is *Enteromorpha intestinalis*, which grew in the N addition experiment, but only in the lowest concentration treatment. This supports earlier findings that demonstrate nutrient uptake and growth are often temporally uncoupled (Duke et al. 1989b, Fong, unpub. data). Both uptake and growth are energy-dependant processes. One explanation for temporal uncoupling of these two processes may be that opportunistic, "nutrient specialist " types of algae allo-

cate 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 3 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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Table 1. Initial tissue nitrogen and phosphorus concentrations and initial water column NO_3 and PO_4 concentrations in 4 separate experiments measuring rates of NO_3 and PO_4 uptake by *Enteromorpha intestinalis* and *Ulva expansa*. Nitrogen data are provided for the nitrogen experiments and phosphorus data are provided for the phosphorus experiments. Values are means ± SE.

Algae	Initial tissue nutrient status (N or P as % dry wt)		Initial water nutrient concentrations (µM NO_3 or PO_4)		
Nitrogen Experiments	Depleted	Enriched	Low	Medium	High
Enteromorpha intestinalis	1.43 ± 0.06	2.18 ± 0.06	65.24 ± 1.25	243.10 ± 18.72	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
Phosphorus Experiments					
Enteromorpha intestinalis	0.12 ± 0.00	0.19 ± 0.00	5.48 ± 0.00	16.21 ± 0.36	56.13 ± 0.40
Ulva expansa	0.15 ± 0.00	0.19 ± 0.00	5.81 ± 0.23	24.52 ± 0.35	48.47 ± 0.36

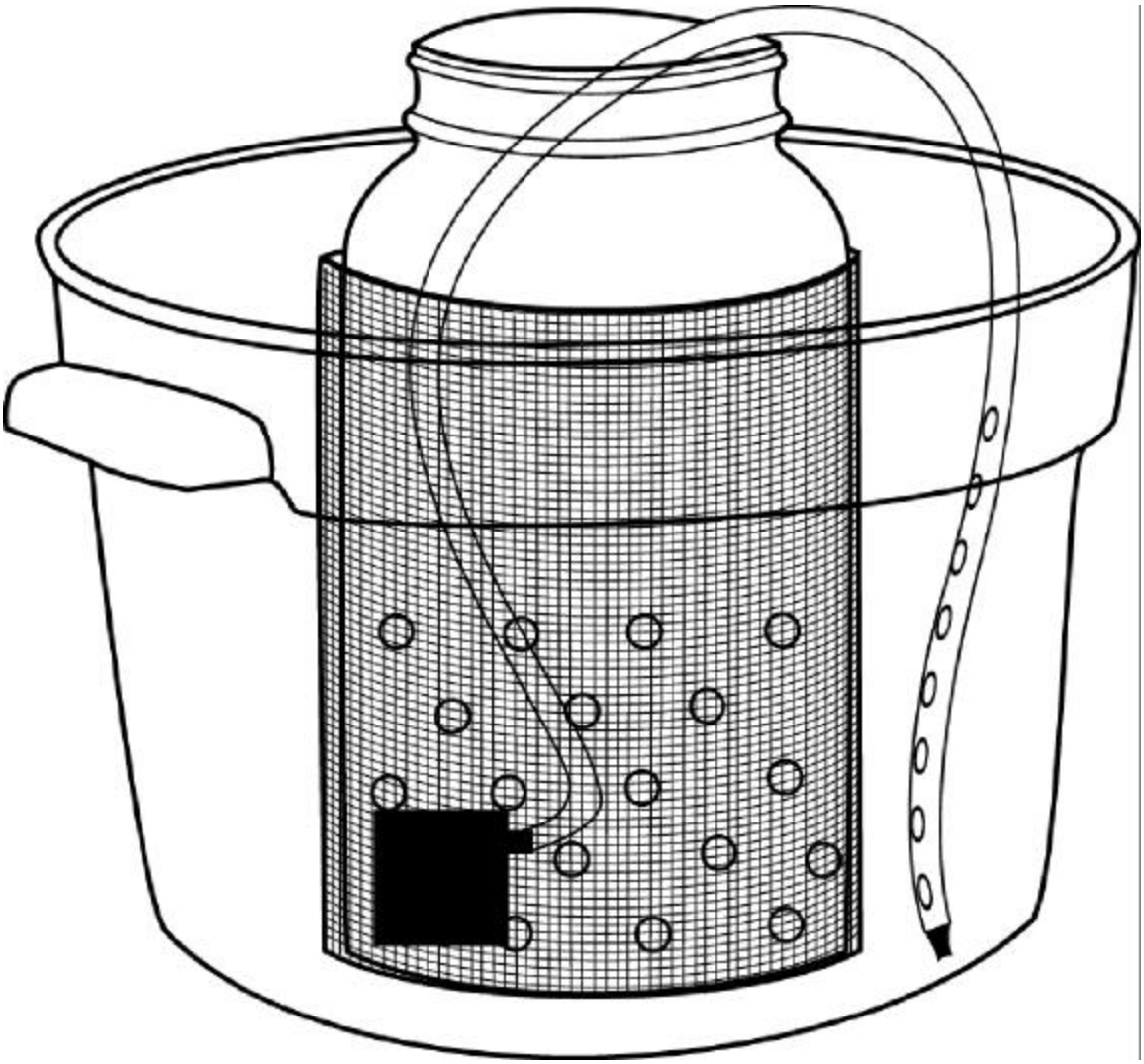

Figure 1. Diagram of experimental units showing the outer container and inner plastic jar with holes to allow passive water flow from the outer flow path to the interior of the jar through the window screening. The inner jar contained an aquarium pump, and plastic irrigation tubing was attached to the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

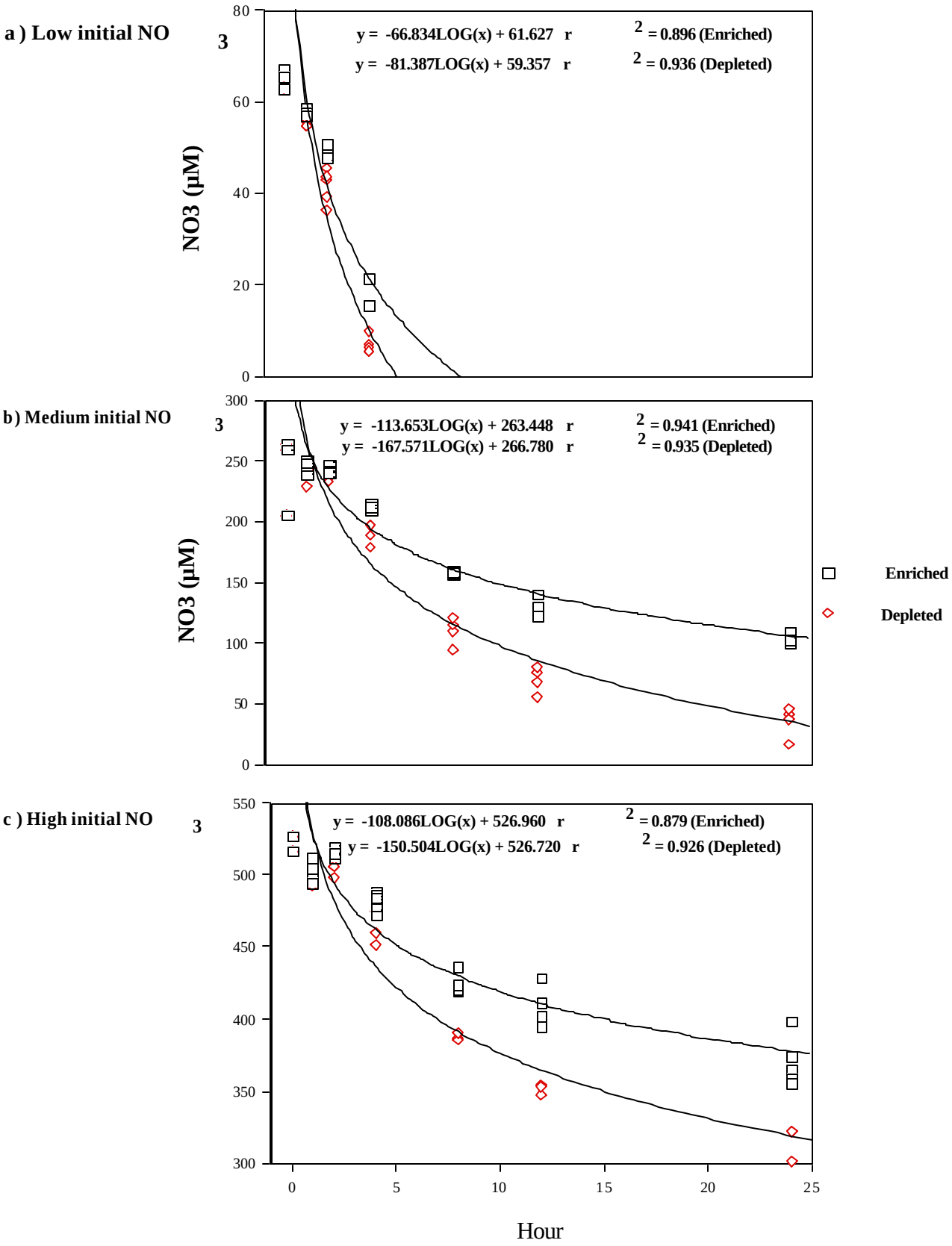

Figure 2. Decrease in water column NO_3 over time in the *Enteromorpha intestinalis* nitrogen uptake experiment for enriched and depleted algae for low (a), medium (b) and high (c) initial water column nutrient concentrations.

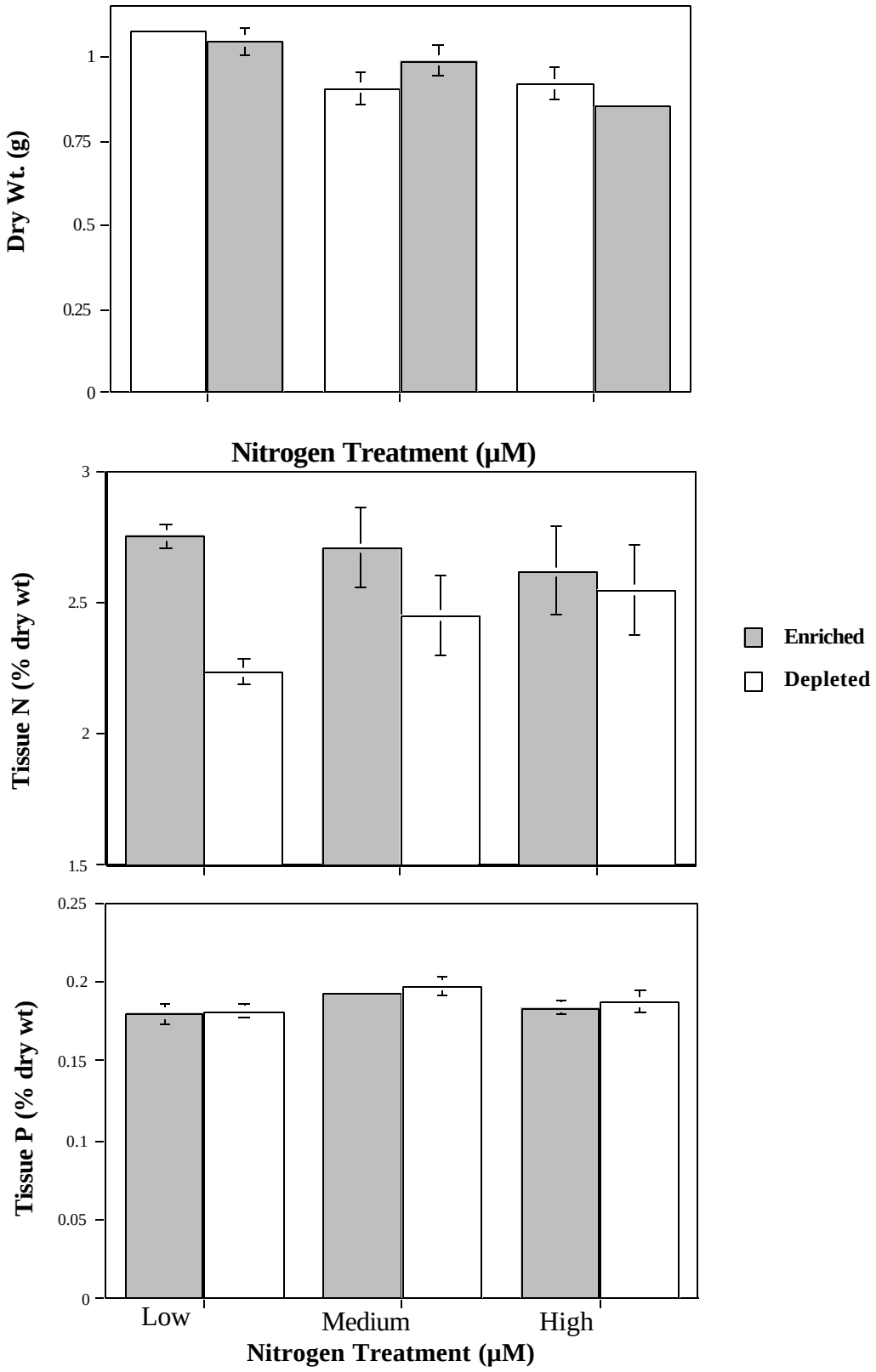

Figure 3. Final dry weight (a), tissue N concentration (b) and tissue P concentration (c) of enriched and depleted *Enteromorpha intestinalis* incubated with low, medium and high initial water column nutrient concentrations in the nitrogen uptake experiment.

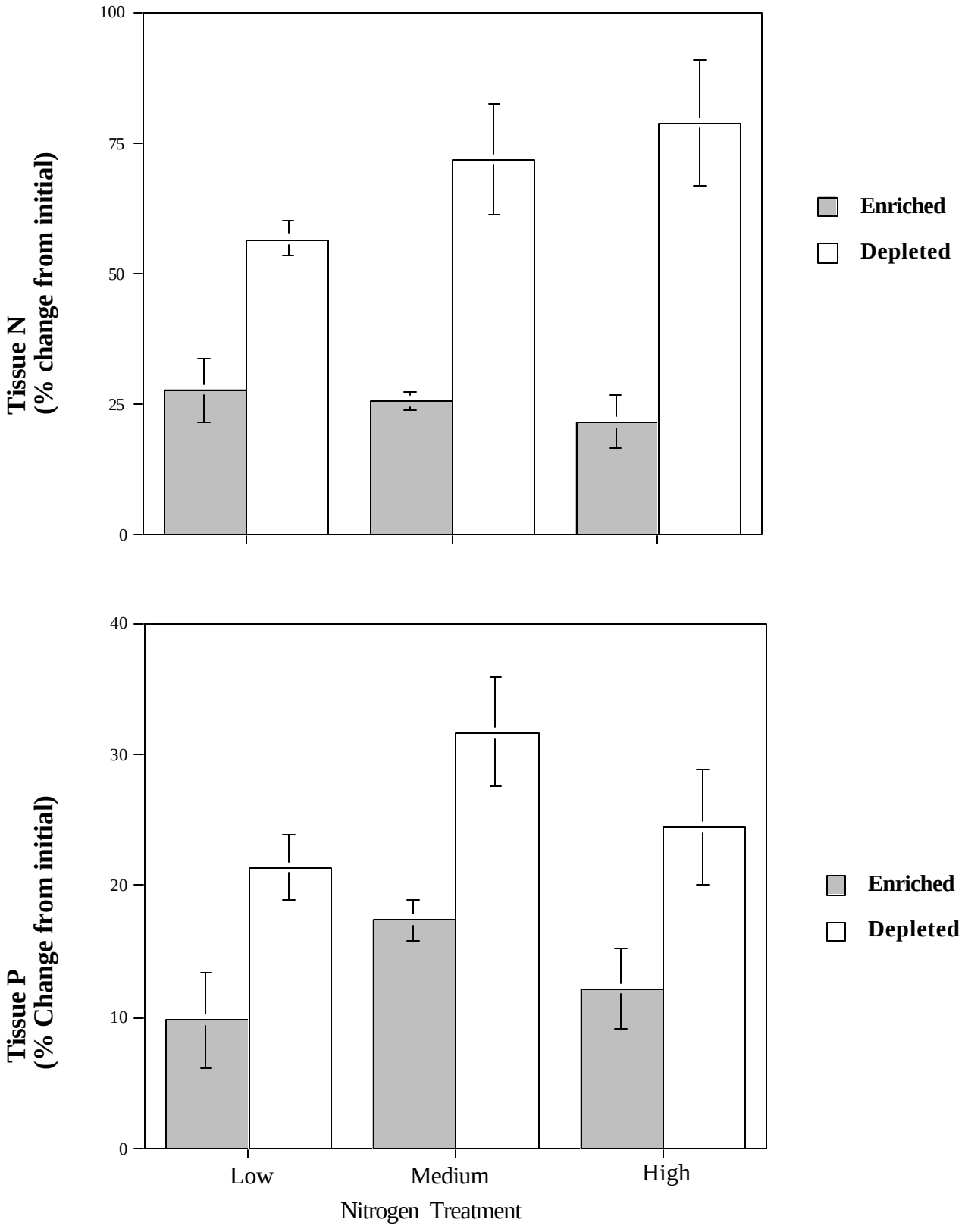

Figure 4. Percent change in *Enteromorpha intestinalis* tissue N (a) and P (b) from initial values in enriched and depleted algae incubated with low, medium and high initial water column nutrient concentrations in the nitrogen uptake experiment.

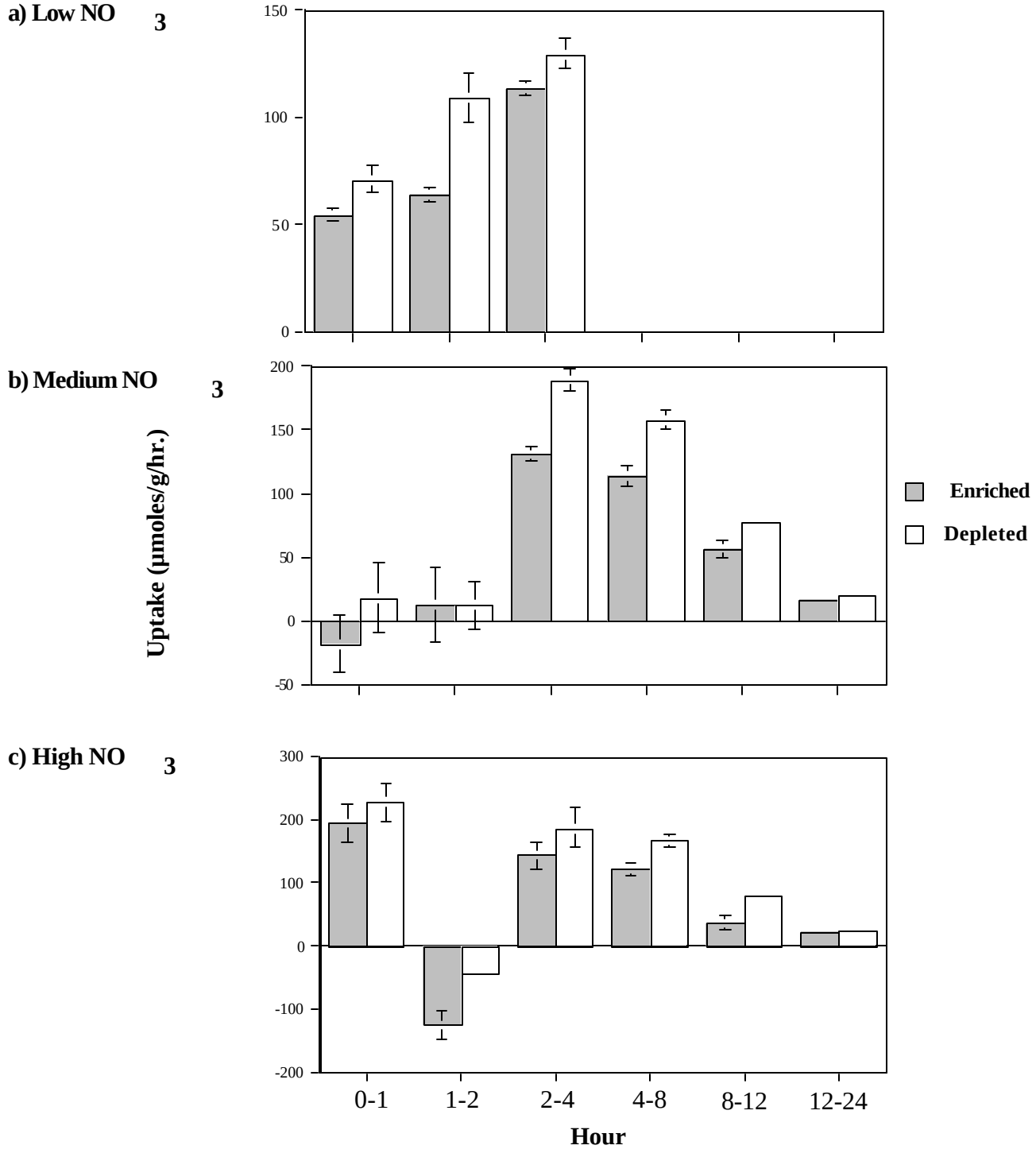

Figure 5. ***Enteromorpha intestinalis*** **NO_3 uptake rates for enriched and depleted algae in low (a), medium (b) and high (c) initial water column nutrient concentrations in the nitrogen uptake experiment.**

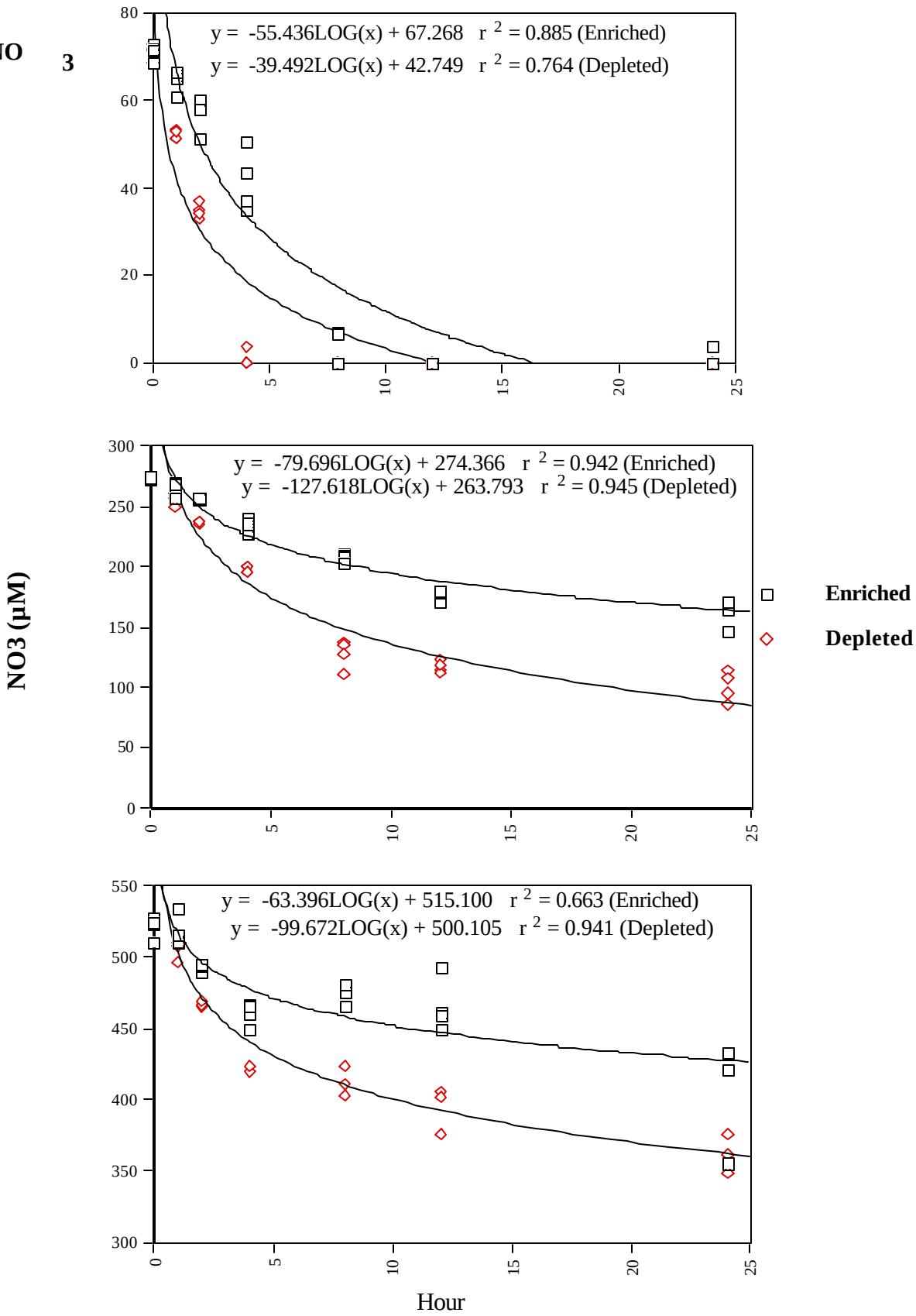

Figure 6. Decrease in water column NO_3 over time in the *Ulva expansa* nitrogen uptake experiment for enriched and depleted algae for low (a), medium (b) and high (c) initial water column nutrient concentrations.

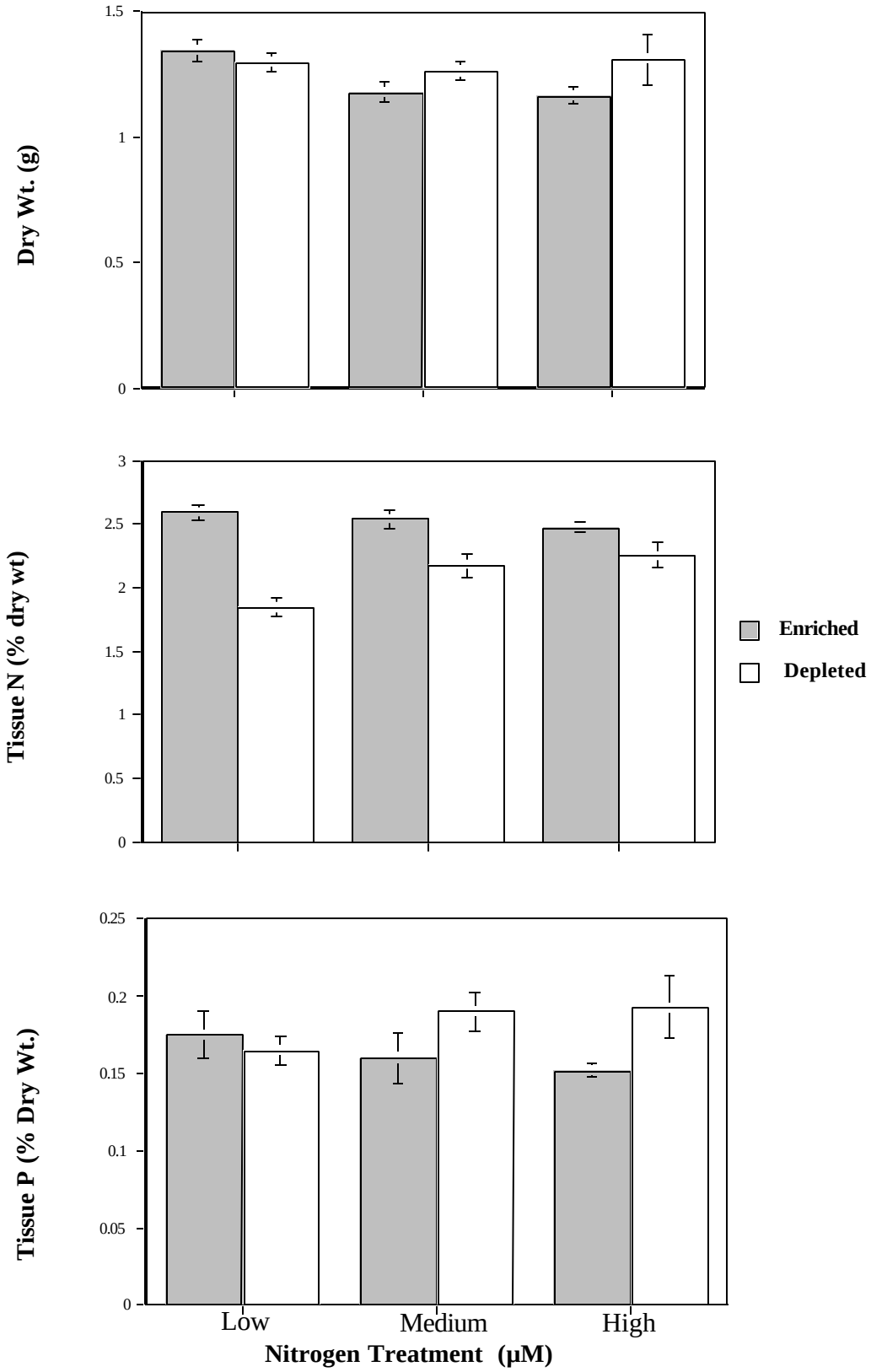

Figure 7. Final dry weight (a), tissue N concentration (b) and tissue P concentration (c) of enriched and depleted *Ulva expansa* incubated with low, medium and high initial water column nutrient concentrations in the nitrogen uptake experiment.

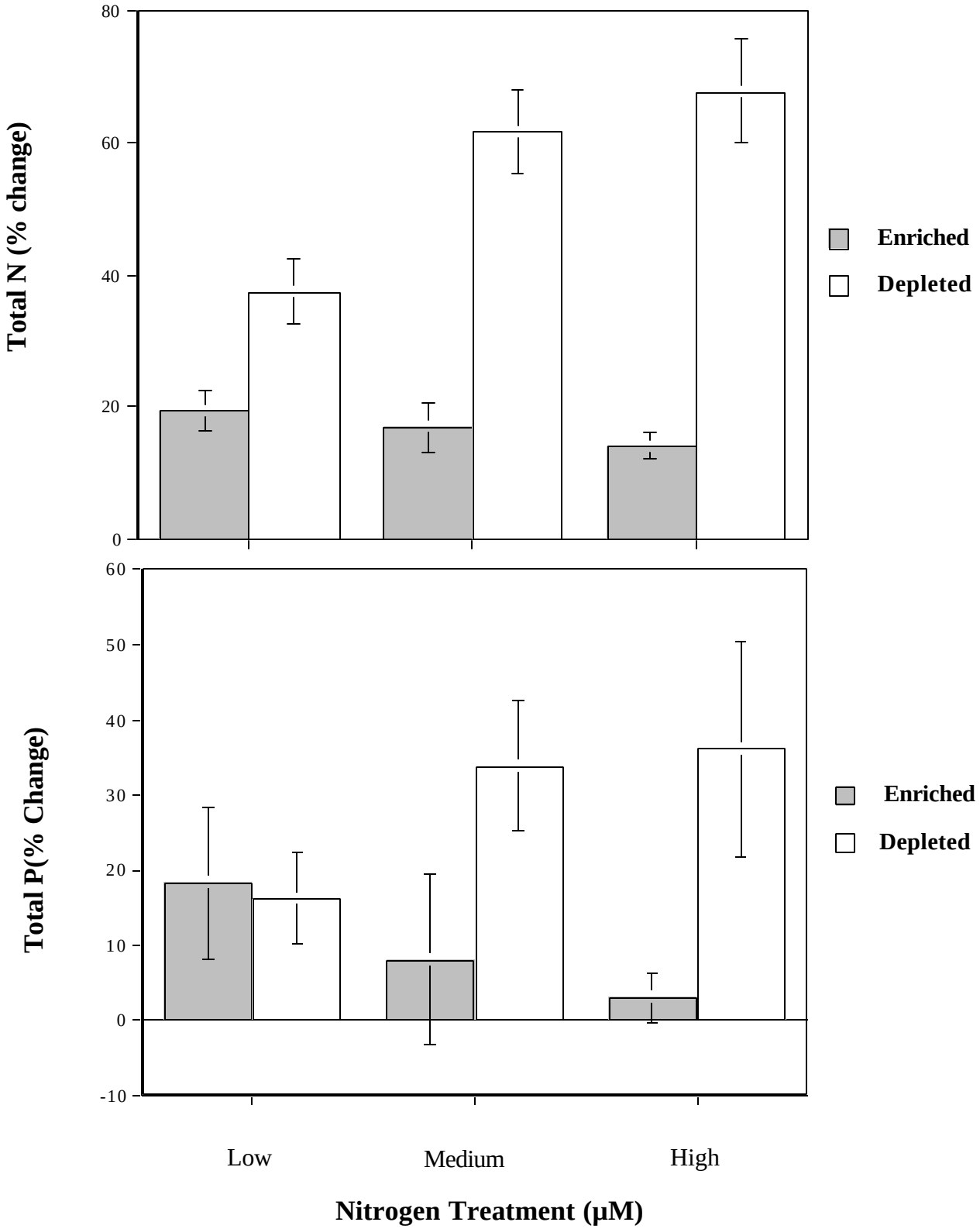

Figure 8. Percent change in *Ulva expansa* tissue N (a) and P (b) from initial values in enriched and depleted algae incubated with low, medium and high initial water column nutrient concentrations in the nitrogen uptake experiment.

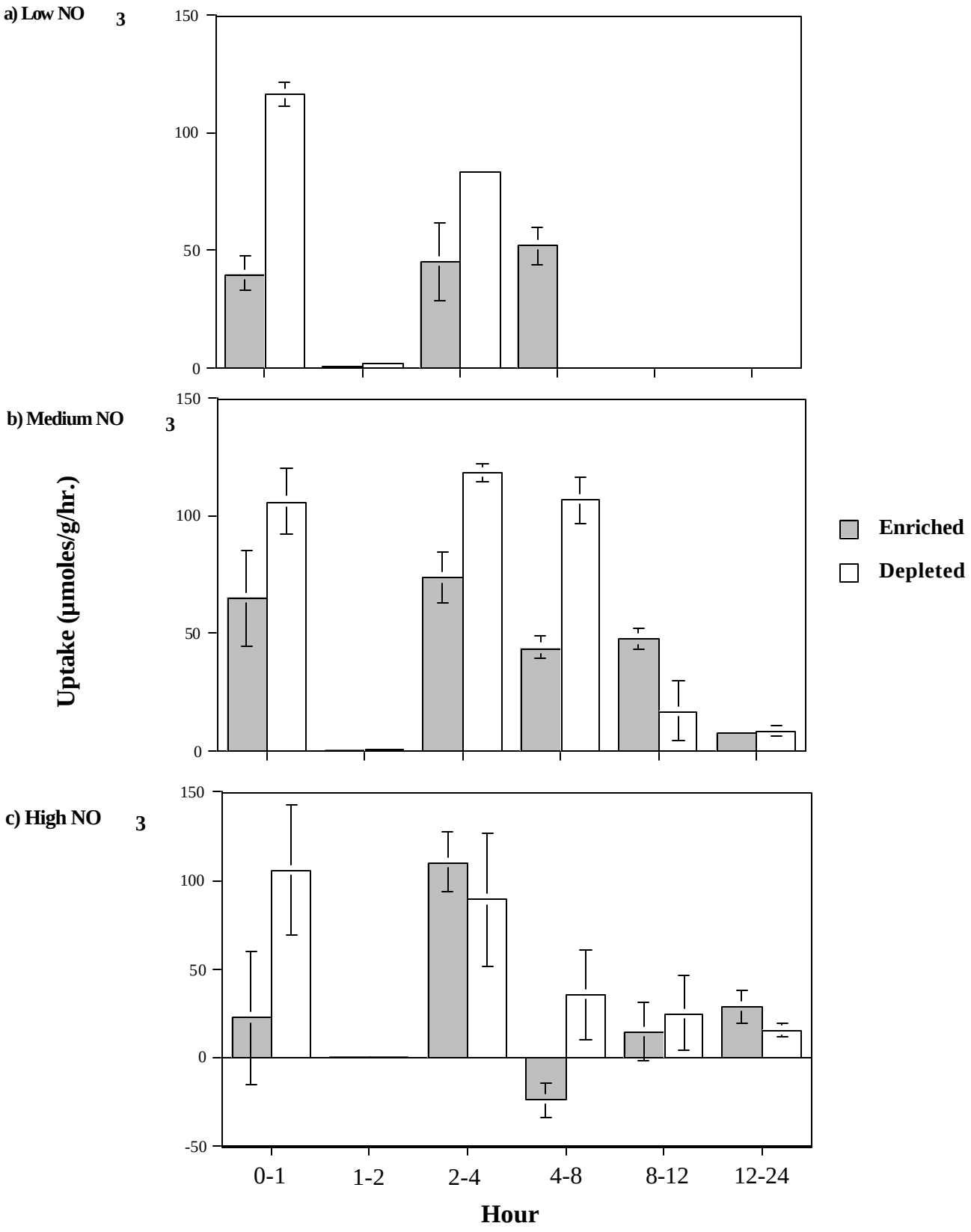

Figure 9. *Ulva expansa* NO_3 uptake rates for enriched and depleted algae in low (a), medium (b) and high (c) initial water column nutrient concentrations in the nitrogen uptake experiment.

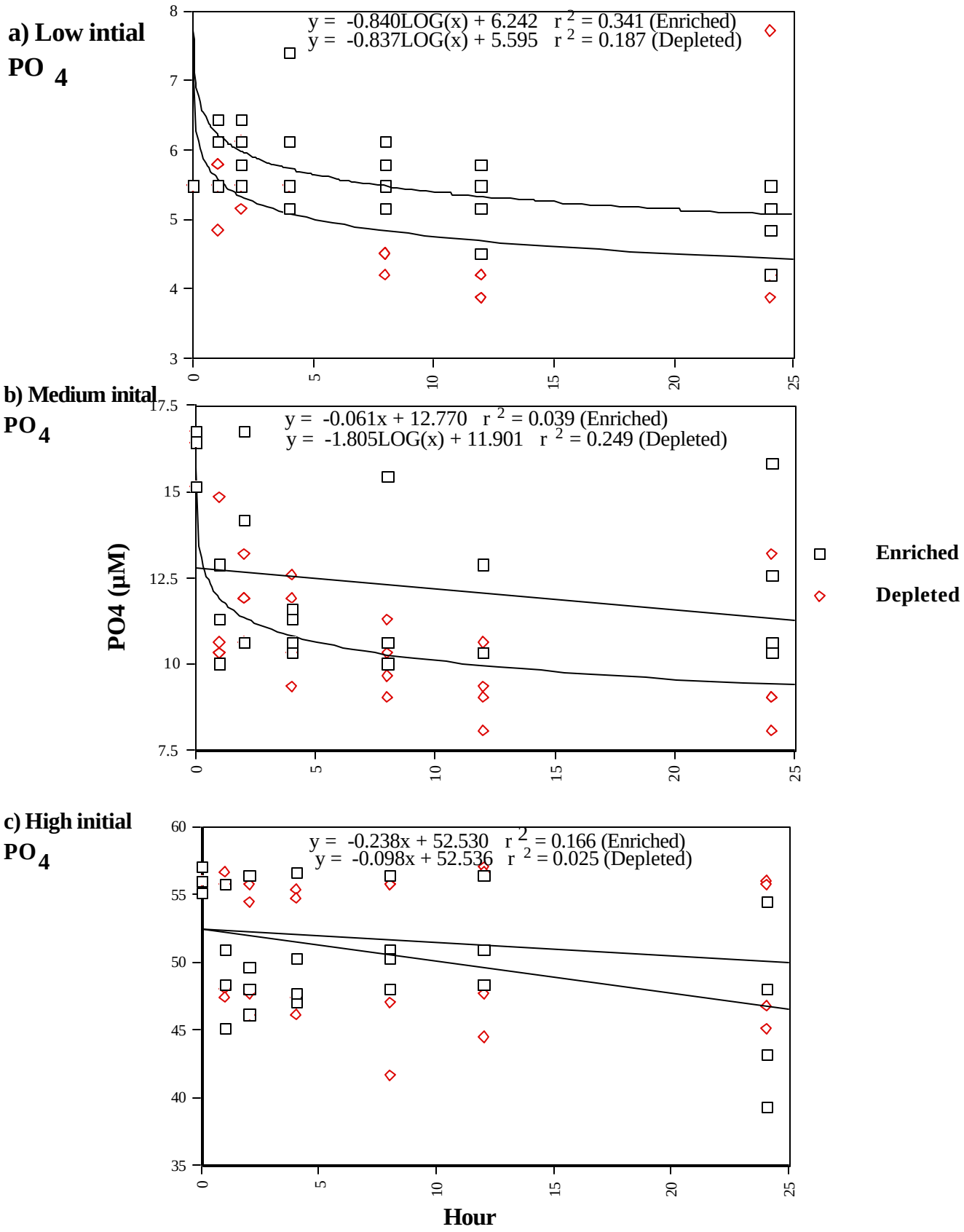

Figure 10. Decrease in water column PO_4 over time in the *Enteromorpha intestinalis* phosphorus uptake experiment for enriched and depleted algae for low (a), medium (b) and high (c) initial water column nutrient concentrations.

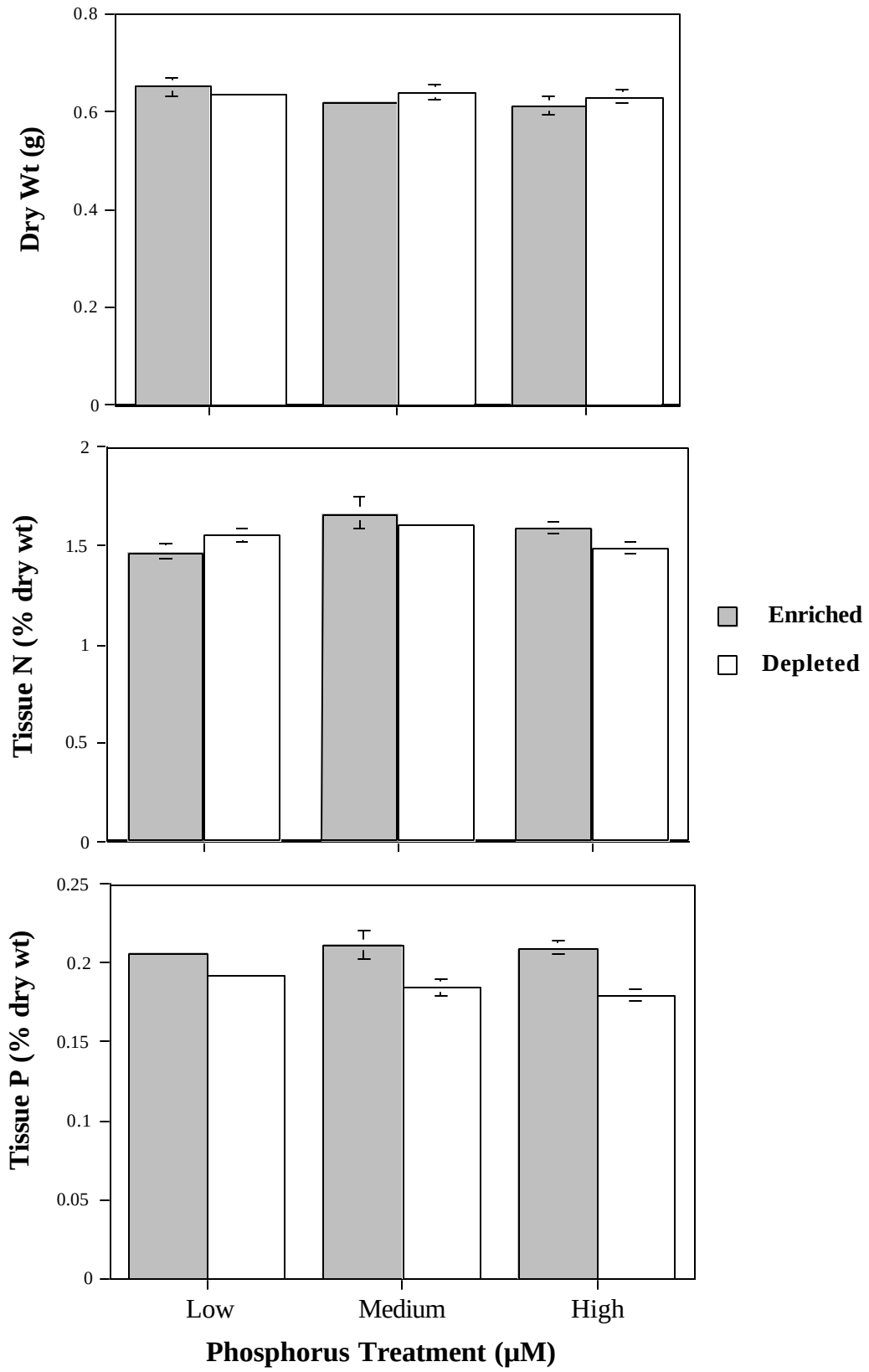

Figure 11. Final dry weight (a), tissue N concentration (b) and tissue P concentration (c) of enriched and depleted *Enteromorpha intestinalis* incubated with low, medium and high initial water column nutrient concentrations in the phosphorus uptake experiment.

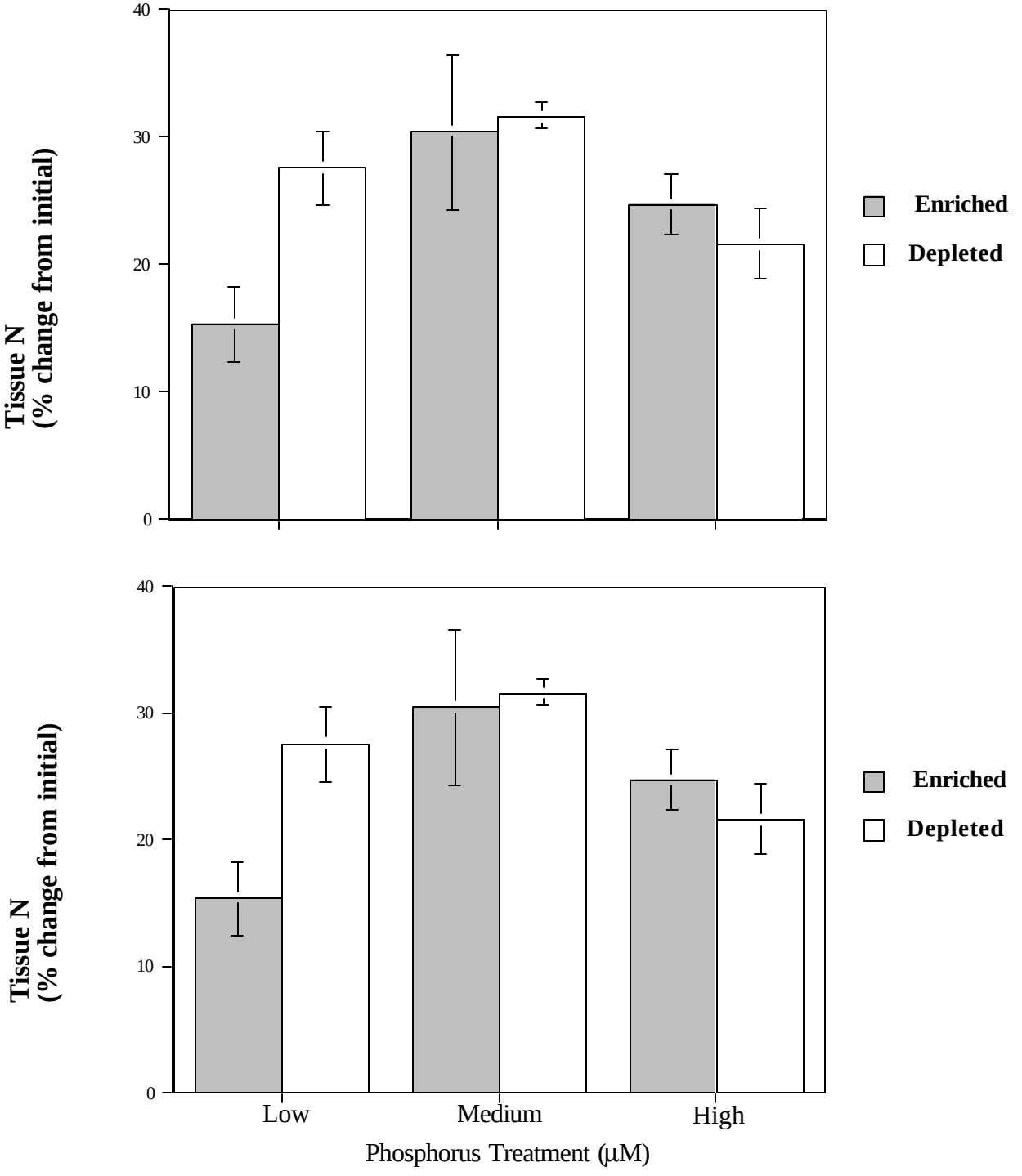

Figure 12. Percent change in *Enteromorpha intestinalis* tissue N (a) and P (b) from initial values in enriched and depleted algae incubated with low, medium and high initial water column nutrient concentrations in the phosphorus uptake experiment.

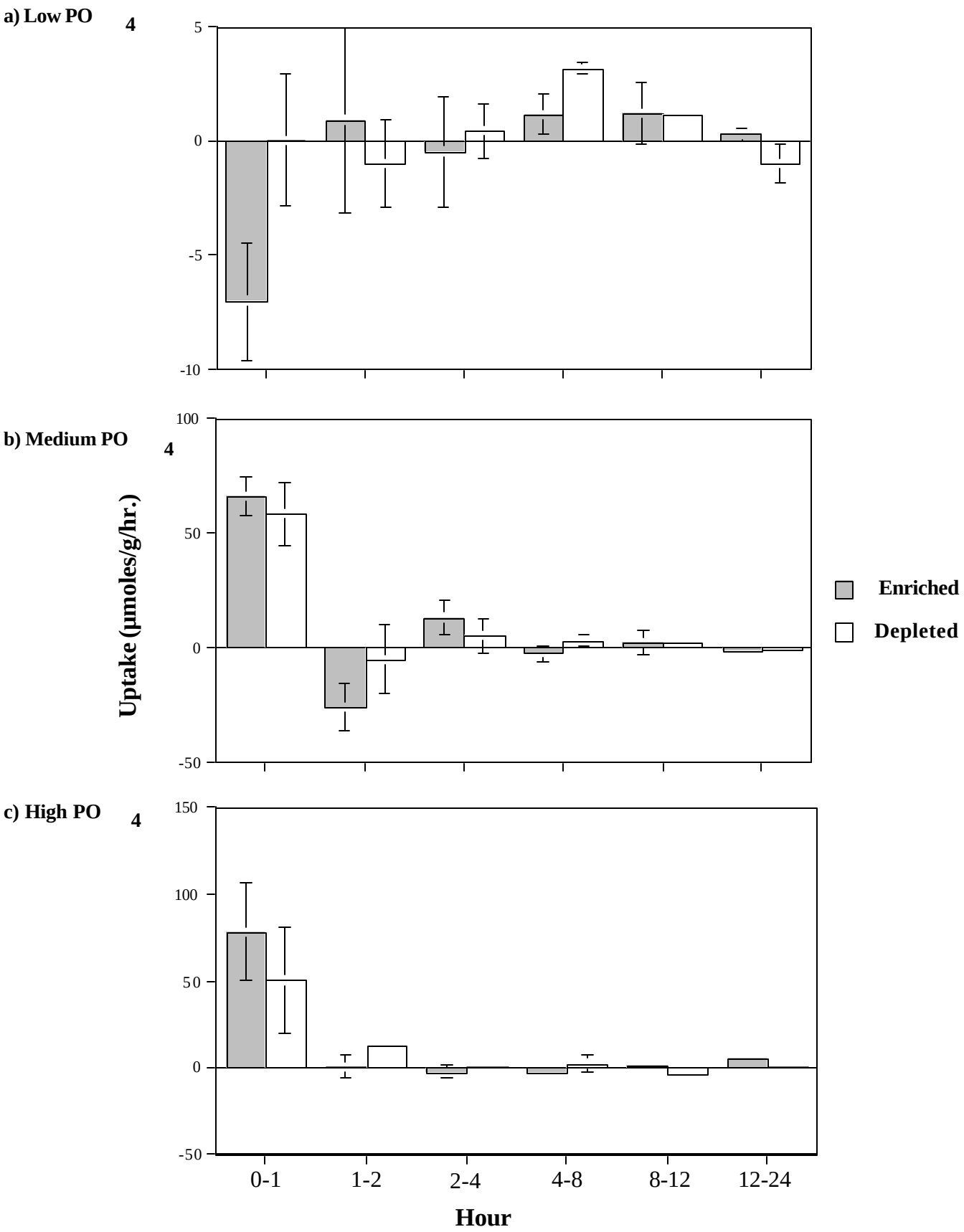

Figure 13. ***Enteromorpha intestinalis*** **PO_4 uptake rates for enriched and depleted algae in low (a), medium (b) and high (c) initial water column nutrient concentrations in the phosphorus uptake experiment.**

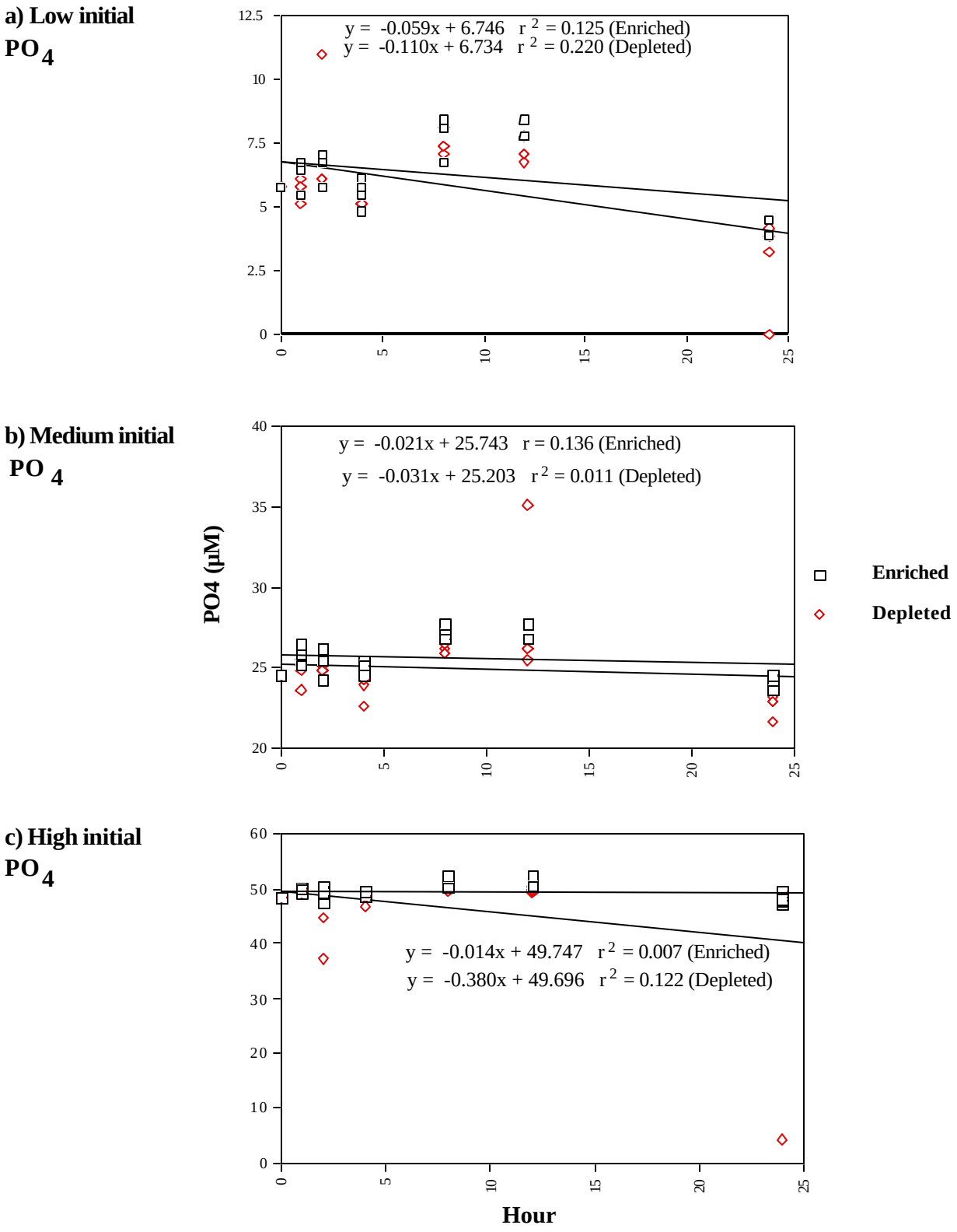

Figure 14. Decrease in water column PO_4 over time in the *Ulva expansa* phosphorus uptake experiment for enriched and depleted algae for low (a), medium (b) and high (c) initial water column nutrient concentrations.

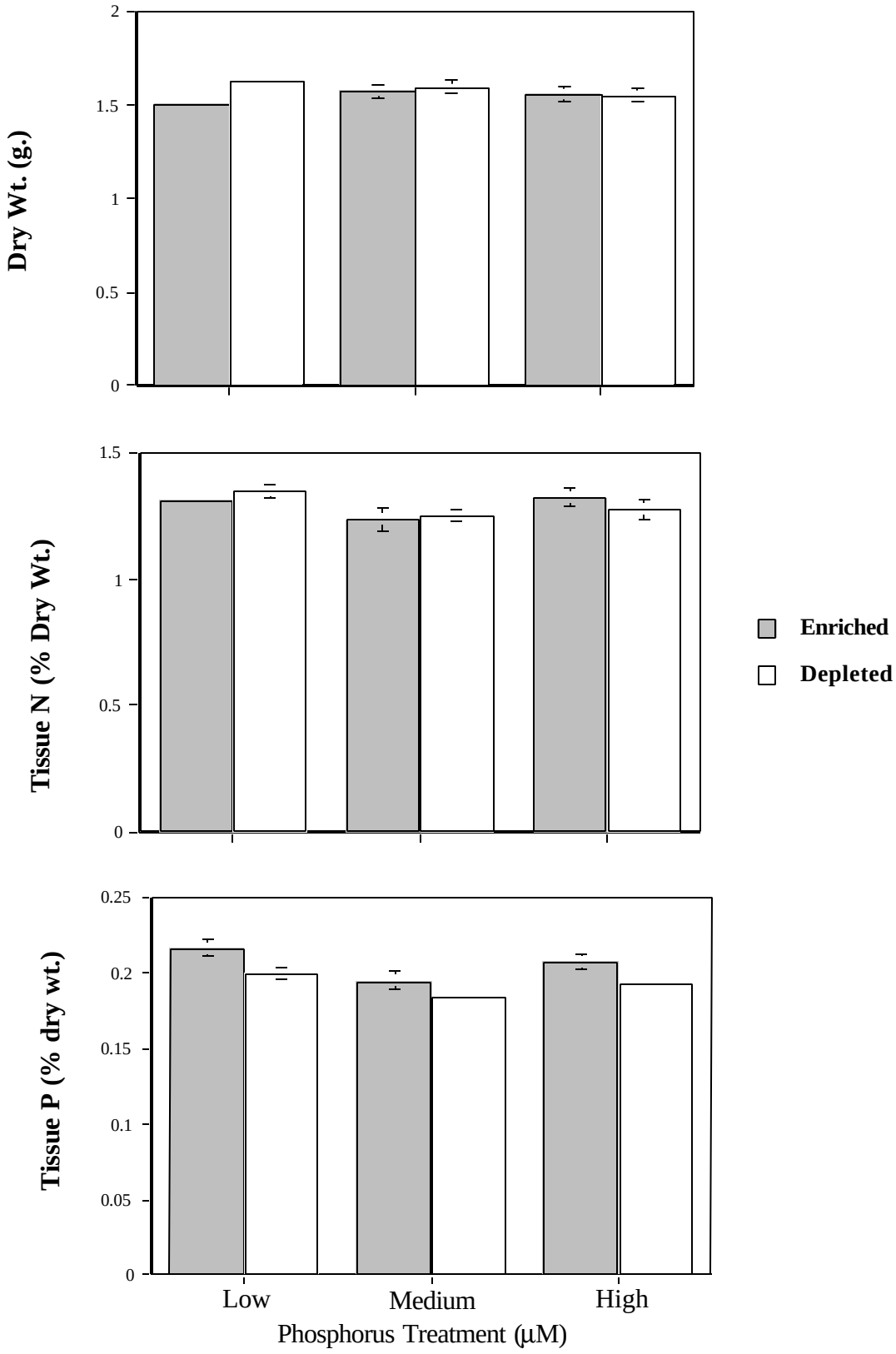

Figure 15. Final dry weight (a), tissue N concentration (b) and tissue P concentration (c) of enriched and depleted *Ulva expansa* incubated with low, medium and high initial water column nutrient concentrations in the phosphorus uptake experiment.

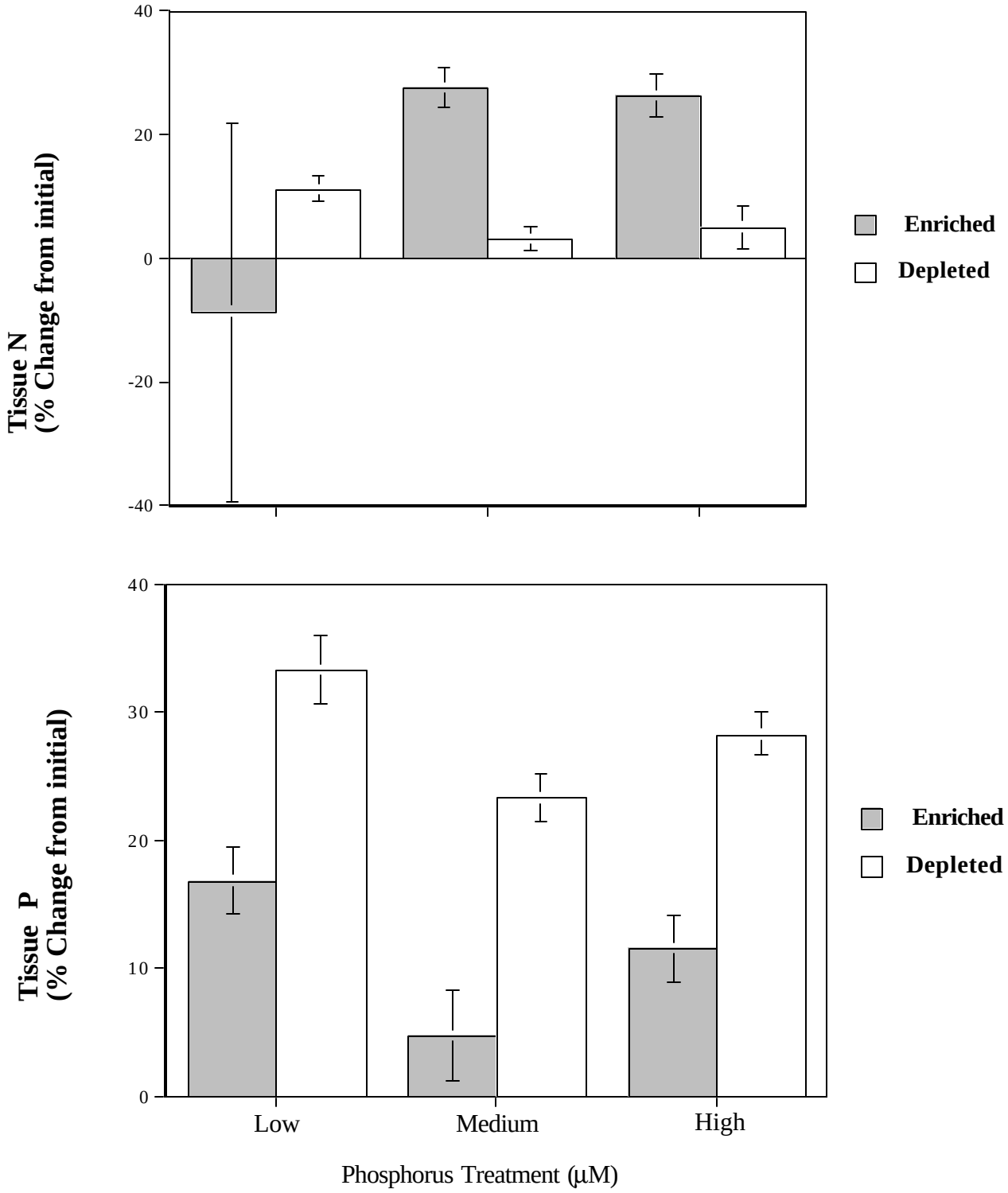

Figure 16. Percent change in *Ulva expansa* tissue N (a) and P (b) from initial values in enriched and depleted algae incubated with low, medium and high initial water column nutrient concentrations in the phosphorus uptake experiment.

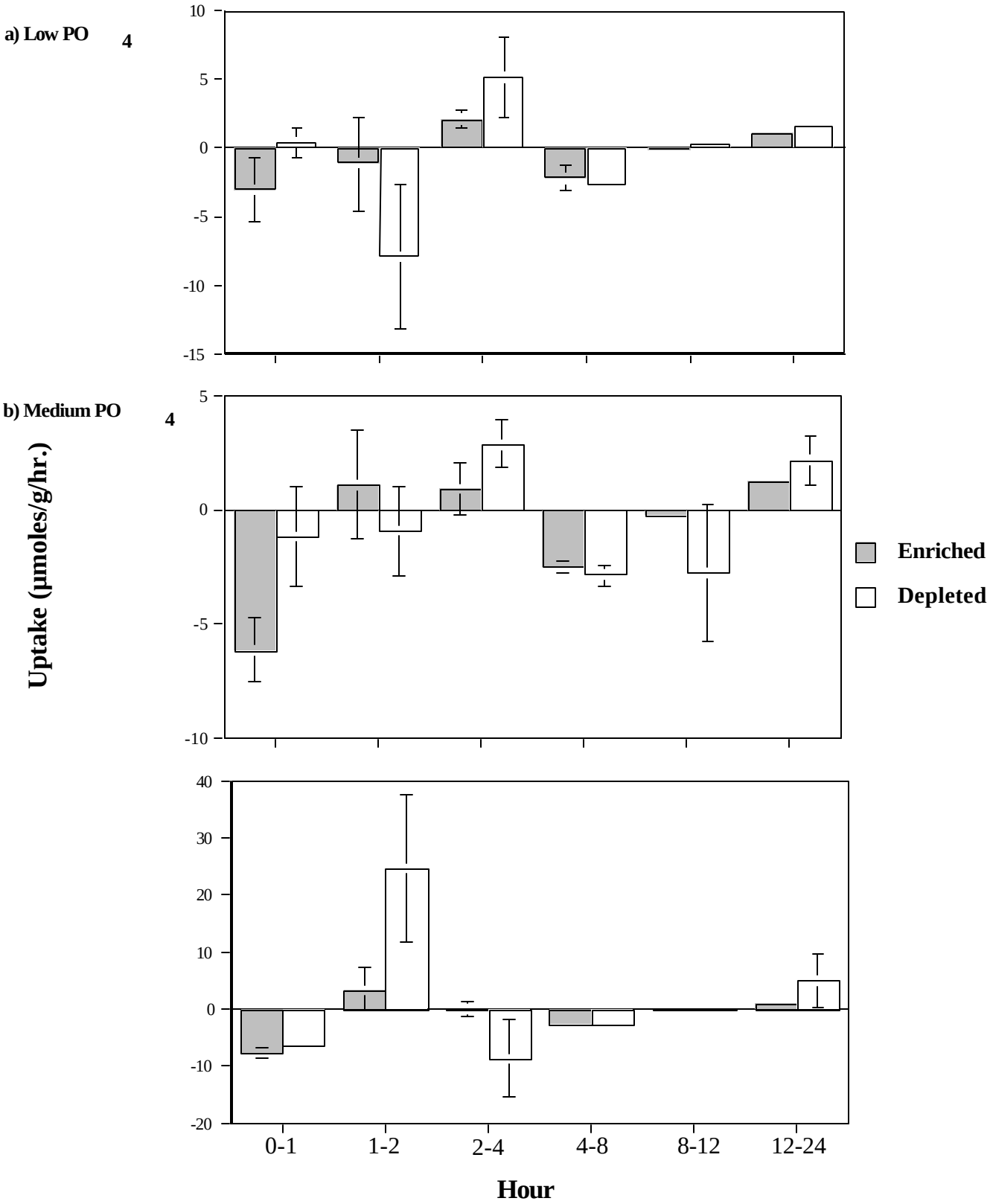

Figure 17. *Ulva expansa* PO_4 uptake rates for enriched and depleted algae in low (a), medium (b) and high (c) initial water column nutrient concentrations in the phosphorus uptake experiment.

APPENDIX D

Equal Loads of Nutrients Administered to Macroalgae Via Pulses of Differing Frequency and Concentration Affect Growth and Tissue Nutrients of *Enteromorpha intestinalis* and *Ulva expansa*

Krista Kamer*, Rachel L. Kennison, Kenneth Schiff and Peggy Fong

*Corresponding author

ABSTRACT

Nutrient inputs to estuaries vary over temporal scales from hours to months, and macroalgae can store nutrients and use reserves for growth when external nutrient supplies are low for periods of up to 2 months. We investigated the effect of frequency and concentration of nutrient (nitrogen (N) and phosphorus (P)) supply on algal growth and tissue nutrient dynamics of *Enteromorpha intestinalis* and *Ulva expansa*, two bloom-forming green macroalgae. Over a 28-day period, *E. intestinalis* and *U. expansa* were each given equal supplies of NO_3-N (28 mg) and PO4-P (6.2 mg) via pulses of different frequency and therefore different concentration. NO_3-N doses given to 10 g wet wt of algae in 1 L seawater were: 1 mg (daily), 7 mg (weekly), 14 mg (bi-weekly), or 28 mg (monthly). Phosphorus was also added in a 10:1 (molar) ratio. *E. intestinalis* and *U. expansa* responded to all nutrient doses used in this study. Growth increased most with daily doses of N, however positive growth was seen in both algae for all frequencies of N doses. Algae were able to store enough nutrients from the large, monthly pulse to continue growing in a low N environment for up to 28 d. Tissue nutrient content was also related to frequency and concentration of N doses. Total mass of N and P in algal tissue (mg $unit^{-1}$) increased as frequency of N doses increased. Overall, tissue N concentrations were greater in *U. expansa*, and tissue P concentrations were greater in *E. intestinalis*. *E. intestinalis* and *U. expansa* removed substantial portions of the nutrient doses. Twenty-nine % to 96% of added nutrients were removed from the water by algae within 24 h. The frequency of nutrient inputs to coastal systems may be critical in determining macroalgal biomass, and temporal scales should be taken into account when regulating nutrient loads in order to minimize macroalgal biomass.

INTRODUCTION

Large blooms of opportunistic green macroalgae such as *Enteromorpha* and *Ulva* spp. occur in estuaries throughout the world (Owens and Stewart 1983, Pregnall and Rudy 1985, Rudnicki 1986, Sfriso et al. 1987, Lavery and McComb 1991, Sfriso et al. 1992, Hernández et al. 1997, Farris and Oviatt 1999, Martins et al. 1999, Pihl et al. 1999, Raffaelli et al. 1999, Kamer et al. 2001, Tyler et al. 2001, Eyre and Ferguson 2002). While these algae are natural components of estuarine systems and play integral roles in estuarine processes (Pregnall and Rudy 1985, Kwak and Zedler 1997, Boyer and Fong in review), 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, Sfriso et al. 1992) leading to changes in species composition, shifts in community structure, and loss of ecosystem function (Raffaelli at al. 1991, Ahern et al. 1995, Thiel and Watling 1998).

Factors that influence macroalgal biomass include, but are not limited to, the availability of nutrients (Valiela et al. 1997, Schramm 1999) such as nitrogen (N) and phosphorus (P). Nutrients have been shown to stimulate macroalgal growth in laboratory and field experiments (Harlin and Thorne-Miller 1981, Lapointe 1987, Fong et al. 1993, Fong et al. 1996, Kamer and Fong 2001), and the occurrence of macroalgal blooms in natural systems is often related to nutrient enrichment (Sfriso et al. 1987, Sand-Jensen and Borum 1991, Valiela et al. 1992, Duarte 1995, Nixon 1995, Hernández et al. 1997, Paerl 1997, Valiela et al. 1997, Paerl 1999, Raffaelli et al. 1999).

Bloom-forming species of macroalgae, such as *Enteromorpha* and *Ulva* spp., typically have high nutrient uptake rates (Fujita 1985, Kennison et al. in prep.). Furthermore, these algae can store sufficient nutrients to maintain positive growth for up to 10 days (Fujita 1985), which likely enables them to succeed in environments with episodic inputs of nutrients. Macroalgae can use nutrient reserves for growth when external nutrient supply

is low (Hanisak 1983). *Gracilaria tikvahiae* grew at maximum rates in low nutrient seawater for up to 2 weeks using internal N pools (Ryther et al. 1981). Similarly, internal N reserves of *Fucus vesiculosis* sustained maximum growth for 12 d and reduced growth for another 34 d (Pedersen and Borum 1996). High growth rates of *Laminaria longicruris* were sustained by tissue N reserves up to 2 months when water column NO_3 was low (Chapman and Craigie 1977).

Nutrient supply to estuarine macroalgae can happen over various temporal scales and is typically not steady-state (Ramus and Venable 1987). Temporal variability in the supply of limiting resources, such as nutrients, may have impacts on macroalgae that differ depending on the degree of variability in the timing of nutrient supply and the nutrient storage capacity of the algae. The nutrient concentrations that macroalgae experience can vary on a daily scale due to tidal influences (Day et al. 1995) and light-associated processes such as nutrient uptake (Litaker et al. 1987). Precipitation events and runoff can cause variation in nutrient supply on the order of days (ca. 4 d; Litaker et al. 1987) to weeks (Day et al. 1995). Nutrient inputs to estuaries also vary over greater periods such as seasons (Boynton et al. 1980, Litaker et al. 1987, Sutula et al. in review), particularly in regions with Mediterranean climates (McComb et al. 1981, McComb et al. 1998) such as southern California (Boyle et al. in prep.).

A limited amount of investigation on the effect of temporal variability in nutrient supply to macroalgae has been conducted. Ramus and Venable (1987) added equal amounts of NH_4 to different experimental treatments over a period of 14 d but varied the frequency and magnitude of the doses. When NH_4 was added in small, frequent doses, growth was greater for *Ulva curvata* than when the same amount of NH_4 was added in larger, more infrequent doses. They determined that algal growth rate was proportional to frequency of NH_4 addition.

Upper Newport Bay (UNB) is a large southern California estuary that receives nutrient-laden runoff from its urbanized watershed (California Regional Water Quality Control Board 1997) and experiences blooms of *Enteromorpha intestinalis* and *Ulva expansa* (AHA 1997, Kamer et al. 2001). The growth of these algae is primarily limited by N throughout much of the Bay (Kamer et al. in prep.). Therefore, temporal variation in the supply of N to *E. intestinalis* and *U. expansa* may affect the biomass of these algae. Our objective was to determine how the frequency and concentration of N supplied to *E. intestinalis* and *U. expansa* affects growth and tissue nutrient dynamics.

MATERIALS AND METHODS

We determined how equal amounts of nutrients (28 mg N, 6 mg P over a 28-day period) supplied to *Enteromorpha intestinalis* and *Ulva expansa* at different frequencies and concentrations affected the growth and tissue nutrient dynamics of these algae. We employed a two factor experimental design. One factor was algal species; we used *E. intestinalis* and *U. expansa* separately, and we varied the second factor, the frequency with which algae received nutrients (N as NO_3, P as PO_4). Therefore, the concentration of N and P in the doses given at different frequencies varied as well. The frequency of nutrient doses was daily, weekly, bi-weekly, or monthly; these treatments were designed to mimic nutrient supply in estuaries subject to temporally variable nutrient inputs.

Enteromorpha intestinalis and *Ulva expansa* were collected from UNB 11 days prior to the beginning of the experiment. Algae were transported to the laboratory within 5 h where biomass of each species was separated and placed in individual shallow pans filled with aerated, low nutrient seawater (<0.05 mg/l NO_3-N, <0.05 mg/l PO_4-P). Pans were kept outdoors in a temperature controlled water bath (20 ± 2ºC) and covered with window screening to reduce incident light. Algae were kept under these conditions to reduce internal nutrient stores and

variability in initial tissue nutrient levels (Fong et al. 1994). Initial *E. intestinalis* N and P concentrations were 1.21 ± 0.01 % dry wt and 0.14 ± 0.00% dry wt, respectively. Initial *U. expansa* N and P concentrations were 1.91 ± 0.02 % dry wt and 0.21 ± 0.00 % dry wt, respectively.

Nutrients were added to seawater taken from the intake pipe of the Redondo Beach Power Plant with background nutrient levels of <0.05 mg/l NH_4-N, 0.35 ± 0.02 mg/l NO_3-N, and <0.05 mg/l PO_4-P to create solutions of ~1, 7, 14 and 28 mg/l NO_3-N (Table 1). These were the initial solutions for the daily, weekly, bi-weekly and monthly treatments, respectively. NO_3-N concentrations were chosen to represent levels of inorganic N that have been previously measured in UNB (Blodgett 1981, USEPA 1998, Boyle et al. in prep., Kamer et al. in prep.). PO_4 was added to solutions in a 10:1 atomic ratio to ensure that P would not become limiting over the course of the experiment. PO_4-P concentrations of the solutions were ~0.21 mg/l (daily), 1.5 mg/l (weekly), 3 mg/l (bi-weekly), and 6 mg/l (monthly) (Table 1).

Glass experimental units were each filled with 1 liter of the appropriate solution. *Enteromorpha intestinalis* and *Ulva expansa* were each placed in nylon mesh bags and each species was spun in a salad spinner for 1 minute to remove excess water. 10.0 ± 0.1 g sub-samples of either *E. intestinalis* or *U. expansa* were added to each unit. The 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 5-fold for a total of 40 units.

The experiment ran for 28 days. Each day, 1 mg of NO_3-N and 0.21 mg of PO_4-P were added to the daily treatments (Table 1, Figure 1). This was done by first stirring each unit and then removing 300 ml of solution with a syringe, taking care to not remove any algae. Three hundred ml of a 3.33 mg/l NO_3-N, 0.72 mg/l PO_4-P solution was added to each unit, thereby reconstituting the volume of the solution to 1 liter and adding 1 mg of N and 0.21 mg of P at the same time. Solutions used to add the daily doses of nutrients were made up periodically throughout the experiment and sampled for nutrient concentrations each time a new solution was used in the experiment to ensure consistent levels of nutrient addition throughout the experiment. Over the course of the experiment, NO_3-N ranged from 3.21 to 3.75 mg/l in individual samples of daily solutions and the mean was 3.51 ± 0.08 mg/l (n=9); PO_4-P ranged from 0.59 to 0.88 mg/l and the mean was 0.72 ± 0.03 mg/l (n=9).

To control for the effects of exchanging 300 ml of seawater to the daily treatments, seawater was exchanged on a daily basis to units in the weekly, bi-weekly and monthly treatments as well (Figure 1). Each unit was stirred, 300 ml of solution was removed from each unit as was done for the daily treatment units, and 300 ml of ambient seawater (<0.05 mg/l NH_4-N, 0.35 ± 0.02 mg/l NO_3-N, and <0.05 mg/l PO_4-P) was added to each unit to reconstitute the volume to 1 liter. At the beginning of the second, third, and fourth weeks of the experiment (days 8, 15, and 22), instead of ambient seawater, 300 ml of a 21 mg/l NO_3-N, 5 mg/l PO_4-P solution was added to each weekly unit, thereby administering the weekly 7 mg N and 1.5 mg P dose (Table 1, Figure 1). At the beginning of the third week (day 15, halfway through the experiment), instead of ambient seawater, 300 ml of a 44 mg/l NO_3-N, 10 mg/l PO_4-P solution was added to each bi-weekly unit, thereby administering the 14 mg N and 3 mg P dose (Table 1, Figure 1).

To monitor water column nutrient levels throughout the course of the experiment, a sample of the 300 ml removed from each unit was processed on specific days for nutrient analyses (Figure 1). Samples were taken on the second day of the experiment to estimate removal of nutrients from the water column by the algae over the 24 h following the first doses of nutrients. These samples were used to calculate the percent by which the initial concentrations of NO_3 and PO_4 were reduced by the algae during the first 24 h of the experiment. Based on 30% removal of available nutrients in each unit due to the exchange of 300 ml each day, and assuming that algae

continued to reduce nutrient concentrations by the same percentage calculated in the first 24 h of the experiment, we calculated the mass of NO_3-N and PO_4-P that we removed from units in each treatment until our calculated amounts dropped below the detection limits of 0.05 mg/l.

Samples of the 300 ml removed were also taken at the beginning of the second week (day 8) to measure the changes in water column nutrients over the first week. Similarly, samples were taken at the beginning of weeks 3 and 4 to measure changes from the previous week. Samples were also taken from all units during the second, third and fourth weeks 24 h after the weekly and bi-weekly doses were added in order to estimate removal of nutrients from the water column in the 24 h following these doses and also to monitor water column nutrient concentrations in the other units over the course of the experiment. A water sample was also taken from each unit on the last day of the experiment.

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 freshwater to remove external salts, dried in a forced air oven at 60ºC to a constant weight, and ground with mortar and pestle for subsequent tissue N and P analysis. N and P content of algae are reported as both concentration (% dry wt) and total mass per unit (mg unit^{-1}), which is calculated by multiplying the nutrient concentration of a sample (as a proportion) by the dry weight of that sample:

$$\text{mg N or P unit}^{-1} = [\%\ \text{tissue N or P}/100] * \text{dry wt (g)} * 1000\ \text{mg/g}$$

Laboratory analyses

Water column nutrients: NO_3 was reduced to NO_2 via cadmium reduction; NO_2 was measured spectrophotometrically after diazotation (Switala 1999, Wendt 1999). NH_4 was heated with solutions of salicylate and hypochlorite and determined spectrophotometrically (Switala 1999, Wendt 1999). 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). PO_4 was determined spectrophotometrically following reaction with ammonium molybdate and antimony potassium under acidic conditions (APHA 1998). These automated methods have detection limits of 0.05 mg/l for all forms of N and P.

Algal tissue nutrients: N was determined using an induction furnace and a thermal conductivity detector (Dumas 1981). 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 analysis

All data were tested for normality and homogeneity of variance. No transformations were necessary. Among treatment differences in final wet biomass, dry biomass, wet:dry wt ratios, algal tissue N and P concentration, algal tissue N and P total mass unit^{-1}, and the percentage of NO_3 and PO_4 removed from the water column in the first 24 h of the experiment were analyzed using 2-factor ANOVA (species x nutrient dose). Following a significant ANOVA, multiple comparisons were used to determine differences among individual treatments (Fisher's Least Significant Difference test (LSD)). No interactions occurred unless otherwise stated.

RESULTS

Final wet algal biomass differed significantly between species (p=0.001, ANOVA) and with nutrient dose (p=0.001, ANOVA). Overall, wet biomass of *Enteromorpha intestinalis* was greater at the end of the experiment than wet biomass of *Ulva expansa* (Figure 2a). For both species, biomass was greatest with daily nutrient doses. Among weekly, bi-weekly, and monthly treatments for *E. intestinalis*, wet biomass was not directly proportional to the frequency of nutrient doses; bi-weekly treatment biomass was greater than both weekly and monthly treatments (p=0.014 and 0.020 respectively, Fisher's LSD). Among the weekly, bi-weekly, and monthly treatments for *U. expansa*, there were no differences in biomass, likely causing a significant interaction between species and nutrient dose (p=0.017).

Final dry biomass was significantly affected by nutrient dose (p=0.001, ANOVA) but not by species (p=0.262, ANOVA), and there was an interaction between the terms (p=0.003). Similar to wet biomass, dry biomass was greatest for both species with daily nutrient doses (Figure 2b). Within *Enteromorpha intestinalis* however, patterns were different from those seen for wet biomass. Among weekly, bi-weekly and monthly treatments, weekly treatment biomass was similar to bi-weekly biomass (p=0.188, Fisher's LSD) and less than monthly biomass (p=0.045, Fisher's LSD). Bi-weekly and monthly biomass were similar (p=0.535, Fisher's LSD). Within *Ulva expansa*, weekly biomass was greater than bi-weekly and monthly biomass (p=0.002 and 0.018, respectively, Fisher's LSD), and bi-weekly and monthly biomass were similar (p=0.340, Fisher's LSD).

Wet:dry wt ratios were significantly affected by species (p=0.001, ANOVA) and nutrient dose (p=0.007, ANOVA) and there was an interaction between the terms (p=0.002). *Enteromorpha intestinalis* wet:dry wt ratios were higher than those of *Ulva expansa* (Table 2). Within *E. intestinalis*, the monthly treatment had the lowest wet:dry wt ratio, which, in part, explains the difference in the pattern between the final wet and dry biomass data. The differences in wet:dry wt ratios between nutrient dose treatments within each species are much less than the overall differences between species.

Tissue N concentration (% dry wt) was significantly affected by species and nutrient dose (p=0.001 for both factors, ANOVA). In general, tissue N concentrations were greater in *Ulva expansa* than *Enteromorpha intestinalis* (Figure 3a), likely reflecting initial differences in N concentration between species. For both species, tissue N concentrations were lowest in the monthly treatments. Within each species, tissue N concentrations of the daily, weekly and bi-weekly treatments were similar.

Algal tissue N content (mg unit^{-1}) was significantly affected by species and nutrient dose (p=0.001 for both factors, ANOVA). Overall, tissue N content was greater for *Ulva expansa* than for *Enteromorpha intestinalis*. The amount of N contained in *E. intestinalis* and *U. expansa* increased with increasing frequency of nutrient doses (Figure 3b). While tissue N concentrations of both species were similar in the daily, weekly and bi-weekly treatments (Figure 3a), differential growth between these treatments (Figure 2 a and b) accounts for the patterns seen in the total mass of N per experimental unit.

Tissue P concentration (% dry wt) was significantly affected by species (p=0.007, ANOVA) but not by nutrient dose (p=0.051, ANOVA). Overall, tissue P concentrations were greater in *Enteromorpha intestinalis* than *Ulva expansa* (Figure 4a).

Algal tissue P content (mg unit^{-1}) was significantly affected by nutrient dose (p=0.001, ANOVA) but not species (p=0.185, ANOVA). The amount of P contained in *Enteromorpha intestinalis* and *Ulva expansa* was greatest when nutrients were added daily (Figure 4b). *E. intestinalis* tissue P from the weekly, bi-weekly and monthly

treatments was similar. *U. expansa* tissue P from the weekly treatment was similar to the monthly treatment (p=0.232, Fisher's LSD) and greater than the bi-weekly treatment (p=0.027, Fisher's LSD). Tissue P in bi-weekly and monthly treatments was similar (p=0.280, Fisher's LSD).

Water column NO_3 concentrations decreased dramatically following nutrient doses. Water column measurements taken 24 h after the beginning of the experiment showed that the % of NO_3 removed by the algae varied significantly with species and nutrient dose (p=0.001 for both factors, ANOVA). Overall, *Ulva expansa* removed a greater percentage of NO_3 from the water column than *Enteromorpha intestinalis* (Figure 5a). For *E. intestinalis*, the percentage of NO_3 removed decreased as the concentration of the dose increased. NO_3-N dropped below the detection limit of 0.05 mg/l in the daily treatment and to 0.70 ± 0.11, 6.03 ± 0.34, and 19.66 ± 0.17 mg/l in the weekly, bi-weekly and monthly treatments, respectively. For *U. expansa*, the percentage of N removed was similar in the daily and weekly treatments (p=0.773, Fisher's LSD), which was different than the pattern seen for *E. intestinalis* and likely caused an interaction between species and nutrient dose (p=0.001, ANOVA). The percentage of NO_3 removed decreased from the daily to the bi-weekly (p=0.002, Fisher's LSD) and monthly treatments (p=0.001, Fisher's LSD) as the concentration of the dose increased. In the daily and weekly treatments, NO_3-N dropped below the detection limit of 0.05 mg/l, and in the bi-weekly and monthly treatments it dropped to 1.18 ± 0.30 and 15.42 ± 0.47 mg/l, respectively.

At the end of the first week, water column NO_3-N was <0.10 mg/l in all experimental units. A portion of the NO_3 in each treatment was removed by the exchange of 300 ml of solution each day (Table 3), and the rest was presumably removed by the algae. NO_3-N remained low in all units for the duration of the experiment; at the end of weeks 2, 3, and 4, NO_3-N was <0.11 mg/l in all units except for one, which measured 1.27 mg/l at the end of week 3. NH_4 concentrations in all units were always <0.17 mg/l NH_4-N.

Water column PO_4 concentrations also decreased following nutrient doses. The percent of PO_4 removed from the water column by the algae in the first 24 h of the experiment varied significantly with species and nutrient dose (p=0.001 for both factors, ANOVA). Overall, *Ulva expansa* removed a greater percentage of PO_4 from the water column than *Enteromorpha intestinalis* (Figure 5b). For *E. intestinalis*, the percentage of PO_4 removed was greatest in the daily treatment and lowest in the weekly treatment (p=0.001, Fisher's LSD). Percent PO_4 removed increased from the weekly to the bi-weekly (p=0.007, Fisher's LSD) and monthly treatments (p=0.001, Fisher's LSD), which were not different from each other (p=0.107, Fisher's LSD). PO_4-P dropped below the detection limit of 0.05 mg/l in the daily treatment and to 0.58 ± 0.06, 0.76 ± 0.06, and 0.94 ± 0.06 mg/l in the weekly, bi-weekly and monthly treatments, respectively. For *U. expansa*, the percentage of PO_4 removed was greatest in the daily and monthly treatments, which were similar (p=0.403, Fisher's LSD), and lowest in the weekly and bi-weekly treatments, which were also similar (p=0.140, Fisher's LSD). PO4-P dropped below the detection limit of 0.05 mg/l in the daily treatment, and in the weekly, bi-weekly and monthly treatments it dropped to 0.33 ± 0.02, 0.44 ± 0.11, and 0.23 ± 0.02 mg/l, respectively.

At the end of the first week, water column PO_4-P was <0.25 mg/l in all experimental units except one, which measured 2.05 mg/l. Though a portion of the PO_4 in each treatment was removed by the exchange of 300 ml of solution each day (Table 3), we calculated that the algae removed a substantial portion of the PO_4 load in the first week.

Throughout the rest of the experiment, PO_4-P was variable, ranging from <0.05 to 0.53 mg/l in all units.

DISCUSSION

Enteromorpha intestinalis and *Ulva expansa* responded to all frequencies of nutrient addition used in this study. Algae grew best with frequent, low concentration inputs of nutrients compared to more episodic, high concentration inputs. However, algae were able to store nutrients from the high concentration doses and use those reserves to support growth over periods of days to weeks when nutrient supply was low.

Ramus and Venable (1987) also investigated the response of macroalgae to pulsed doses of NH_4 that varied in frequency and therefore concentration, similar to our experimental design. They found that while growth of *Ulva curvata* increased with increased frequency of nutrient addition, *U. curvata* was able to maintain growth (0.12 ± 0.01 g g^{-1} d^{-1}) up to 14 d following a single NH_4 pulse. Therefore, while pulsed inputs of nutrients to estuarine systems may appear to be transient in the water column (Fong et al. 1998), the storage of pulsed nutrients in macroalgal tissue means that the effects of nutrient pulses may be longer lasting, on the order of days to weeks.

The presence of sediments in natural systems compared to our sediment-free experimental system may prolong the impacts of large pulses of nutrients on macroalgae. Nutrients may flux into the sediments during periods of high water column concentration and flux back out when water column concentrations decrease (Boynton et al. 1980, Rizzo and Christian 1996). Macroalgae can take up nutrients fluxing from the sediments (Thybo-Christesen et al. 1993, Bierzychudek et al. 1993) and utilize them for growth (Lavery and McComb 1991, Kamer et al. in prep.). Through these processes, large pulses of nutrients in natural systems may stimulate macroalgal growth for periods longer than those that we investigated in this study.

Algae were better able to assimilate nutrients when they were added on a daily basis compared to more episodic pulses. Though all treatments in this experiment were given the same total loads of N and P over the course of the experiment, the greater amounts of N and P in algal tissue from the daily treatments compared to the monthly treatments indicate that the algae were able to retain proportionally more of the pulsed nutrient additions when they were administered more frequently. This is supported by the differential removal of NO_3 from the water column in the first 24 h of the experiment; the smaller the dose, the greater the percentage of it that was taken up by the algae.

Opportunistic macroalgae often experience "surge" or enhanced nutrient uptake in response to sudden increases in nutrient availability (Rosenberg and Ramus 1984). These algae can temporarily increase their nutrient uptake rates during pulses of nutrients in order to compensate for reduced uptake during periods of low N availability (Fujita 1985, Pedersen and Borum 1997). This may have helped the algae remove significant amounts of N and P from the high concentration doses in a very short time. Surge uptake may be an evolutionary response to temporally dynamic environments; when nutrient inputs are episodic, increased nutrient uptake rates may allow an alga to persist longer in periods of low nutrient supply by utilizing internal stores of nutrients, as we saw *Enteromorpha intestinalis* and *Ulva expansa* do in this study.

Overall, *Ulva expansa* had a greater affinity for N and P than did *Enteromorpha intestinalis*. After keeping both species in low-nutrient seawater for 11 d prior to the beginning of the experiment, the tissue N and P content of *E. intestinalis* was less than that of *U. expansa*. In the first 24 h of the experiment, *U. expansa* removed greater percentages of the nutrient doses than *E. intestinalis* did. Furthermore, at the end of the experiment, *U. expansa* had a greater concentration of tissue N and a greater amount of N in its tissue than *E. intestinalis*, even though dry weights were similar.

In a natural system, macroalgae such as *Enteromorpha intestinalis* and *Ulva expansa* may be able to assimilate the majority of low-grade, chronic nutrient inputs. This study also demonstrates that significant portions of large nutrient pulses may also be assimilated by macroalgae, stored, and used for growth during periods of low nutrient supply. Furthermore, sediments in natural systems may serve as a secondary storage mechanism and release nutrients when water column supplies are low, thereby prolonging the effects of large pulses. Due to storage in algal tissue and estuarine sediments, effects of large nutrient pulses may be as important and persistent in natural systems as chronic, low grade nutrient inputs.

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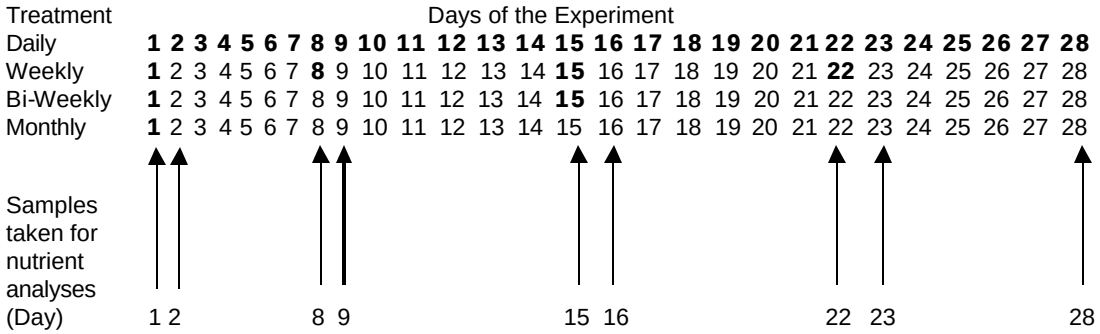

Figure 1. Days of the experiment on which 300 ml of solution in experimental treatments was exchanged, with the exception of day 1. Days in boldface represent days on which nutrient doses listed in Table 1 were added to individual units in each treatment. Days in plaintype represent days on which 300 ml ambient seawater were added to unit in each treatment. Arrows indicate days on which the 300 ml removed from each unit was processed for nutrient analysis.

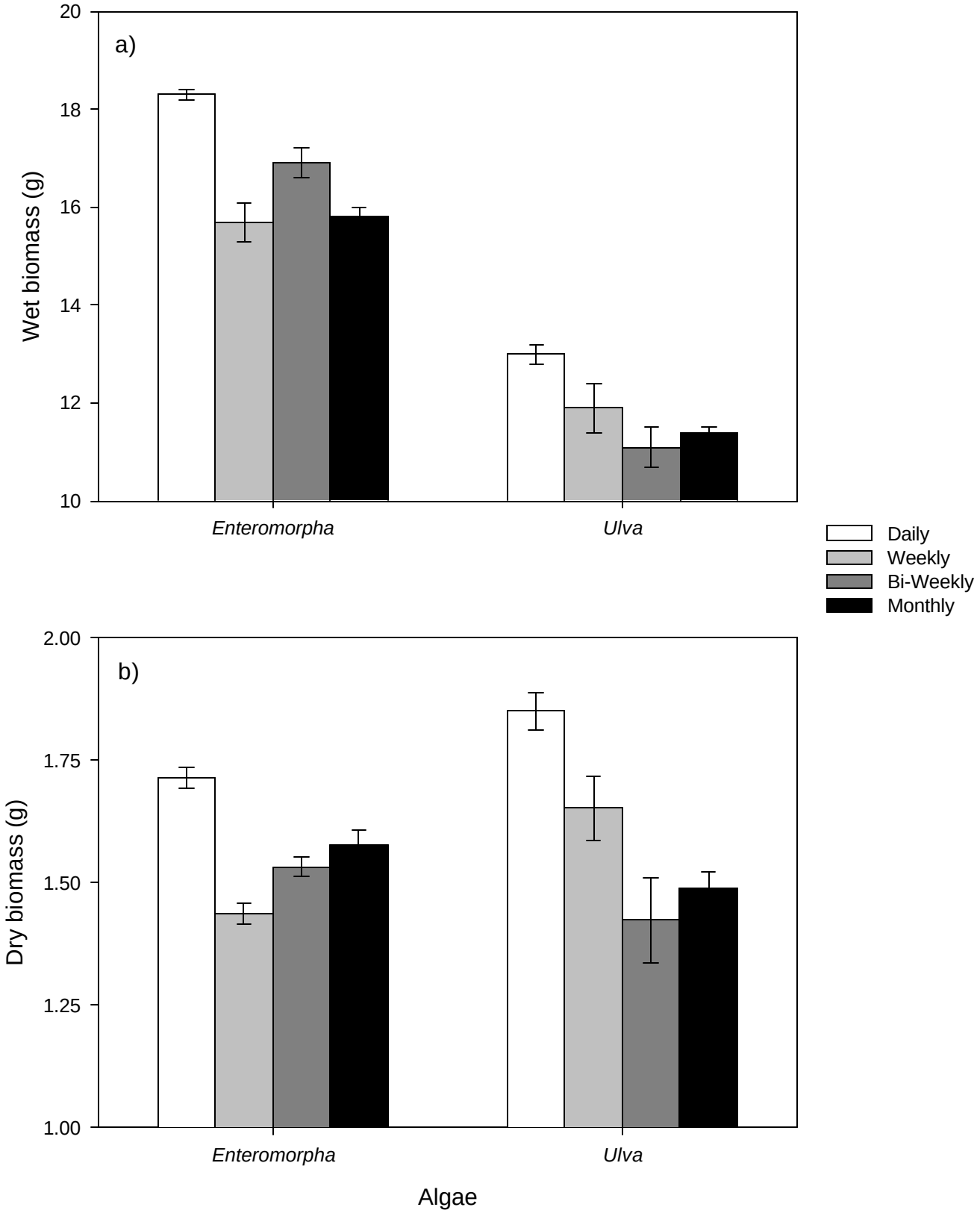

Figure 2. Final average wet biomass (a) and dry biomass (b) of *Enteromorpha intestinalis* and *Ulva expansa* grown for 28 d with daily, weekly, bi-weekly and monthly nutrient additions (bars are ± 1 SE).

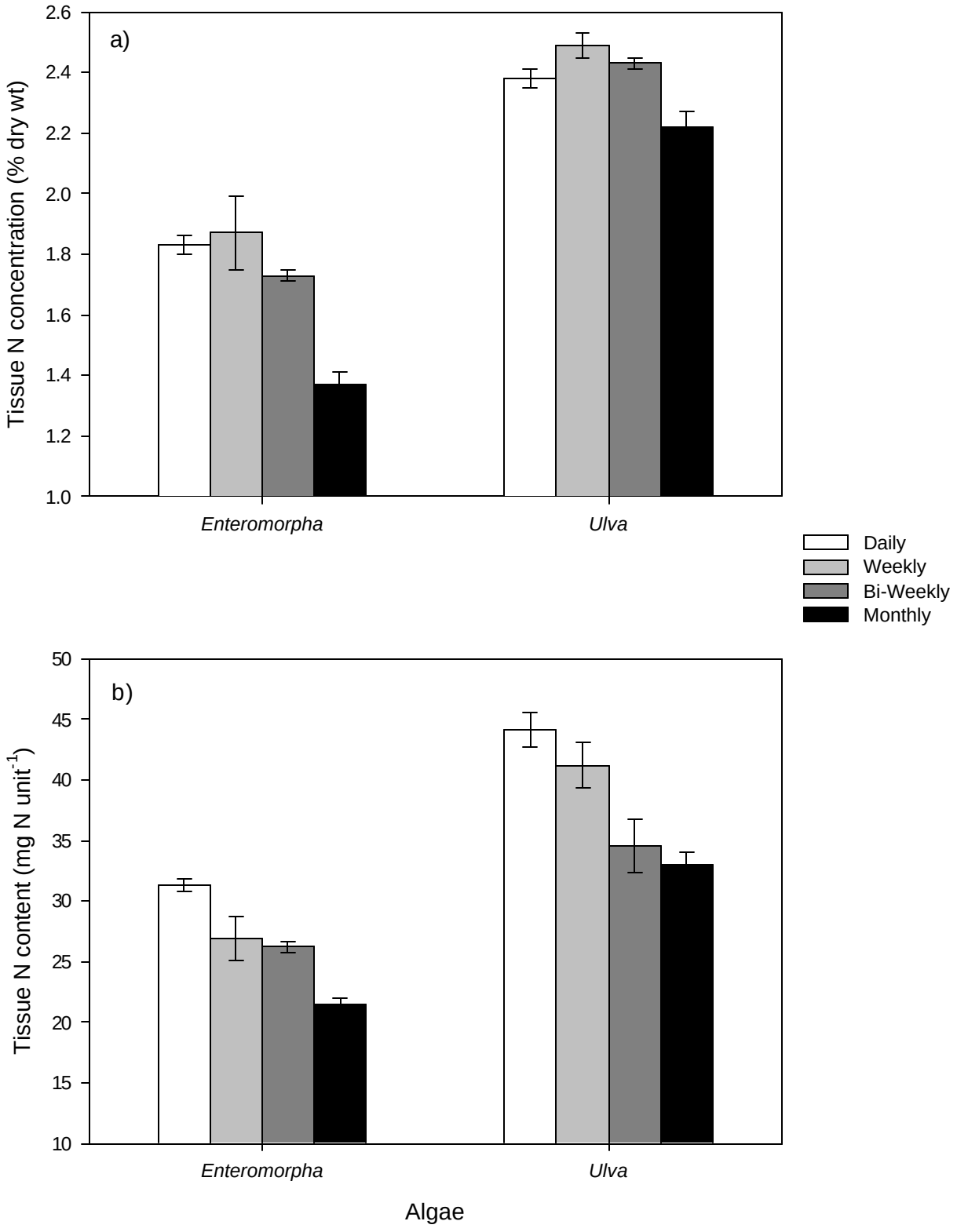

Figure 3. Final average tissue N concentration (as % dry wt) (a) and content (mg N unit^{-1}) of *Enteromorpha intestinalis* and *Ulva expansa* grown for 28 d with daily, weekly, bi-weekly and monthly nutrient additions (bars are ± 1 SE).

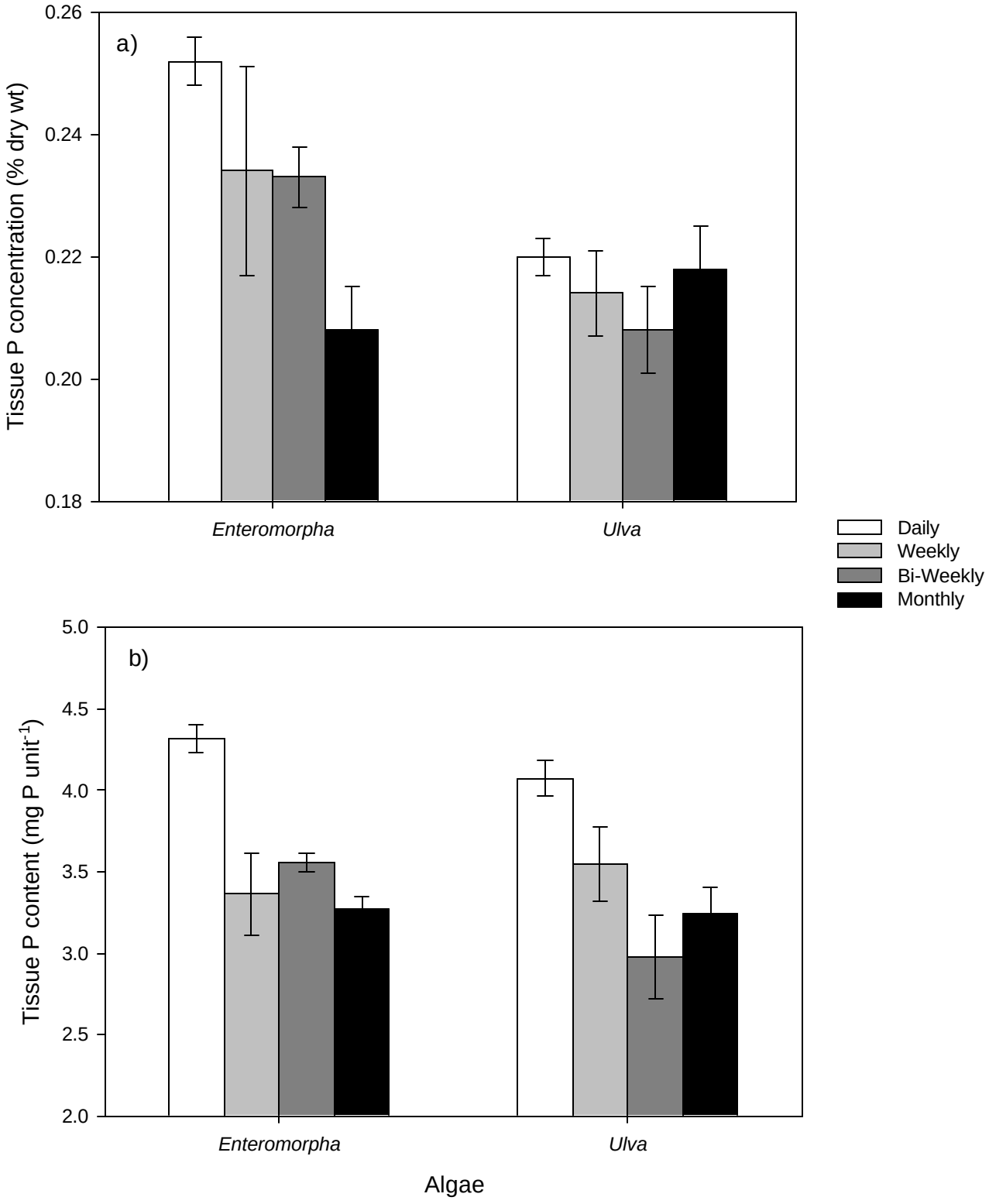

Figure 4. Final average tissue P concentration (as % dry wt) (a) and content (mg P unit^{-1}) of *Enteromorpha intestinalis* and *Ulva expansa* grown for 28 d with daily, weekly, bi-weekly and monthly nutrient additions (bars are ± 1 SE).

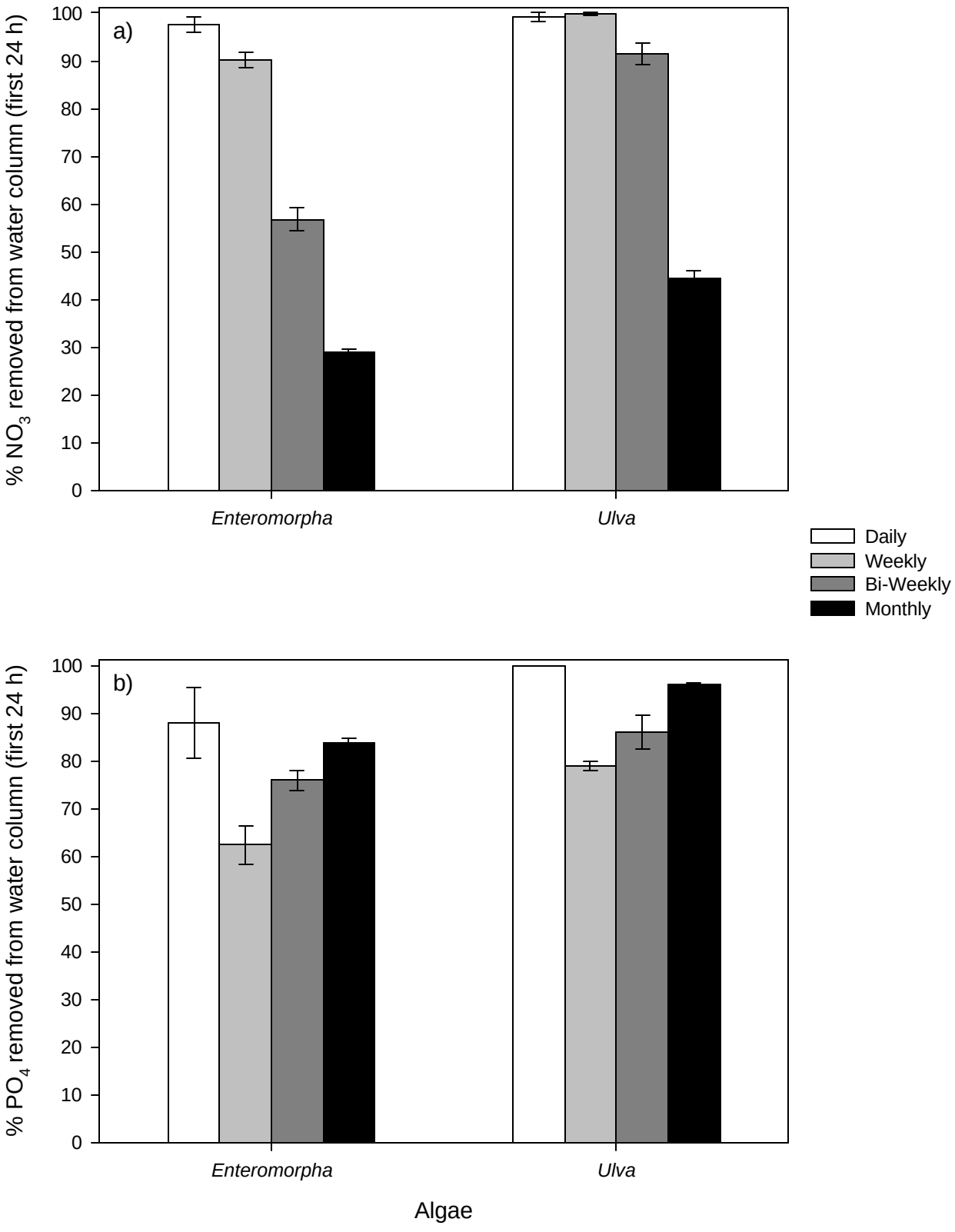

Figure 5. Percent of initial concentration of NO_3-N (a) and PO_4-P (b) removed by *Enteromorpha intestinalis* and *Ulva expansa* in the first 24 h of the experiment.