The relative importance of sediment and water column supplies of nutrients to the green macroalga *Enteromorpha intestinalis* across an estuarine resource gradient

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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 nutrients from the water column, but estuarine sediments may also be an important nutrient source. 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 three sites in Upper Newport Bay estuary, California, that varied greatly in water column nutrient concentrations. For each site, there were three treatments: sediments + water, sediments + water + *Enteromorpha intestinalis* (algae), and inert sand + water + algae. Water in units was exchanged weekly, simulating the low turnover characteristic of poorly flushed estuaries. 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 dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) levels were low, estuarine sediments increased *E. intestinalis* growth and tissue nutrient content. 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. However, salinity was low in these units and may have inhibited algal growth. Water column DIN was depleted each week of 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. Depletion of water column DIN in sediment + water units indicated that the sediments acted as a nutrient sink in the absence of algae in this experiment. Previous studies have demonstrated the potential importance of sediments as a source of nutrients to primary producers; our data provide direct experimental evidence that macroalgae utilize and ecologically benefit from nutrients stored in estuarine sediments.

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INTRODUCTION

Large blooms of opportunistic green macroalgae such as *Enteromorpha* and *Ulva* spp. occur in estuaries throughout the world (e.g., Sfriso *et al.* 1987, 1992; Schramm and Nienhuis 1996; Raffaelli *et al.* 1999), often in response to increased nutrient loads from developed watersheds (Valiela *et al.* 1992, Nixon 1995, 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 2002), 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 and 1992), leading to changes in species composition, shifts in community structure (Raffaelli *et al.* 1991, Ahern *et al.* 1995, Thiel and Watling 1998), and loss of ecosystem function.

Biomass of *Enteromorpha* and *Ulva* spp. is often regulated by nutrient availability (Sfriso *et al.* 1987, Hernández *et al.* 1997, Schramm 1999). These algae efficiently remove nitrogen (N) from the water column (Fujita 1985; O'Brien and Wheeler 1987; Duke *et al.* 1989a, 1989b). 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). 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. The release of nitrogen and phosphorus (P) from sediments is well established (e.g., Boynton *et al.* 1980, Nixon 1981, Clavero *et al.* 2000, Grenz *et al.* 2000). Flux of nutrients from sediments is believed to increase the availability of water column nutrients to primary producers; a number of studies have constructed nutrient budgets in which N and P fluxing from sediments may potentially meet a portion of the nutrient requirement of the system's primary producers (Boynton *et al.* 1980, Blackburn and Henriksen 1983, Trimmer *et al.* 1998, 2000). However, these studies infer the primary producers' use of nutrients fluxing from sediments rather than providing direct evidence.

Several field studies provide correlative evidence that macroalgae take up nutrients fluxing from estuarine sediments. 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. Thybo-Christesen *et al.* (1993) showed decreases in water column N and P from the sediment surface toward floating algal mats, indicating uptake by the macroalgae of nutrients fluxing from the sediments. These studies indirectly support the hypothesis that macroalgae utilize nutrients fluxing from sediments, but they do not provide direct experimental evidence.

Several laboratory studies have also demonstrated the ability of algal mats to intercept nutrients fluxing from sediments. However, McGlathery *et al.* (1997) simulated benthic nutrient flux by using nutrient-enriched seawater in the bottom compartment of an incubation chamber separated from the overlying water by a 0.7 µm filter paper to simulate sediments rather than using real estuarine sediments. Tyler *et al.* (2001) measured uptake by *Ulva lactuca* of urea released from estuarine sediments, but uptake was only measured over a 12 h period and longer term ecological effects such as tissue nutrient status and growth were not assessed. These studies confirm macroalgal uptake of nutrients fluxing from artificial and estuarine sediments, yet there is still a need to study the effect of sediment nutrients on the long-term ecology of macroalgae.

Lavery and McComb (1991) found that estuarine sediments increased the growth of *Chaetomorpha linum* over a three-week period. This is the only study to date that documents a long-term effect of estuarine sediments on macroalgal biomass. However, sediments from different depths were homogenized to reconstruct sediment profiles. Therefore, ecological effects of nutrients fluxing from undisturbed estuarine sediments to macroalgae have not been determined. Furthermore, neither differential effects of nutrient content of sediments on macroalgal growth nor effects of sediment-derived nutrients on macroalgal nutrient dynamics have been investigated.

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). The role of estuarine sediments as a source of nutrients to macroalgae may be particularly important in systems where nutrient inputs are episodic and availability of nutrients in the water column fluctuates over short time scales (Litaker *et al.* 1987, Day *et al.* 1995), or where strong spatial gradients in water column nutrient availability exist (Rizzo and Christian 1996, Nedwell *et al.* 2002). Furthermore, the potential contribution of nutrients from sediments to macroalgae may be greater in poorly flushed estuaries, where water circulation is greatly reduced as the result of physical modifications made to the system (Fong and Zedler 2000). The installation of culverts and tidal gates often restrict flow, creating areas that only experience circulation when tidal amplitude reaches a threshold (A. Armitage, personal communication) or preventing drainage of some areas (Fong and Zedler 2000). In such cases, water column nutrients in stagnant, ponded areas may be depleted by algal uptake much faster than in areas with increased circulation where water column nutrients are continuously supplied. Further work is needed to understand the ecological significance of sediment nutrient efflux to macroalgae across a range of water column nutrient concentrations.

The objective of this study was to determine the relative importance of the water column versus the sediments as sources of 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 or in poorly flushed, ponded areas. Our experimental study modeled Upper Newport Bay (UNB), a large southern California estuary subject to blooms of

Enteromorpha intestinalis and *Ulva expansa* (Kamer *et al.* 2001). In UNB, water column nutrient concentrations are 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 2002), while sediment nutrient concentrations vary throughout the estuary (0.034-0.166% dry wt TKN, 0.044-0.072% dry wt P) with no clear spatial pattern (Boyle 2002). The UNB also exemplifies estuaries with altered hydrology due to physical modifications. The highly developed and modified Lower Bay (seaward of Pacific Coast Highway, Figure 1) separates the natural portion of the estuary (UNB) from the Pacific Ocean, and the permanently established mouth alters the hydrodynamics from those that would occur with a naturally migrating mouth. Although the Upper Bay is largely natural, several major berms (just above Middle site, Figure 1) reduce circulation and create ponded areas.

METHODS

To test the relative importance of the water column versus sediments as a source of nutrients to macroalgae, sediment cores and water were collected from three sites in UNB across a water column nutrient gradient (Figure 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), and inert sand + water + algae. The sediment + water cores served to eliminate algae as a nutrient source/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 site nearest the head of the estuary (Head, Figure 1) was at the mouth of San Diego Creek, a large freshwater and nutrient source to UNB. This site had the lowest salinity; the greatest water column DIN, mostly in the form of NO_3; the lowest Total Kjeldhal nitrogen (TKN) and soluble reactive phosphorus (SRP); and was intermediate in terms of sediment P, sand, and silt content (Table 1). The second site (Middle) was mid-way between the head and the seaward end of the estuary and had intermediate salinity and water column DIN, the highest initial sediment P, the least sand, and the highest silt content (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 situated at the lower boundary of the estuarine system (Kamer *et al.* 2001). This site had the highest salinity and the lowest water column DIN; its TKN and SRP concentrations were similar to the Middle site; and it had the sandiest sediments, the least silt content, and the lowest sediment P (Table 1). All three sites had similar clay content, and there was a non-significant trend toward greatest sediment N at the Middle site (Table 1).

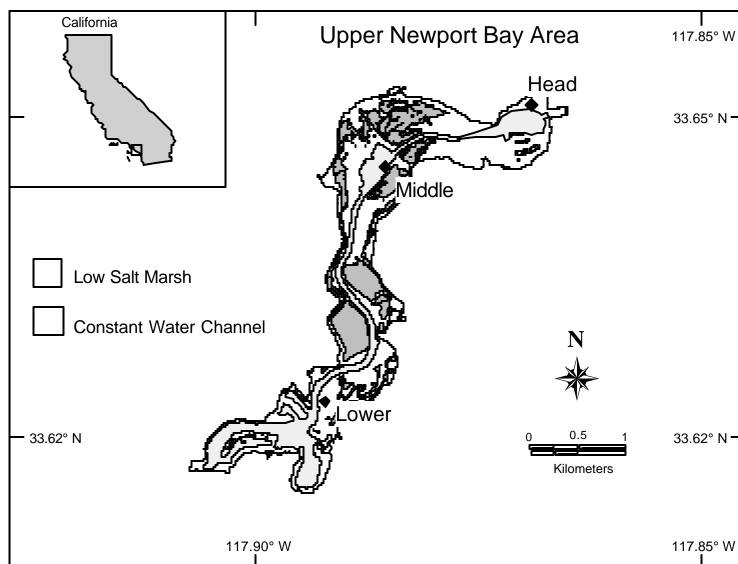

Figure 1. Map of Upper Newport Bay estuary, California, with three sites (Lower, Middle, and Head sites) from which water and sediments were collected to construct experimental units.

At each site, 10 individual sediment cores were taken 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. The edge of the vegetation was used as an elevational guide to ensure sampling of similar elevation among sites. The bottoms of the cores were capped and sealed in the field, and the tops were left open. Care was taken not to disturb the vertical stratification of the cores.

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

Table 1. Salinity, mean initial water column nutrients, mean initial sediment nitrogen and phosphorus concentrations, and grain size distribution from three sites in Upper Newport Bay and inert sand used in sand + water + algae treatments. Mean values that are significantly different from each other ($p<0.05$, Fisher's LSD following significant 1-factor ANOVA) are indicated with superscripts. For water column nutrient data, n=9; mean values were calculated from triplicate samples from Weeks 1, 2, and 3. For sediment data, n=5. Inert sand was not included in nutrient content statistical analyses as N and P were below detection limits or grain size statistical analyses as data were invariant. Values given are means (SE) except for salinity (n=1).

	Water Column Nutrients (uM)					Sediment Nutrients (percent dry weight)			Percent by Sediment Type		
Site (Salinity)	NO_3	NH_4	DIN	TKN	SRP	Total N	Total P	N:P (Molar)	Sand	Silt	Clay
Head (8 psu)	414 (8.0)[a]	11.4 (2.3)[a]	422 (5.8)[a]	33.3 (6.6)[a]	<1.61	0.066 (0.004)[a]	0.050 (0.000)[a]	2.92	61 (3)[a,b]	25 (2)[a]	14 (1)[a]
Middle (25 psu)	101 (3.4)[b]	21.0 (0.5)[b]	122 (3.4)[b]	61.9 (4.3)[b]	5.95 (2.37)[a]	0.072 (0.002)[a]	0.058 (0.002)[b]	2.76	53 (3)[b]	32 (2)[b]	15 (1)[a]
Lower (30 psu)	49 (1.8)[c]	23.2 (3.7)[b]	72 (4.5)[c]	55.6 (6.9)[b]	3.41 (0.19)[a]	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)

each polycarbonate tube containing an estuarine sediment core to construct the sediment + water portion of the experimental units. Enough water was added so that 300 mL overlaid 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. To complete the experimental units, *Enteromorpha intestinalis* was added (collected from a single site 10 d prior to the initiation of the experiment) to 5 tubes from each site containing estuarine sediments. *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. Initial tissue N was $1.19 \pm 0.02\%$ dry wt (n=5, mean ± SE) and initial tissue P was $0.11 \pm 0.00\%$ dry wt (n=5).

To separate the contribution of nutrients to macroalgae from the estuarine sediment and the contribution from the water column, five inert sand + water + algae experimental units were constructed per site. These were identical to the sediment + water + algae units with the exception of an 8 cm deep layer of sand in the bottom of each instead of estuarine sediment. The sand 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. Initial N of the sand was below the detection limit of 0.05% dry wt, and P was below the detection limit of 0.01% dry wt (Table 2). The sand simulated the physical presence of the estuarine sediments, yet had no measurable nutrients to contribute to the macroalgae. This treatment allowed us to compare the response of the algae with sediments and water to the response of algae with nutrient-free sand and water, thereby determining the effects of the sediments on algae.

Experimental units were placed outdoors in a temperature-controlled water bath (20 ± 2°C) 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 five-fold replication for a total of 45 experimental 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, the water in each experimental unit was sampled for nutrient analysis. Algae were removed from the tubes, and all of the water was removed from each unit with a 60 cc syringe, 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 sub-sample 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 SRP. Water levels in two units, each in different treatments, indicated that water was leaking out, and these units were excluded from analysis. The NO_3 was reduced to NO_2 via cadmium reduction; NO_2 was measured spectrophotometrically after diazotation (Switala 1999, Wendt 1999). The NH_4 was heated with solutions of salicylate and hypochlorite and determined spectrophotometrically (Switala 1999, Wendt 1999). The TKN was determined by the wet oxidation of nitrogen using sulfuric acid and a digestion catalyst. The procedure converts organic nitrogen to NH_4, which is subsequently determined (Carlson 1978). The SRP 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, each unit was refilled with 300 mL of water from each site that was collected at the beginning of the experiment. Water was added such that it did not disturb the sediment surface. The weekly exchange of water in all units simulated low turnover in poorly flushed estuaries characteristic of southern California (Zedler 1982, Zedler 1996, Fong and Zedler 2000). The water was stored in the dark at 6°C and triplicate samples of water from each site were analyzed weekly for nutrient concentrations. Algae were replaced in the appropriate units, and the units were re-randomized in the water bath.

At the end of the experiment, *Enteromorpha intestinalis* was 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 with mortar and pestle and analyzed for tissue N and P. The N was determined using an induction furnace and a thermal conductivity detector (Dumas 1981). The 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). The N and P content of algae are reported as total mass $unit^{-1}$, which is calculated by multiplying the nutrient concentration of a sample (percent dry wt), 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 sub-sample of each core was dried in a forced air oven at 60°C to a constant weight, ground with mortar and pestle, and analyzed for N and P. The 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). The 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 the percent of change from initial concentration.

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 analyzed statistically, as they were all below the detection limits of 0.05% dry wt and 0.01% dry wt, respectively.

The 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. The SRP 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 SRP 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). The TKN data

from the end of each week were 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), where initial sediment nutrients were highest, 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 site (p=0.834, Fisher's LSD),

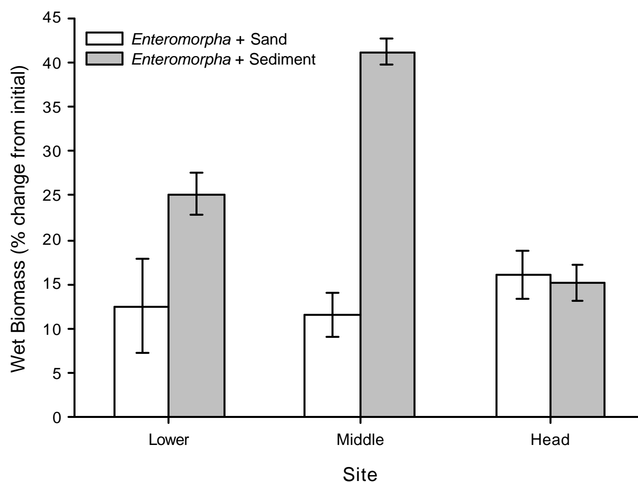

Figure 2. *Enteromorpha intestinalis* biomass (as percent of change from initial concentration) grown with either inert sand or estuarine sediment from three sites in Upper Newport Bay. Bars represent ± 1 SE.

and growth in these units was low, possibly due to low salinity conditions. Algal biomass was similar among sites when incubated with sand (p>0.20 for all comparisons, Fisher's LSD), increasing 12-16% from initial weight in three 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 site (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 site (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 area (p=0.002, Fisher's LSD) and then the Head area (p=0.028, Fisher's LSD). Within sand treatments, tissue P did not vary with site (p=0.50 for all comparisons, 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 3.57 µM in all experimental units except the sediment + water treatments from the Head area. The 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 low during each week of the experiment. Water column SRP was BDL (1.61 µM) in 29 of 43 of units at the end of Week 1, and 31 of 43 units at the end of Week 2. Mean SRP (± 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 Week 3, SRP was detected in 35 of 43 units. The SRP was significantly affected by site (p=0.011, ANOVA) but not treatment (p=0.512, ANOVA). Mean SRP for all units with measurable values was 5.43 ± 0.48 µM

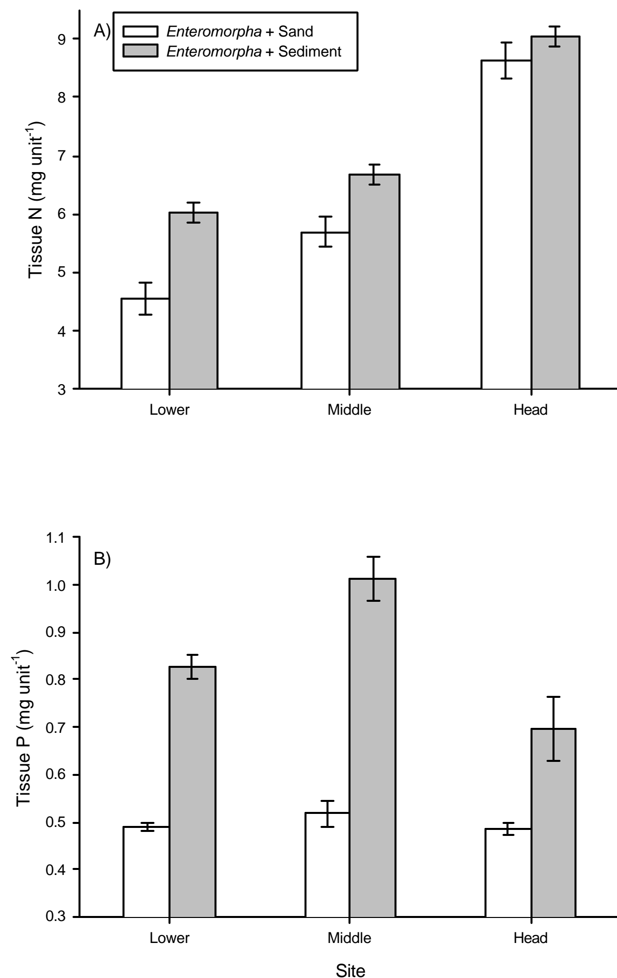

Figure 3. Mass of N (A) and P (B) in *Enteromorpha intestinalis* tissue grown with either inert sand or estuarine sediment from three sites in Upper Newport Bay. Bars represent ± 1 SE.

from the Lower site, 6.26 ± 0.79 µM from the Middle site, and 3.55 ± 0.29 µM from the Head site. The SRP was similar in units from the Lower and Middle sites (p=0.516, Fisher's LSD), and SRP in units from the Head site was lower than in units from the Lower site (p=0.020, Fisher's LSD).

Final sediment N (percent of change from the initial measurement) 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 (percent of change from the initial measurement) varied significantly with site (p=0.001, ANOVA). Sediment P increased in units from the Lower site and decreased in units from the Middle site (Figure 4B). In units from the Head site, there was a trend toward sediment P increasing when algae were not present. However, there was no change from initial levels of sediment P when algae were present, and there was no significant effect of algae on sediment P percent of change (p=0.186, ANOVA).

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 compared to when water column N was high. 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.

Sediments can be an important nutrient source to macroalgae when water column nutrients are low, which occurs when large amounts of algae deplete the water column of nutrients. Often, there is a poor correlation between macroalgal biomass and water column nutrient concentrations (Fong *et al.* 1998), and water column nutrients in natural systems are often periodically depleted to the low levels obtained in our experiment. Fong and Zedler (2000) found NO_3 and PO_4 values as low as 0.82 and 0.75 µM, respectively, in Famosa Slough, California, a poorly flushed estuary, during a large macroalgal bloom. In UNB, NO_3 concentrations in poorly flushed tidal creeks were up to 10 times lower than concentrations in the deeper, well-circulated main channel; and several macroalgal species were more abundant in the creeks (Boyle 2002).

When water column N was high, such as in units from the Head area, 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 nutrient 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

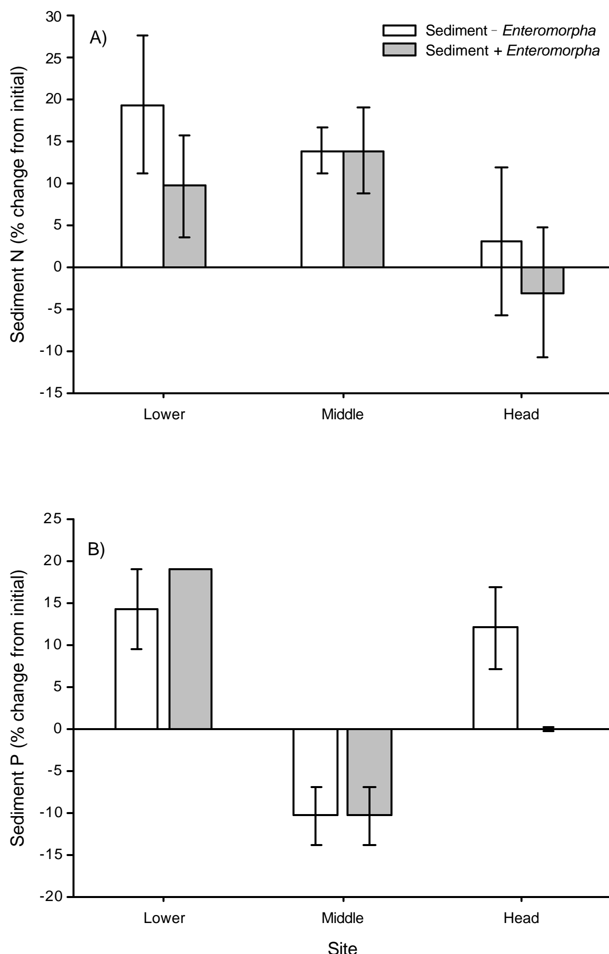

Figure 4. Sediment N (A) and P (B) (as percent of change from initial concentration) from three sites in Upper Newport Bay incubated in the presence or absence of *Enteromorpha intestinalis*. Bars represent ± 1 SE.

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 nutrients 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. The greater N supply to these algae presumably led to the observed greater biomass. When water column N was high, the presence of sediments did not affect tissue N, nor did low salinity conditions impair the algae's ability to remove N from the water column. *E. intestinalis* tissue N levels were greatest overall in units from the Head of the estuary, probably due to the greater supply of N available in the water column. Algae in these units must have derived most, if not all, of their N from the water column as tissue N content was the same between treatments with and without estuarine sediments.

Enteromorpha intestinalis tissue P content was greatly enhanced by the presence of sediments in units from all three sites. Additionally, tissue P was influenced by the magnitude of sediment P availability; the highest tissue P came from 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.

Estuarine sediments were also a nutrient sink, as indicated by the decrease in water column nutrients in the sediment + water units, which contained no algae. In units containing algae, reduction of water column nutrients was attributed to algal uptake. In units containing only estuarine sediments and water, reduction of water column nutrients is most logically explained by diffusion of nutrients into the sediment porewaters as has been often measured in other studies (Boynton *et al.* 1980, Nowicki and Nixon 1985, Cowan and Boynton 1996, Trimmer *et al.* 1998 and 2000, Magalhães *et al.* 2002). Dissolved nutrients are not known to volatilize (Ryther *et al.* 1981), and there were no signs of significant phytoplankton, microphytobenthic, or bacterial production in our units.

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, 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 utilize 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, Nowicki and Nixon 1985, Cowan and Boynton 1996), few studies have investigated whether algae are able to use these sediment-derived nutrients. Lavery and McComb (1991), Thybo-Christesen *et al.* (1993), McGlathery *et al.* (1997), and Tyler *et al.* (2001) provided evidence that macroalgal mats can intercept nutrients fluxing from sediments. Our study furthers the understanding of sediment-macroalgal nutrient dynamics by demonstrating that these nutrients are of ecological significance to the algae by their enhancement of growth rates and tissue nutrient content. Therefore, we recommend that nutrient loads from the sediments as well as from the watershed be incorporated into assessments of sources of nutrients to primary producers.

LITERATURE CITED

Ahern, J., J. Lyons, J. McClelland and I. Valiela. 1995. Invertebrate response to nutrient-induced changes in macrophyte assemblages in Waquoit Bay. *Biological Bulletin* 189: 241-242.

American Public Health Association, American Water Works Association and Water Environmental Federation. 1998. Flow injection analysis for orthophosphate. pp. 4 -149 to 4-150 *in*: L.S. Clesceri , A.E. Greenberg and A.D. Eaton (eds.), Standard Methods for the Examination of Water and Wastewater, 20th edition. American Public Health Association. Washington, DC.

Birch, P.B., D. M. Gordon and A. J. McComb. 1981. Nitrogen and phosphorus nutrition of *Cladophora* in the Peel-Harvey estuarine system, western Australia. *Botanica Marina* 24: 381-387.

Blackburn, T.H. and K. Henriksen. 1983. Nitrogen cycling in different types of sediments from Danish waters. *Limnology and Oceanography* 28: 477-493.

Boyer, K.E. 2002. Linking community assemblages and ecosystem processes in temperate and tropical coastal habitats. Dissertation. University of California, Los Angles, Los Angeles, CA.

Boyle, K.A. 2002. Investigating nutrient dynamics and macroalgal community structure in an eutrophic southern California estuary: results of field monitoring and microcosm experiments. Ph.D. Thesis. University of California, Los Angeles, Los Angeles, CA.

Boynton, W.R., W.M. Kemp and C.G. Osborne. 1980. Nutrient fluxes across the sediment-water interface in the turbid zone of a coastal plain estuary. pp. 93-109 *in*: V.S. Kennedy (ed.), Estuarine Perspectives. Academic Press. New York. NY.

Carlson, R.M. 1978. Automated separation and conductimetric determination of ammonia and dissolved carbon dioxide. *Analytical Chemistry* 50: 1528-1531.

Clavero, V., J.J. Izquierdo, J.A. Fernandez and F.X. Niell. 2000. Seasonal fluxes of phosphate and ammonium across the sediment-water interface in a shallow small estuary (Palmones River, southern Spain). *Marine Ecology Progress Series* 198: 51-60.

Cowan, J.L. and W.R. Boynton. 1996. Sediment-water oxygen and nutrient exchanges along the longitudinal axis of Chesapeake Bay: Seasonal patterns, controlling factors and ecological significance. *Estuaries* 19: 562-580.

Day, J.W., Jr., D. Pont, P.F. Hensel and C. Ibanez. 1995. Impacts of sea-level rise on deltas in the Gulf of Mexico and the Mediterranean: The importance of pulsing events to sustainability. *Estuaries* 18: 636-647.

Duke, C.S., W. Litaker and J. Ramus. 1989. Effect of temperature on nitrogen-limited growth rate and chemical composition of *Ulva curvata* (Ulvales: Chlorophyta). *Marine Biology* 100: 143-150.

Duke, C.S., W. Litaker and J. Ramus. 1989. Effects of temperature, nitrogen supply, and tissue nitrogen on ammonium uptake rates of the chlorophyte seaweeds *Ulva curvata* and *Codium decorticatum. Journal of Phycology* 25: 113-120.

Dumas, J.B. 1981. Sur les procedes de l'analyse organique. *Annals de Chimie* XLVII: 195-213.

Fong, P., K.E. Boyer and J.B. Zedler. 1998. Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic alga, *Enteromorpha intestinalis* (L. Link). *Journal of Experimental Marine Biology and Ecology* 231: 63-79.

Fong, P. and J.B. Zedler. 2000. Sources, sinks and fluxes of nutrients (N+P) in a small highly modified urban estuary in southern California. *Urban Ecosystems* 4: 125-144.

Fujita, R.M. 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. *Journal of Experimental Marine Biology and Ecology* 92: 283-301.

Grenz, C., J.E. Cloern, S.W. Hager and B.E. Cole. 2000. Dynamics of nutrient cycling and related benthic nutrient and oxygen fluxes during a spring phytoplankton bloom in South San Francisco Bay (USA). *Marine Ecology Progress Series* 197: 67-80.

Hernandez, I., G. Peralta, J.L. Perez-Llorens, J.J. Vergara and F.X. Niell. 1997. Biomass and dynamics of growth of *Ulva* species in Palmones River estuary. *Journal of Phycology* 33: 764-772.

Kamer, K., K.A. Boyle and P. Fong. 2001. Macroalgal bloom dynamics in a highly eutrophic southern California estuary. *Estuaries* 24: 623-635.

Kamer, K. and P. Fong. 2000. A fluctuating salinity regime mitigates the negative effects of reduced salinity on the estuarine macroalga, *Enteromorpha intestinalis* (L.) link. *Journal of Experimental Marine Biology and Ecology* 254: 53-69.

Kwak, T.J. and J.B. Zedler. 1997. Food web analysis of southern California coastal wetlands using multiple stable isotopes. *Oecologia* 110: 262-277.

Lavery, P.S. and A.J. McComb. 1991. Macroalgal-sediment nutrient interactions and their importance to macroalgal nutrition in a eutrophic estuary. *Estuarine Coastal and Shelf Science* 32: 281-295.

Litaker, W., C.S. Duke, B.E. Kenney and J. Ramus. 1987. Short-term environmental variability and phytoplankton abundance in a shallow tidal estuary. I. Winter and summer. *Marine Biology* 96: 115-121.

Magalhaes, C.M., A.A. Bordalo and W.J. Wiebe. 2002. Temporal and spatial patterns of intertidal sediment-water nutrient and oxygen fluxes in the Douro River estuary, Portugal. *Marine Ecology Progress Series* 233: 55-71.

McGlathery, K.J., D. Krause-Jensen, S. Rysgaard and P.B. Christensen. 1997. Patterns of ammonium uptake within dense mats of the filamentous macroalga *Chaetomorpha linum. Aquatic Botany* 59: 99-115.

Meyer, G.A. and P.N. Keliher. 1992. An overview of analysis by inductively coupled plasma-atomic emission spectrometry. pp. 473-505 *in*: A. Montaser and D.W. Golightly (eds.), Inductively Coupled Plasmas in Analytical atomic spectrometry. VCH Publishers Inc. New York. NY.

Nedwell, D.B., A.S. Sage and G.J.C. Underwood. 2002. Rapid assessment of macroalgal cover on intertidal sediments in a nutrified estuary. *Science of the Total Environment* 285: 97-105.

Nixon, S.W. 1981. Remineralization and nutrient cycling in coastal marine ecosystems. pp. 111-138 *in*: B.J. Neilson and L.E. Cronin (eds.), Estuaries and Nutrients. Humana Press. Clifton, NJ.

Nixon, S.W. 1995. Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia* 41: 199-219.

Nowicki, B.L. and S.W. Nixon. 1985. Benthic nutrient remineralization in a coastal lagoon ecosystem. *Estuaries* 8: 182-190.

O'Brien, M.C. and P.A. Wheeler. 1987. Short term uptake of nutrients by *Enteromorpha prolifera* (Chlorophyceae). *Journal of Phycology* 23: 547-556.

Paerl, H.W. 1999. Cultural eutrophication of shallow coastal waters: Coupling changing anthropogenic nutrient inputs to regional management approaches. *Limnologica* 29: 249-254.

Pregnall, A.M. and P.P. Rudy. 1985. Contribution of green macroalgal mats (*Enteromorpha* spp.) to seasonal production in an estuary. *Marine Ecology Progress Series* 24: 167-176.

Raffaelli, D., P. Balls, S. Way, I.J. Patterson, S. Hohmann and N. Corp. 1999. Major long-term changes in the ecology of the Ythan estuary, Aberdeenshire, Scotland; How important are physical factors? *Aquatic Conservation* 9: 219-236.

Raffaelli, D., J. Limia, S. Hull and S. Pont. 1991. Interactions between the amphipod *Corophium volutator* and macroalgal mats on estuarine mudflats. *Journal of the Marine Biological Association of the United Kingdom* 71: 899-908.

Rizzo, W.M. and R.R. Christian. 1996. Significance of subtidal sediments to heterotrophically-mediated oxygen and nutrient dynamics in a temperate estuary. *Estuaries* 19: 475-487.

Ryther, J.H., N. Corwin, T.A. DeBusk and L.D. Williams. 1981. Nitrogen uptake and storage by the red alga *Gracilaria tikvahiae* (McLachlan, 1979). *Aquaculture* 26: 107-115.

Schramm, W. 1999. Factors influencing seaweed responses to eutrophication: Some results from EU-project EUMAC. *Journal of Applied Phycology* 11: 69-78.

Schramm, W. and P.H. Nienhuis, (eds.). 1996. Marine Benthic Vegetation: Recent changes and the effects of eutrophication. Springer-Verlag. New York. NY.

Sfriso, A., A. Marcomini and B. Pavoni. 1987. Relationships between macroalgal biomass and nutrient concentrations in a hypertrophic area of the Venice Lagoon, Italy. *Marine Environmental Research* 22: 297-312.

Sfriso, A., B. Pavoni, A. Marcomini and A.A. Orio. 1992. Macroalgae, nutrient cycles, and pollutants in the Lagoon of Venice. *Estuaries* 15: 517-528.

Svensson, J.M., G.M. Carrer and M. Bocci. 2000. Nitrogen cycling in sediments of the Lagoon of Venice, Italy. *Marine Ecology Progress Series* 199: 1-11.

Switala, K. 1999. Determination of ammonia by flow injection analysis. QuikChem Method 10-107-06-1-A. Lachat Instruments. Milwaukee, WI.

Thiel, M. and L. Watling. 1998. Effects of green algal mats on infaunal colonization of a New England mud flat - long-lasting but highly localized effects. *Hydrobiologia* 375/376: 177-189.

Thybo-Christesen, M., M.B. Rasmussen and T.H. Blackburn. 1993. Nutrient fluxes and growth of *Cladophora sericea* in a shallow Danish bay. *Marine Ecology Progress Series* 100: 273-281.

Trimmer, M., D.B. Nedwell, D.B. Sivyer and S.J. Malcolm. 1998. Nitrogen fluxes through the lower estuary of the river Great Ouse, England: The role of the bottom sediments. *Marine Ecology Progress Series* 163: 109-124.

Trimmer, M., D.B. Nedwell, D.B. Sivyer and S.J. Malcolm. 2000. Seasonal organic mineralisation and denitrification in intertidal sediments and their relationship to the abundance of *Enteromorpha* sp. and *Ulva* sp. *Marine Ecology Progress Series* 203: 67-80.

Tyler, A.C., K.J. McGlathery and I.C. Anderson. 2001. Macroalgae mediation of dissolved organic nitrogen fluxes in a temperate coastal lagoon. *Estuarine Coastal and Shelf Science* 53: 155-168.

Valiela, I., K. Foreman, M. LaMontagne, D. Hersh, J. Costa, P. Peckol, B. DeMeo-Andreson, C. D'Avanzo, M. Babione, C.H. Sham, J. Brawley and K. Lajtha. 1992. Couplings of watersheds and coastal waters sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. *Estuaries* 15: 443-457.

Valiela, I., J. McClelland, J. Hauxwell, P.J. Behr, D. Hersh and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* 42: 1105-1118.

Wendt, K. 1999. Determination of nitrate/nitrite by flow injection analysis (low flow method). QuikChem Method 10-107-04-1-A. Lachat Instruments. Milwaukee, WI.

Zedler, J.B. 1982. The ecology of southern California coastal salt marshes: A community profile. FWS/OBS-81/54. U.S. Fish and Wildlife Service, Biological Services Program. Washington, DC.

Zedler, J.B., Principal Author. 1996. Tidal wetland restoration: A scientific perspective and southern California focus. T-038. California Sea Grant College System, University of California. La Jolla, CA.

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