INTRODUCTION

Many environmental factors influence the growth and biomass accumulation of aquatic primary producers. In shallow marine and estuarine systems, macroalgae are important primary producers (Zedler 1980, Duarte 1995). They are often extremely abundant (Valiela et al. 1997), function as both a sink and a source of nutrients (Sfriso et al. 1987, Sfriso et al. 1992) and are an important food source for higher trophic levels (Kwak & Zedler 1997). Macroalgal abundance can be affected by a suite of factors including temperature, light, salinity and nutrient availability (Bird et al. 1979, Lapointe 1987, Lapointe 1989, Friedlander 1992, Valiela et al. 1997, Schramm 1999).

Salinity in estuaries fluctuates depending on the relative influences of terrestrial derived freshwater runoff and oceanic seawater. Southern California has a Mediterranean climate with distinct wet and dry seasons. Historically, estuaries received freshwater primarily during the wet season (November to April) and functioned as marine embayments during the dry season (May to October) (Onuf 1987). However, human activities such as agriculture, sewage treatment and municipal water use in populated watersheds generate a large amount of freshwater that enters coastal estuaries year-round (Zedler 1996), and estuarine salinity <10 psu has been measured in at least 1 local estuary from winter through summer (K. A. Boyle et al. pers. comm.). To maintain osmotic balance with the external environment, algae regulate their internal solute concentration, which requires energy (Kirst 1989). As a result, algal growth and productivity may be reduced (Bird et al. 1979, Bird & McLachlan 1986, Edwards et al. 1988, Murthy et al. 1988, Thomas et al. 1988, Karsten & Kirst 1989, Friedlander 1992).

ABSTRACT: In southern California estuaries, the green macroalga Enteromorpha intestinalis experiences wide fluctuations in both nitrogen (N) supply and salinity. We investigated the effects of simultaneous variation in N and salinity on the growth, biomass accumulation and tissue nutrient dynamics of E. intestinalis. We conducted a fully crossed 2-factor experiment in which we varied N enrichment (low, medium and high) and salinity (15, 25 and 35 psu). Overall, addition of N enhanced algal growth while salinity reduction decreased growth. High N enrichment mitigated the negative effects that reduced salinity had on dry biomass, wet:dry biomass ratios, tissue nutrients and ability to remove phosphorus from the water column. Largely, E. intestinalis abundance was governed by N availability rather than salinity, indicating that blooms of macroalgae will likely continue to proliferate in estuaries unless nutrient loading is reduced.

KEY WORDS: Enteromorpha intestinalis · Macroalgae · Nitrogen · Salinity · Estuaries · Southern California

Enteromorpha intestinalis is an opportunistic alga with high nutrient uptake rates (Fujita 1985, Kamer et al. unpubl. data) and a large internal storage capacity (Fong et al. 1994). These characteristics enable it to take advantage of nutrient pulses and to proliferate in areas such as estuaries where nutrient inputs may be episodic. E. intestinalis is also generally considered a euryhaline species (Reed & Russell 1979, Ritchie & Larkum 1985, Edwards et al. 1987, Young et al. 1987). However, evidence shows that salinity outside its optimal range may negatively affect productivity and growth (Martins et al. 1999, Kamer & Fong 2000) or may even cause death (Ritchie & Larkum 1985, Edwards et al. 1988, Martins et al. 1999). E. intestinalis is an important primary producer in southern California estuaries (Zedler 1980) and plays an important role in food webs of southern California estuaries (Kwik & Zedler 1997). E. intestinalis is a food source for lined shore crabs (K. Boyer pers. comm.) and topsmelt (E. Logothetis unpubl. data). However, excessive blooms of macroalgae can be detrimental to estuarine ecosystems. Large algal masses can deplete oxygen in the water column (Sfriso et al. 1987, Valiela et al. 1992) and sediments, resulting in fish and invertebrate mortality (Sfriso et al. 1992) and changes in infaunal community structure (Raffaelli et al. 1991, Ahern et al. 1995).

Fong et al. (1996) examined the effects of nutrient enrichment and salinity stress on growth of Enteromorpha intestinalis in southern California. In separate, single factor experiments, they found that E. intestinalis responded positively to N addition but negatively to prolonged periods of reduced salinity. However, these environmental parameters vary together in the field. Nutrient pulses generally accompany freshwater inputs in winter following seasonal storms (Boyle et al. pers. comm.), and because of human influences in southern California, freshwater inputs to estuaries are often enriched with nutrients throughout the year (Onuf 1987, Zedler 1996, Fong & Zedler unpubl. data). We do not have an understanding of how E. intestinalis responds to the simultaneous variations in N and salinity that it may experience in southern California estuaries. To address this question, we performed a fully crossed factorial experiment modeling field conditions in which N enrichment and salinity varied within known field conditions for southern California.

**MATERIALS AND METHODS**

We used a 2-factor experimental design to test the effects of N enrichment and salinity on biomass accumulation and nutrient dynamics of Enteromorpha intestinalis. Three levels of N (as NO$_3$) enrichment (low, medium and high) were fully crossed with 3 levels of salinity (15, 25 and 35 psu) while PO$_4$ was held high and constant across all treatments.

Enteromorpha intestinalis was collected from Mugu Lagoon, Ventura County, CA, in January 1997. Mugu Lagoon is a shallow estuary subject to freshwater flow and high nutrient loads from surrounding agriculture and has year-round proliferation of macroalgae. Algae were kept for 6 d in a greenhouse at the University of California, Los Angeles (UCLA) in shallow pans covered with window screening to reduce light and temperature. Light was usually above saturation and temperature was ambient for coastal conditions. Pans were filled with aerated low nutrient seawater (<3.57 µM NO$_3$, <1.56 µM PO$_4$). Algae were kept in batch culture to expose them all to the same nutrient levels and therefore equalize internal nutrient stores (Fong et al. 1994). Initial tissue N levels were 1.531 ± 0.055% dry wt (mean ± SE) and initial phosphorus (P) levels were 0.124 ± 0.007% dry wt.

Nine individual solutions were mixed. Thirty-five psu low nutrient (<3.57 µM NO$_3$, <1.56 µM PO$_4$) filtered seawater (Whatman GF/C glass fiber filters) was diluted with distilled water to reduce salinity to 15 and 25 psu. Three levels of N were added to each salinity for mean initial concentrations of 18.57 ± 0.41 µM NO$_3$ (low NO$_3$), 80.47 ± 2.75 µM NO$_3$ (medium NO$_3$) and 151.90 ± 1.19 µM NO$_3$ (high NO$_3$). These concen-
trations are within the range measured in a southern California estuary in winter (K. A. Boyle et al. pers. comm.) Mean initial PO$_4$ was 76.66 ± 3.24 µM across all treatments. NH$_4$ was not added; mean initial NH$_4$ values were <3.57 µM. Mean initial total Kjeldahl nitrogen (TKN) was 56.35 ± 9.55 µM. TKN is a measure of all forms of N except NO$_3$.

Replicate 3 g (±0.1 g) subsamples (weighed as described below) of Enteromorpha intestinalis were placed in experimental units (350 ml total volume), each filled with 200 ml of the appropriate solution. Units were placed in a randomized array in a greenhouse at UCLA. Air temperature in the greenhouse fluctuated daily between 16 and 22°C. Replication was 5-fold for a total of 45 experimental units. Solutions were changed weekly and salinity was monitored daily with a hand held refractometer (precision: ±0.5 psu). Distilled water was added to compensate for evaporation. Units were maintained for 4 wk. Each of the 9 solution batches was sampled again at the end of the experiment to ensure that nutrient levels did not change during the course of the experiment.

Enteromorpha intestinalis wet biomass was measured at the end of the experiment. Algae were removed from experimental units, placed in individually labeled nylon mesh bags, spun in a salad spinner for 1 min to remove excess water and weighed. Algae were then rinsed briefly in distilled water to remove external salts and dried to a constant weight at 60°C. Algal dry weights were recorded and wet:dry biomass ratios were calculated.

Dried Enteromorpha intestinalis was ground with a mortar and pestle and then analyzed for tissue N and P content by the Division of Agriculture and Natural Resources (DANR) Analytical Laboratory of the University of California (UC), Davis. Total N in algal tissue was analyzed by N gas analyzer using induction furnace and thermal conductivity (Sweeney 1989). Total P in algal tissue was quantitatively determined by atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry (ICP-AES) (Franson 1985) following a nitric acid/hydrogen peroxide microwave digestion (Johnson & Ulrich 1959). These automated methods have detection limits of 3.57 µM for N and 1.56 µM for P.

Algal condition was estimated in 2 ways. First, wet:dry biomass ratios provide an estimate of condition because algae in poor condition usually have the lowest dry biomass but not necessarily the lowest wet biomass (Kamer & Fong 2000). As Enteromorpha intestinalis deteriorates it loses structural integrity, and less water per unit algal biomass is removed by centrifugation than for algae in good condition (K.K. pers. obs.). This leads to an overestimation of wet biomass and higher wet:dry biomass ratios for algae in poor condition. Second, the algae’s ability to take up and store nutrients is a useful indicator of algal condition. Thus, we considered algae that removed more nutrients from the water column and had greater stored tissue nutrients to be in better condition.

Among treatment differences in biomass measurements, tissue nutrient content and amounts, and final water column nutrient concentrations (NH$_4$, TKN and total P) were analyzed with 2-factor ANOVA (N enrichment × salinity level). Histograms of data and plots of residuals versus fitted Y values were examined to determine whether data complied with ANOVA assumptions of normality and homogeneity of variance. Unequal variances in algal tissue N % dry wt and mg P in algal tissue-experimental unit$^{-1}$ data were corrected by transforming the data with a $1/x$ calculation. Unless otherwise stated, no significant interactions occurred between factors of the ANOVA. Final water column NO$_3$ values from 36 of the 45 experimental units were below the detection limit of the DANR Analytical Laboratory (lower limit of detection is 3.57 µM for all N analyses). The remaining values that were reported were not statistically analyzed.

RESULTS

Dry biomass of Enteromorpha intestinalis decreased as salinity decreased but increased as N enrichment increased (Fig. 1a). Biomass was significantly affected by N enrichment (p = 0.0002) and by salinity level (p = 0.0001). The effect of N enrichment seemed to be greatest under 15 psu conditions; the effect of reduced salinity was ameliorated by high N enrichment.

Enteromorpha intestinalis wet:dry biomass ratios increased as salinity decreased, but N enrichment alleviated this effect (Fig. 1b). Ratios were significantly affected by N enrichment (p = 0.0064) and by salinity
level ($p = 0.0001$). For low and medium levels of N enrichment, ratios were higher under 15 psu conditions than they were under 35 psu conditions. For the high level of N enrichment, ratios between 15 and 35 psu conditions were more similar.

The highest Enteromorpha intestinalis tissue N content was found in algae from the lowest salinity treatments (Fig. 2a). Tissue N was significantly affected by salinity level ($p = 0.0001$) but not N enrichment ($p = 0.4816$). Algal tissue N content (as % dry wt) decreased as salinity increased, showing an opposite pattern from dry biomass.

Enteromorpha intestinalis tissue P content under the lowest salinity level was enhanced by N enrichment ($p = 0.0001$) but not N enrichment ($p = 0.4816$). Algal tissue N content (as % dry wt) decreased as salinity increased, showing an opposite pattern from dry biomass.

Enteromorpha intestinalis tissue P content under the lowest salinity level was enhanced by N enrichment (Fig. 2b). Tissue P was significantly affected by both N enrichment ($p = 0.0093$) and salinity level ($p = 0.0001$). Algal tissue P content (as % dry wt) decreased as salinity decreased at low and medium N enrichment treatments. Under high N enrichment, tissue P content for all 3 salinity levels was similar.

Nitrogen enrichment ($p = 0.0001$) and salinity level ($p = 0.0027$) each significantly affected the mass of N in Enteromorpha intestinalis tissue per experimental unit. N unit$^{-1}$ increased with salinity at low and medium N enrichment treatments (Fig. 3a) but under high N enrichment treatments, N unit$^{-1}$ was similar among the 3 salinity levels. With regard to salinity level, N unit$^{-1}$ showed a pattern opposite to that of algal tissue N content (as % dry wt; Fig. 2a). N per experimental unit also increased as N enrichment increased.

The mass of P in Enteromorpha intestinalis tissue per experimental unit showed a pattern similar to tissue N unit$^{-1}$. At low and medium N enrichment levels, P unit$^{-1}$ increased as salinity increased (Fig. 3b), while under high N enrichment conditions, P unit$^{-1}$ was highly variable and not different between salinities. There was significant interaction between N enrichment and salinity level in the analysis of the concentration of P in E. intestinalis tissue per experimental unit ($p = 0.0019$).

Removal of P from the water column by Enteromorpha intestinalis was greatest under high N enrichment conditions (Fig. 4). Final water column total P was significantly affected by N enrichment ($p = 0.0024$) but not by salinity level ($p = 0.9040$). P was never removed from the water column to the degree that it would have limited algal growth. Final P values from low and
Enteromorpha intestinalis medium N enrichment treatments were similar to initial values. Regardless of NO3 load, final water column NO3 values were very low. Eighty percent of experimental units had final water column NO3 values below the detection limit of 3.57 µM. Reported values range from 3.57 to 5.71 µM and at least 3 of the 5 replicates for each treatment were below detection limit.

There were no significant effects of N enrichment or salinity level on either final water column NH4 or TKN (p > 0.100 for each factor for both variables). Final NH4 was low and variable across all treatments; means ranged from < 3.57 µM to 11.1 µM. Final TKN values ranged from 98.6 to 150.0 µM, which were 2 to 3 times higher than initial values. For both NH4 and TKN, there were no consistent patterns with regard to either N enrichment or salinity level.

**DISCUSSION**

Nitrogen enrichment ameliorated the negative effects of reduced salinity on Enteromorpha intestinalis. Under low salinity conditions, E. intestinalis had the greatest biomass, lowest wet: dry biomass ratios, most N and P stored in its tissues, and the greatest ability to pull nutrients out of the water column when N enrichment was highest. High N enrichment also enhanced these parameters under 25 and 35 psu, but the differences were not as dramatic as under 15 psu, indicating that N supply was most critical when salinity was reduced. When N loading to coastal estuaries is high, macroalgae such as E. intestinalis will likely proliferate even if salinity fluctuates moderately, such as within the ranges presented here. Macroalgal blooms can be expected to continue unless nutrient loads are reduced.

Enteromorpha intestinalis was negatively affected by salinity reduction. Previous research has shown that E. intestinalis can tolerate a wide range of salinity (Reed & Russell 1979, Ritchie & Larkum 1985, Edwards et al. 1987, Young et al. 1987) but cannot survive prolonged exposure to 0 psu (Ritchie & Larkum 1985, Edwards et al. 1988, Martins et al. 1999, Kamer & Fong 2000). The degree of salinity reduction in this experiment was not as extreme as 0 psu but still affected E. intestinalis growth and condition. Similarly, Kamer and Fong (2000) found that exposure to 15 psu conditions for 5 d periods decreased E. intestinalis growth. Ulva lactuca growth has been reduced by salinity between 20 and 30 psu (Murthy et al. 1988, Friedlander 1992), and other less closely related bloom-forming species (Gracilaria spp., Cladophora spp.) have also been negatively affected by reduced salinity (Bird & McLachlan 1986, Thomas et al. 1988). Enteromorpha intestinalis in the lowest salinity levels with the highest wet: dry biomass ratios also had the highest tissue N content. Tissue nutrient content and algal condition are not always linearly related because tissue nutrients can be diluted as an alga grows (Duke...
et al. 1989). Increased tissue N can indicate good condition when an alga is growing well and has an abundant supply of N, but tissue N may also increase when an alga takes up and stores N but does not grow due to limitation by other environmental conditions. This phenomenon has also been seen in bloom-forming species of 
Gracilaria
 and 
Ulva
 when light and temperature were limiting (Lapointe & Tenore 1981, Rosenberg & Ramus 1982, Lapointe & Duke 1984, Duke et al. 1989). Algae from the reduced salinity treatments were deteriorating by the end of the experiment, and none had grown >23% in 4 wk. However, even in its worst condition, 
E. intestinalis
 appeared to be scavenging and storing N. 
E. intestinalis
 may have evolved strategies for N uptake and storage even when environmental conditions do not promote growth.

We did not see evidence of the same phenomenon for 
P. P remained in the water column at the end of both experiments and did not appear to limit 
Enteromorpha intestinalis
 growth. Unlike N, P was not sequestered in 
E. intestinalis
 tissue when the algae were in poor condition. The same strategies for conservation of P may not have evolved if P scarcity has not occurred as often or for as long in algal evolution.

These data further our understanding of how 
Enteromorpha intestinalis
 responds to the various salinity and nutrient conditions it may experience in southern California estuaries at different times through out the year. Our results show that N enrichment can ameliorate the negative effects of reduced salinity on the growth and condition of 
E. intestinalis
. This implies that algae may proliferate in winter when inputs of N and freshwater to estuaries are high. In summer, when freshwater inputs are generally less, if N loading still occurs, growth of macroalgae may be even greater. Blooms of macroalgae will likely continue to proliferate in estuaries in southern California and around the world unless nutrient loading from surrounding watersheds is reduced. Development of methods for greater removal of nutrients from treated wastewater, and agricultural and urban runoff may be the key to reducing the magnitude and frequency of macroalgal blooms in coastal estuaries.

Acknowledgements. This work was funded by the California Water Resources Center and EPA Water and Watersheds Program no. R825381. We thank Karleen Boyle, Risa Cohen, Jennie Lee, Steve Lee and Tonatiuh Trejo for assistance with this project.

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Editorial responsibility: Victor de Jonge (Contributing Editor), Haren, The Netherlands

Submitted: August 11, 2000, Accepted: March 22, 2001
Proofs received from author(s): July 16, 2001