
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 Kennison¹,
Peggy Fong¹, and Kenneth Schiff

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-d period, *E. intestinalis* and *U. expansa* were each given equal supplies of NO₃-N (28 mg) and PO₄-P (6.2 mg) via pulses of different frequencies and therefore different concentrations. The NO₃-N doses given to 10 g wet weight (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 percent to ninety-six percent 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 (Sfriso *et al.* 1987, Lavery and McComb 1991, Sfriso *et al.* 1992, Hernández *et al.* 1997, 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), blooms are of ecological concern because they can reduce the habitat quality of an estuary. They can deplete the water column and sediments of oxygen (Sfriso *et al.* 1987, Valiela *et al.* 1992), leading to changes in species composition and shifts in community structure (Raffaelli *et al.* 1991, Bolam *et al.* 2000).

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), and the occurrence of macroalgal blooms in natural systems is often related to nutrient enrichment (Sfriso *et al.* 1987, Nixon 1995, Valiela *et al.* 1997).

Nutrient supply to estuarine macroalgae can occur 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 may vary on a daily scale due to tidal influences (Day *et al.* 1995) and light-associ-

¹Department of Organismic Biology, Ecology, and Evolution,
University of California, Los Angeles, 621 Charles E. Young Drive
Los Angeles, CA 90095

ated processes such as nutrient uptake (Litaker *et al.* 1987). Precipitation events and runoff may 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), particularly in regions with Mediterranean climates (McComb *et al.* 1981, McComb *et al.* 1998), such as southern California.

Macroalgae can take up and store nutrients, and nutrient reserves may be used for growth during periods when external nutrient supply is low (Hanisak 1983). *Enteromorpha* spp. can store sufficient nutrients to maintain positive growth for up to 10 d (Fujita 1985), which likely enables this alga to succeed in environments with episodic inputs of nutrients. *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 vesiculosus* 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).

To date, only limited investigation has been conducted on the effect of temporal variability in nutrient supply to macroalgae. 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. This study, like many other algal physiology studies, used NH_4 as it is the form of N easiest for algae to take up. However, of available DIN in coastal marine and estuarine waters, NO_3 is usually more abundant than NH_4 (Sharp 1983). Therefore, it would be of interest to determine whether temporal variability in NO_3 supply has the same effect on macroalgae as variability in NH_4 supply. Additionally, Ramus and Venable (1987) used only one species of algae, and different species may respond differently to temporal variation in nutrient supply.

Southern California has a Mediterranean climate with distinct wet and dry seasons, and nutrient supply to estuaries is often pulsed and aperiodic (Peters *et al.* 1985). Southern California estuaries are charac-

terized by seasonal blooms of macroalgae, often comprised of *Enteromorpha* and *Ulva* spp. (Peters *et al.* 1985, Kamer *et al.* 2001), and NO_3 concentrations are often much greater than NH_4 , sometimes by up to 10-fold (Page *et al.* 1995). There are currently intense efforts to improve water quality and reduce the occurrence and extent of macroalgal blooms in estuaries throughout southern California. With greater understanding of the effect of temporal variability in nutrient supply on macroalgae, estuaries can be modeled and managed effectively. Our objective was to determine how variation in the frequency and concentration of nutrients supplied to *E. intestinalis* and *U. expansa* affects macroalgal growth and tissue nutrient dynamics.

METHODS

We employed a two-factor experimental design to determine how equal amounts of nutrients (28 mg N, 6 mg P over a 28-d period) supplied to *Enteromorpha intestinalis* and *Ulva expansa* at different frequencies and concentrations affected the growth and tissue nutrient dynamics of these algae. The first factor varied was the frequency with which algae received nutrient doses (N as NO_3 , P as PO_4), and therefore the concentrations of nutrients in the doses 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, and the amounts and concentrations of nutrients in the doses were reflective of those measured in southern California estuaries (Page *et al.* 1995, Fong and Zedler 2001). The second factor varied was algal species. Both *E. intestinalis* and *U. expansa* were used in the experiment as these are two of the dominant, bloom-forming species in southern California. These algae exhibited different nutrient uptake rates and growth dynamics in previous research and therefore may respond differently to temporal variation in nutrient supply.

Enteromorpha intestinalis and *Ulva expansa* were collected from Upper Newport Bay estuary in Orange County, California, 11 d prior to the beginning of the experiment. Algae were transported to the laboratory within 5 h, where each species was 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 ($20 \pm 2^\circ\text{C}$) 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 $\text{NH}_4\text{-N}$, 0.35 ± 0.02 mg/L $\text{NO}_3\text{-N}$, and <0.05 mg/L $\text{PO}_4\text{-P}$ to create solutions of ~1, 7, 14, and 28 mg/L $\text{NO}_3\text{-N}$ (Table 1). These were the initial solutions used for the daily, weekly, bi-weekly, and monthly treatments, respectively. 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. $\text{PO}_4\text{-P}$ 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. Sub-samples (10.0 ± 0.1 g) 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 ($20 \pm 2^\circ\text{C}$) and covered with window screening to reduce incident light. Replication was five-fold for a total of 40 units.

The experiment was conducted for 28 d. Each day, 1 mg of $\text{NO}_3\text{-N}$ and 0.21 mg of $\text{PO}_4\text{-P}$ 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 not to remove any algae. Three hundred milliliters of a 3.33 mg/L $\text{NO}_3\text{-N}$, 0.72 mg/L $\text{PO}_4\text{-P}$ 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 were made up periodically throughout the experiment and were sampled for nutrient concentrations before use to ensure consistent levels of nutrient addition throughout the experiment. Over the course of the experiment, $\text{NO}_3\text{-N}$ ranged from 3.21 to 3.75 mg/L in individual samples of daily solutions, and the mean value was 3.51 ± 0.08 mg/L ($n=9$); $\text{PO}_4\text{-P}$ ranged from 0.59 to 0.88 mg/L, and the mean value was 0.72 ± 0.03 mg/L ($n=9$).

To control for the effects of exchanging 300 mL of seawater in the daily treatments, seawater was exchanged on a daily basis 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 $\text{NH}_4\text{-N}$, 0.35 ± 0.02 mg/L $\text{NO}_3\text{-N}$, and <0.05 mg/L $\text{PO}_4\text{-P}$) 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 $\text{NO}_3\text{-N}$, 5 mg/L $\text{PO}_4\text{-P}$ 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 $\text{NO}_3\text{-N}$, 10 mg/L $\text{PO}_4\text{-P}$ 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 analysis (Figure 1).

Table 1. Schedule and amounts of nutrient ($\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$) doses administered to *Enteromorpha intestinalis* and *Ulva expansa* daily, weekly, bi-weekly, and monthly.

Treatment	Dose (mg)		Given on Day X (28 total days in experiment)	Number of Doses	Total Load (mg)	
	$\text{NO}_3\text{-N}$	$\text{PO}_4\text{-P}$			N	P
Daily	1	0.21	Every day	28	28	6
Weekly	7	1.5	1, 8, 15, 22	4	28	6
Bi-Weekly	14	3	1, 15	2	28	6
Monthly	28	6	1	1	28	6

Statistical Analysis

Among-treatment differences in final wet biomass, dry biomass, wet:dry weight ratios, algal tissue N and P concentration, algal tissue N and P total mass unit⁻¹, and the percentage of NO₃ and PO₄ removed from the water column within the first 24 h of the experiment were analyzed using two-factor analysis of variance (ANOVA) (nutrient dose x species). Following a significant ANOVA, multiple comparisons were used to determine differences among individual treatments (Fisher's Least Significant Difference [LSD] test). No interactions occurred unless otherwise stated. All data were tested for normality and homogeneity of variance prior to analysis and no transformations were necessary.

RESULTS

Final wet algal biomass differed significantly with nutrient dose ($p=0.001$, ANOVA) and between species ($p=0.001$, 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).

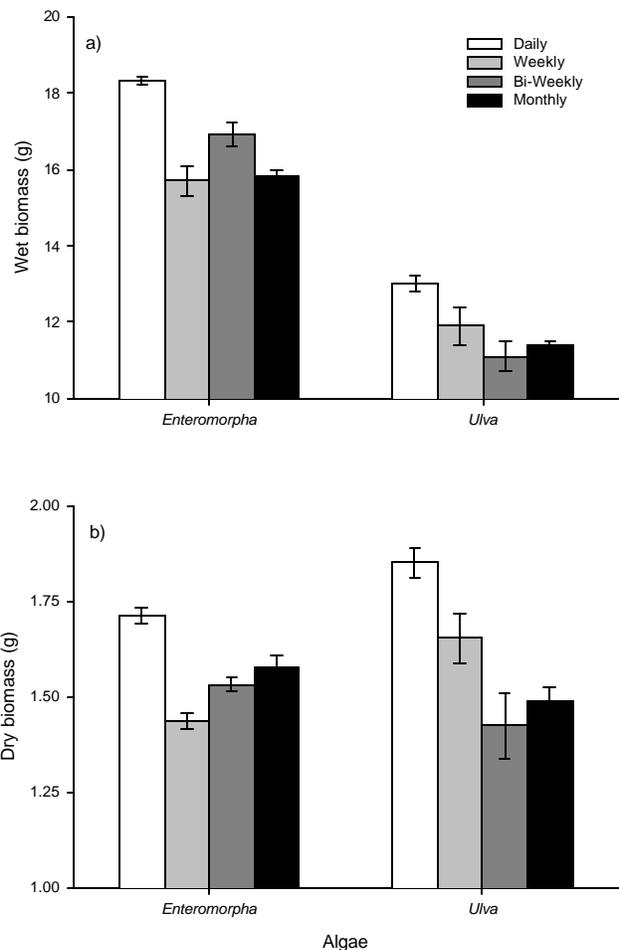


Figure 2. Final average wet biomass (a) and dry biomass (b) of *Enteromorpha intestinalis* and *Ulva expansa* grown for 28 d with daily, weekly, bi-weekly, and monthly nutrient additions (bars are ± 1 SE).

Wet:dry weight ratios were significantly affected by nutrient dose ($p=0.007$, ANOVA) and species ($p=0.001$, ANOVA), and there was an interaction between the terms ($p=0.002$). *Enteromorpha intestinalis* wet:dry weight ratios were higher than those of *Ulva expansa* (Table 2). Within *E. intestinalis*, the monthly treatment had the lowest wet:dry weight ratio, which, in part, explains the difference in the pattern between the final wet and dry biomass data. The differences in wet:dry weight 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 nutrient dose and species ($p=0.001$ for both factors, ANOVA). In general, tissue N concentrations were greater in *Ulva expansa* than *Enteromorpha intestinalis* (Figure 3a). For both

Table 2. Average wet:dry weight ratios of *Enteromorpha intestinalis* and *Ulva expansa* treated with four different frequencies of N addition. Values in () are SE.

N Additions	Algae	
	<i>Enteromorpha intestinalis</i>	<i>Ulva expansa</i>
Daily	10.66 (0.13)	7.03 (0.15)
Weekly	10.91 (0.26)	7.21 (0.11)
Bi-weekly	11.03 (0.07)	7.89 (0.27)
Monthly	10.01 (0.14)	7.69 (0.17)

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 nutrient dose and species ($p=0.001$ 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 (Figures 2a and 2b) 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 percentage 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

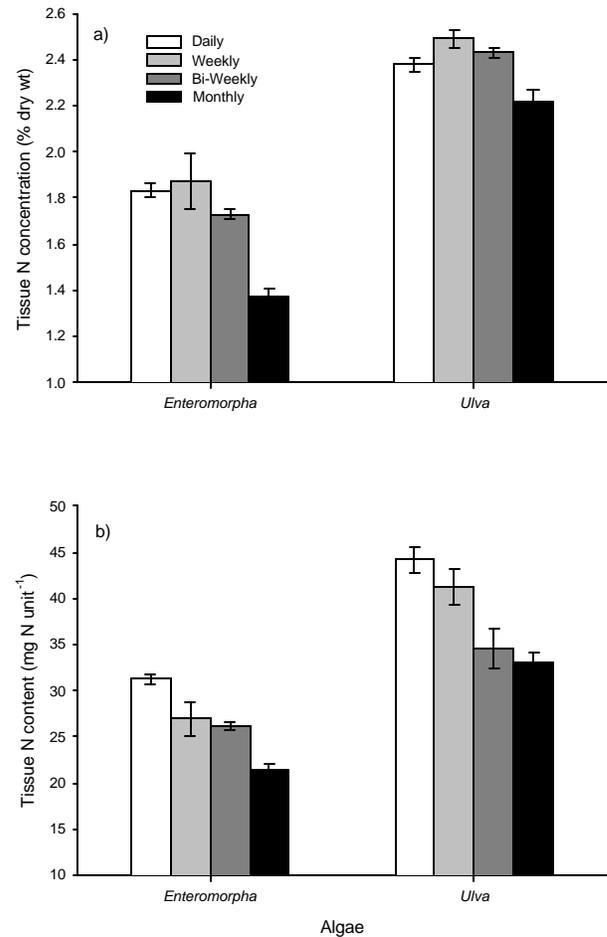


Figure 3. Final average tissue N concentration (as percent of dry wt) (a) and content (mg N unit^{-1}) (b) of *Enteromorpha intestinalis* and *Ulva expansa* grown for 28 d with daily, weekly, bi-weekly and monthly nutrient additions (bars are ± 1 SE).

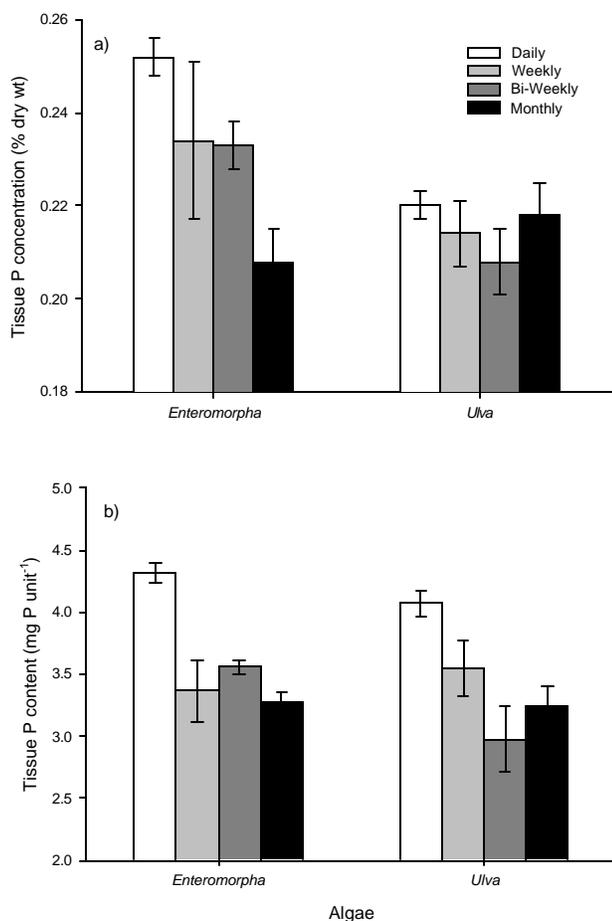


Figure 4. Final average tissue P concentration (as % dry wt) (a) and content (mg P unit⁻¹) (b) of *Enteromorpha intestinalis* and *Ulva expansa* grown for 28 d with daily, weekly, bi-weekly and monthly nutrient additions (bars are ± 1 SE).

decreased as the concentration of the dose increased. NO₃-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 from the pattern seen for *E. intestinalis* and likely caused an interaction between species and nutrient dose (p=0.001, ANOVA). The percentage of NO₃ 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, NO₃-N dropped below the detection limit of 0.05 mg/L; 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 NO₃-N was <0.10 mg/L in all experimental units. A portion of the NO₃ 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. NO₃-N remained low in all units for the duration of the experiment; at the end of Weeks 2, 3, and 4, NO₃-N was <0.11 mg/L in all units except for one, which measured 1.27 mg/L at the end of Week 3. NH₄ concentrations in all units were always <0.17 mg/L NH₄-N.

Water column PO₄ concentrations also decreased following nutrient doses. The percentage of PO₄ 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 PO₄ from the water column than *Enteromorpha intestinalis* (Figure 5b). For *E.*

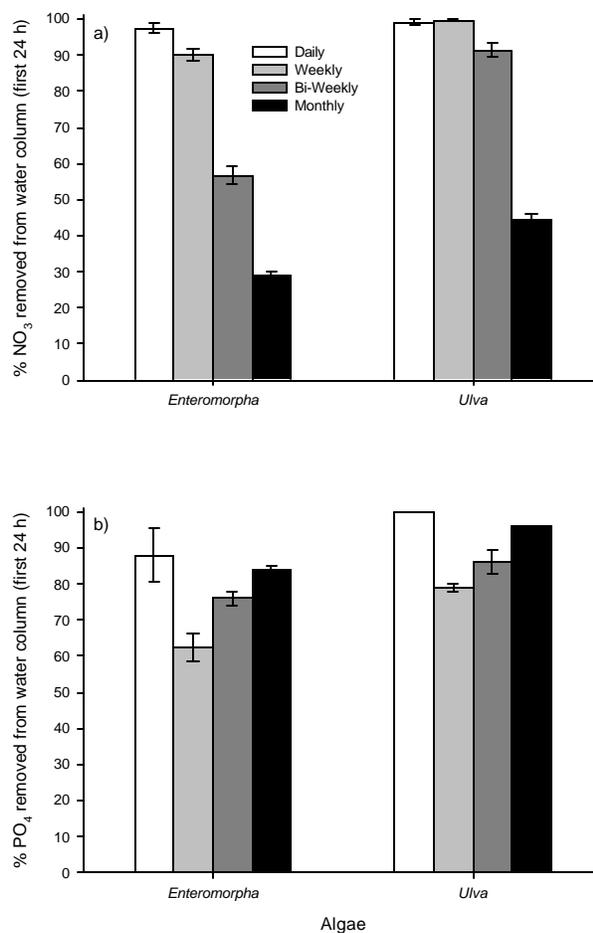


Figure 5. Percentage of initial concentration of NO₃-N (a) and PO₄-P (b) removed by *Enteromorpha intestinalis* and *Ulva expansa* in the first 24 h of the experiment (bars are ± 1 SE).

Table 3. Calculated amounts of NO₃-N and PO₄-P removed from experimental treatments in the first week of the experiment by the exchange of 300 mL of solution daily and by uptake by the algae.

Algae and Nutrient Dose	Calculated Amount Removed in First Week by Daily 300 mL Solution Exchange (mg)		Calculated Amount Removed by Algae (mg)	
	NO ₃ -N	PO ₄ -P	NO ₃ -N	PO ₄ -P
<i>Enteromorpha intestinalis</i>				
Daily	0.07	0.05	6.86	1.42
Weekly	0.21	0.22	6.79	1.28
Bi-Weekly	2.65	0.28	11.35	2.72
Monthly	11.77	0.32	16.23	5.68
<i>Ulva expansa</i>				
Daily	0.05	0.05	6.95	1.42
Weekly	0.05	0.09	6.95	1.41
Bi-Weekly	0.40	0.14	13.60	2.86
Monthly	7.73	0.07	20.27	5.93

intestinalis, the percentage of PO₄ removed was greatest in the daily treatment and lowest in the weekly treatment (p=0.001, Fisher's LSD). The percentage of PO₄ 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). PO₄-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 PO₄ 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 also were similar (p=0.140, Fisher's LSD). In the daily treatment, PO₄-P dropped below the detection limit of 0.05 mg/L, 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 PO₄-P was <0.25 mg/L in all experimental units except one, which measured 2.05 mg/L. Although a portion of the PO₄ 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 PO₄ load in the first week. Throughout the rest of the experiment, PO₄-P was variable, ranging from <0.05 to 0.53 mg/L in all units.

DISCUSSION

Enteromorpha intestinalis and *Ulva expansa* responded most to daily nutrient additions. Algae grew the most with frequent, lower concentration inputs of nutrients compared to more episodic, higher concentration inputs. Since *E. intestinalis* and *U. expansa* in the daily treatments were able to remove practically all of the N and most of the P in the doses, these algae likely retained a greater portion overall of the total nutrient load administered over the course of the experiment. This would translate into greater tissue N and P content of the algae in the daily treatments, which contributes to greater growth of these algae. Similar results were obtained by Ramus and Venable (1987), who also investigated the response of macroalgae to pulsed doses of NH₄ that varied in frequency and concentration. They found that growth of *Ulva curvata* increased with increased frequency of nutrient addition.

Biomass of *Enteromorpha intestinalis* and *Ulva expansa* increased with all frequencies of nutrient addition, however. Algae were able to store nutrients from the pulsed, high-concentration doses and use those reserves to increase and sustain biomass over periods of days to weeks when nutrient supply was low, as suggested by Hanisak (1983). Ramus and Venable (1987) found that *U. curvata* was able to maintain growth (~12% d⁻¹) up to 14 d following a single NH₄ pulse. Likewise, McGlathery *et al.* (1996) found that *Chaetomorpha linum*, another green macroalgal species, could use its tissue N reserves to sustain positive growth for up to 13 d in

the absence of external N supply. Our results are also similar to those of Fujita (1985) and Ryther *et al.* (1981), who determined that *Enteromorpha* and *Gracilaria* could grow off of stored nutrients for 10 and 14 d, respectively. 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 the present study's 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 concentrations 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 review). 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. Although 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 observed with *Enteromorpha intestinalis* and *Ulva expansa* in this study.

The condition of the algae at the beginning of the experiment may have influenced the results. Initial tissue N and P levels of *Enteromorpha intestinalis* and *Ulva expansa* were at the low end of the range of tissue N and P levels measured in these algae collected from the field (Kamer *et al.* 2001), such as may occur in summer when growth and temperature are optimal for maximum growth and the blooms are most prolific. Thus, algae used in this study had a high demand for nutrients at the beginning of the experiment. When algal tissue nutrient levels are greater than those in this experiment, the effects of temporal variation in nutrient supply on macroalgae may not be as dramatic as those seen in our experiment.

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. This was likely a result of both greater initial tissue N concentrations and greater N uptake throughout the experiment. The relationship between increased *U. expansa* tissue N concentration and decreased growth suggests temporal decoupling between nutrient uptake and growth as resources are reallocated from growth to nutrient uptake, which can be energetically expensive (McGlathery *et al.* 1996).

Our results indicate that chronic, frequent, low-grade nutrient supply leads to the greatest increases in macroalgal biomass and potential for growth due to build-up of N and P in algal tissue. Therefore, the frequency of nutrient inputs to coastal systems may be critical in determining macroalgal biomass. However, due to storage of nutrients in algal tissue, and possibly estuarine sediments, effects of large, periodic nutrient pulses may be as important and persistent in natural systems as chronic, low-grade nutrient inputs. This implies that the overall nutrient load to a system

may be more important in governing macroalgal biomass than instantaneous water column nutrient concentrations. Furthermore, temporal scales should be taken into account when regulating nutrient loads in order to minimize macroalgal biomass.

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ACKNOWLEDGEMENTS

This work was partially funded by the Santa Ana Regional Water Quality Control Board. All nutrient analyses were conducted by the Department of Agriculture and Natural Resources Analytical Laboratory, University of California, Davis. We thank the California Department of Fish and Game and John Scholl for access to UNB. We thank Dario Diehl, Lauri Green, Brian Dolan, Risa Cohen, and Anna Armitage for their assistance in the laboratory and field.