

Journal of Experimental Marine Biology and Ecology, 206 (1996) 203-221

JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

Salinity stress, nitrogen competition, and facilitation: what controls seasonal succession of two opportunistic green macroalgae?

Peggy Fong*, Katharyn E. Boyer, Julie S. Desmond, Joy B. Zedler

Pacific Estuarine Research Laboratory, San Diego State University, San Diego, CA 92182-4614, USA

Received 7 November 1995; revised 1 April 1996; accepted 22 April 1996

Abstract

Differential tolerance of low salinity, competition for nitrogen (N), and facilitation by altering N supply all may act to determine the pattern of seasonal succession of Enteromorpha intestinalis (L.) Link and Ulva expansa (Setch) S. and G. in estuaries and lagoons of southern California. Low salinity negatively affected both of these algae. However, when N was in sufficient supply, salinities of 15 ppt favored E. intestinalis while oceanic salinity (35 ppt) favored U. expansa; neither alga had a clear advantage at 25 ppt. When starved of N, E. intestinalis and U. expansa competed directly for nutrients. When grown alone, they had similar N uptake and growth rates; when grown together, E. intestinalis was the superior competitor, negatively affecting growth of U. expansa. In addition, U. expansa facilitated the growth of E. intestinalis when N was in short supply; when grown together, there was a positive effect of U. expanse on E. intestinalis. The mechanism of this effect may have been the release or 'leaking' of DON when U. expansa no longer had sufficient tissue N to grow. Thus, E. intestinalis would be favored immediately after a rain, but would be replaced by U. expansa when N is available and tidal action reestablishes oceanic salinity. However, at the end of the rainy season when N becomes scarce, E. intestinalis would outcompete U. expansa. We hypothesize that U. expansa may facilitate the dominance of E. intestinalis by leaking N that can be assimilated by E. intestinalis.

Keywords: Succession; Salinity; Nitrogen; Competition; Facilitation; Macroalgae

^{*}Corresponding author. Present address: Department of Biology, University of California Los Angeles, 405 Hilgard Avenue, Los Angeles CA 90024-1606, USA. Tel.: (310) 825-5444; Fax: (310) 206-3987; e-mail: pfong@biology.ucla.edu

1. Introduction

Competition, disturbance, and responses to environmental stress are important processes that determine the distribution and abundance of organisms within a community (Dayton, 1971; Connell, 1978; Paine, 1979; Hay, 1981; Paine and Levin, 1981; Roughgarden, 1983). Maintenance and changes in the structure of benthic communities of marine macroalgae are attributed to each of these factors (for reviews, see Carpenter, 1990; Paine, 1990; Olson and Lubchenco, 1990; Maggs and Cheney, 1990). In this paper, we assess the potential for stress tolerance and competition between two dominant macroalgal species with similar life-history characteristics to control seasonal succession in an environmentally variable system subjected to minimal physical disturbance (*sensu* Sousa, 1984).

Opportunistic green macroalgae form extensive mats in shallow estuaries and coastal lagoons in many geographical locations, including southern California (Peters et al., 1985; Fong, 1986; Rudnicki, 1986), Rhode Island (Lee and Olsen, 1985), Western Australia (Gordon and McComb, 1989), Scotland (Raffaelli et al., 1991), and Italy (Sfriso et al., 1987). Under certain conditions, these macroalgal mats detach and float (Smith, 1947; Pickett-Heaps, 1975; Moss and Marshland, 1976). The dominant macroalgae are usually foliose members of the Division Chlorophyta, in the genera *Enteromorpha*, *Ulva*, or *Cladophora*. These genera have simple thallus structures (filamentous, sheet-like, or tubular) and life-history characteristics that favor success in a variable environment, including rapid nutrient uptake and growth rates (Gordon et al., 1981; Rosenberg and Ramus, 1984; Fujita, 1985; Fujita et al., 1988; Duke et al., 1989).

Estuaries or lagoons where opportunistic green macroalgae dominate show wide variation in salinity, temperature and available light or nutrients (Fillet, 1995; Peckol and Rivers, 1995a,b; Rivers and Peckol, 1995), and recent studies have correlated rates of algal photosynthesis or growth to environmental conditions (Fillet, 1995; Rivers and Peckol, 1995) or experimentally determined optimum conditions of one or more variables (Fong and Zedler, 1993; Fong et al., 1993a; Peckol and Rivers, 1995b). In southern California systems, salinity is especially variable, ranging from fresh to brackish in the wet season and up to 80 ppt in the dry season, depending on tidal influence (Zedler, 1982; Zedler and Onuf, 1984; Peters et al., 1985; Fong, 1986; Rudnicki, 1986). *Enteromorpha intestinalis* is known to form floating mats in the wet season, when salinities are low (Peters et al., 1985; Rudnicki, 1986). However, in recent years, both *E. intestinalis* and *Ulva expansa* have dominated in some systems throughout the year (Boyer and Fong, pers. obs.), suggesting that both species may be euryhaline.

Other studies suggest that *Enteromorpha* is more tolerant of reduced salinity than *Ulva*. *E. prolifera* cultured in salinities of 0.1–56.0 ppt had an optimum of 24.5 ppt (Soe–Htun et al., 1986); in the field, it occupied sites ranging from 7–23 ppt (Rijstenbil et al., 1993). Similarly, *E. intestinalis* proliferated through a wide range of salinities (20–31 ppt) in the field, although there was some evidence that sexual reproduction was suppressed in lower (< 23 ppt) salinities (Pringle, 1986). In contrast, *U. lactuca* in culture was more tolerant of hyper- than hyposalinity (Murthy et al., 1988). However, *Ulva rotundata* was present in the field in salinities ranging from 26–36 ppt (De-Casabianca–Chassany, 1989), and *Ulva rigida* bloomed in summer when salinity

remained above 20 ppt (Fillet, 1995). In a comparative study, at 20 ppt growth of U. *lactuca* but not *E. compressa* was reduced (Friedlander, 1992).

These findings led us to test whether *Enteromorpha intestinalis* tolerates low salinity better than *Ulva expansa*. Because these species are taxonomically similar, but morphologically and potentially physiologically different, it is possible that they have different optima. We also asked whether there is any interaction between the two species under different salinity conditions.

Competition among macroalgae has been established or suggested in many algaldominated benthic habitats (Paine and Vadas, 1969; Dayton et al., 1984; Hay, 1986; Paine, 1990). In a recent review, Carpenter (1990)Carpenter, 1990 noted that while competition was frequently invoked as the process controlling algal community structure the underlying mechanisms of competition were not addressed in most studies. In Waquoit Bay, an enriched system in Massachusetts, nitrogen (N) was thought to be in sufficient supply year round to support blooms of Cladophora vagabunda and Gracilaria tikvahiae (Peckol and Rivers, 1995a), although there was some evidence that N was secondarily limiting Ulva lactuca in summer (Rivers and Peckol, 1995). In southern California, large pulses of N associated with runoff and sewage spills enter coastal systems during the wet season (Peters et al., 1985; Fong, 1986; Rudnicki, 1986). Earlier studies have shown that, despite seasonally high loading rates, N limits growth and biomass of Enteromorpha spp. during much of the year (Fong et al., 1993a, 1994a,b). In addition, a microcosm experiment demonstrated that opportunistic green macroalgae competed for N with phytoplankton and benthic mats of cynaobacteria across a wide range of N supply rates (Fong et al., 1993b).

We previously observed that *Enteromorpha intestinalis* (L.) Link usually dominates intertidal to subtidal channels immediately after rain events in Sweetwater Marsh National Wildlife Refuge, San Diego, CA (Boyer and Fong, pers. obs.). Within 2 to 3 wk after each rain storm, *E. intestinalis* was replaced by *Ulva expansa*. In systems that received N enrichment throughout the year (Sweetwater Marsh, Tijuana Estuary), *Ulva. expansa* (Setch) S & G dominated subtidal areas during the dry season while *E. intestinalis* was patchily distributed. In contrast, in systems with no regular N supply in the dry season (Batiquitos Lagoon, San Elijo Lagoon), *E. intestinalis* either continued to dominate, forming floating rafts, or macroalgae disappeared entirely and phytoplankton dominated (pers. obs.; Peters et al., 1985).

In this study, our objective was to assess the ability of *Enteromorpha intestinalis* and *Ulva expansa* to compete for a limiting resource (N) across a resource gradient. In addition, we explicitly investigated the underlying mechanism of competition: consumption and sequestering of a limiting nutrient.

2. Methods

2.1. Variable salinity experiment (N sufficient)

We tested the hypothesis that salinity reduction significantly affected growth and biomass of *Enteromorpha intestinalis* and *Ulva expansa* under initially N sufficient conditions. In addition, we tested whether there was any interaction between the two algae across the salinity gradient tested. Salinity treatments were 35, 25, and 15 ppt, well within the range found in many southern California estuaries and lagoons (Zedler, unpubl. data Peters et al., 1985; Fong, 1986; Rudnicki, 1986). Within each salinity treatment, U. expansa (U) and E. intestinalis (E) were grown alone and together (U + E). Thus, there were nine experimental treatments; replication was 4-fold for a total of 36 experimental units.

Seawater was obtained from Scripps Pier in La Jolla, CA, on 16 January 1995. Initial salinity was 35‰; salinity was reduced by addition of deionized water. Subsamples (n = 3) of the initial water were filtered with Whatman GF/C microfiber filters, and analyzed for NH₄, NO₃⁻ + NO₂, and TKN (Quik Chem Method 10-107-06-1-B for ammonia, revision 19 Sept. 1991; Quik Chem Method 10-107-04-1-C for Nitrate/Nitrite, revision 19 Sept. 1991; Quik Chem Method 10-107-06-2-E, revision 27 March 1990) on a Lachat AutoAnalyzer (model #2100-000). Mean dissolved inorganic N (NH₄ + NO₃⁻ + NO₂) in the initial seawater was 14.9 μ M (SE = 0.9). Ammonium nitrate was added to the deionized water to make the mean inorganic N in the reduced salinity treatments the same as in the seawater treatments (mean = 14.5 μ M, SE = 0.7; ANOVA comparing inorganic N concentration among the water mixed for the three salinity treatments, p > 0.05).

Several large sheets (up to 1.5 m long) of *U. expansa* and benthic mats of *E. intestinalis* were collected from the same channel in Sweetwater Marsh National Wildlife Refuge, a constructed marsh at San Diego Bay, on 17 January 1995. Only apparently healthy algae were taken; algae that had begun to desiccate or decay were avoided. Algae were placed into a cooler partially filled with estuarine water, and all samples were transported to a greenhouse at San Diego State University within an hour of collection.

Thirty-six shallow circular bowls (diameter = 12 cm, maximum depth = 5 cm) placed in an array upon a table in a greenhouse were the experimental units. Salinity and algal treatments were randomly assigned a location within the array. Replication was 4-fold. In each bowl, we placed 200 ml of water (~4 cm depth) with the appropriate salinity. During the course of the experiment, deionized water was used daily to counter evaporation and adjust salinity to $\pm 5\%$ of the treatment levels. Temperatures in the greenhouse were regulated by an evaporative cooling system. At this time of year (January), day time temperatures in the greenhouse were similar to ambient outdoor temperatures (range = 13-23°C); the shallow, often isolated pools of water that support these species of algae are frequently subjected to fluctuations in temperature that follow ambient outdoor temperatures. Minimum temperatures at night were 1-2°C warmer in the greenhouse than outside.

In treatments where algae were grown alone, 4 g wet weight of tissue was added to each experimental unit. For mixed-species treatments, 2 g wet weight of each species was added. Wet weights were measured after algal tissue was sorted and rinsed free of visible epiphytes and animals. To get a standardized wet-weight of each sample, a hand-operated centrifuge (salad spinner) was used to remove excess water. As we did not filter the seawater, ambient abundances of oceanic phytoplankton were included in each experimental unit. However, these remained low throughout the experiment and therefore we assumed they did not contribute significantly to N uptake; earlier work demonstrated that *Enteromorpha* spp. is a superior competitor for N through a broad range of N supplies (Fong et al., 1993b).

The N content of macroalgal tissue at the beginning of the experiment was measured on five subsamples of each of the algae; at the end of the experiment, the N content of tissue from each experimental unit was determined. N content was determined on dried tissue (briefly rinsed in freshwater and dried in a forced air oven at 60°C until constant mass). Tissue was ground with a Wiley mill, subjected to a Kjeldahl digestion, and total Kjeldahl nitrogen (TKN) was measured using standard methods (Quik Chem Method 13-107-06-2-D, revision 28 July 1988) on a Lachat nutrient autoanalyzer (model #2100-000). Mean initial tissue N was 3.5% (SE = 0.3) of dry wt for *U. expansa* and 3.0% (SE = 0.4) for *E. intestinalis*.

The experiment lasted 17 days. After 8 and 17 days (determined *a priori*), algal tissue was removed from each experimental unit, placed in individual mesh bags (knee-high nylon stockings), spun for thirty s in the salad spinner, and weighed. Algal growth was estimated by measuring changes in wet weight over time. Growth or loss rates were calculated as percentage change in wet weight during a sampling interval, and then divided by the number of days per interval to determine the daily percent change.

Among treatment differences in mean algal growth were determined with ANOVA (SuperANOVA version 1.11 for Macintosh); alpha < 0.05 were considered significant. Standard tests of ANOVA assumptions (normality and homogeneity of variances) were conducted. A two factor ANOVA (factors = salinity and the presence or absence of the other species) found no significant effects of the presence of the other species on growth or N content of either *E. intestinalis* or *U. expansa*. Thus, growth rates of algae in the same salinity (\pm the other alga) were pooled. After a significant one factor ANOVA, a PLSD test was used to determine which means were different for each alga. Then *t*-tests were used to compare mean growth of *E. intestinalis* and *U. expansa* at each salinity. We controlled our experiment-wise error for *t*-tests by reducing our acceptable alpha to 0.01 for each test.

2.2. Variable N experiment (salinity constant)

We tested the hypotheses that N addition affected growth, biomass accumulation, tissue N content, and N uptake rate of each alga under initial conditions of N limitation. We also tested whether the outcome of competition for N between these two algae changed across the nutrient gradient tested with U. expanse and E. intestinalis grown alone and together in each nutrient treatment. N treatments were: no-N addition (ambient seawater), medium and high N addition rate. Thus there were nine experimental treatments with 5-fold replication for a total of 45 experimental units.

To mimic an initially N-limited system, we collected algae as in the first experiment, but grew it in low N seawater (35% salinity) for 4 wk prior to the experiment. This caused the tissue N to be reduced from over 3% dry weight to 0.94% (SE = 0.03) for *U. expansa* and 0.99% (0.02) for *E. intestinalis* (n = 3 for each) by the time the experiment was initiated. The N enrichment treatments were applied as a pulse on day 1 and 7 of the experiment, similar in magnitude of N pulses following rainfall in natural systems (Peters et al., 1985). To accomplish this, we used ambient, low-N seawater obtained

from Scripps Pier in La Jolla amended with no added (mean = 14.7 μ M, SE = 0.5), medium (mean = 250.0 μ M, SE = 20.2), and high (mean = 840.0 μ M, SE = 14.3) concentrations of NO₃⁻ (*n* = 3 for each mean); NO₃⁻ was added as NaNO₃. These represented N levels that were comparable to 1) tidal water, 2) N concentrations after a rainfall, and 3) N concentrations after a rainfall combined with a sewage spill (Peters et al., 1985; Fong, 1986, Zedler, unpubl. data). Water in each experimental unit was changed weekly. For the no-N addition treatment, water was replaced weekly with unamended seawater. For N treatments, enriched water was used on day 1 and 7 to mimic two rainfalls a week apart. On day 14 and 21, water was replaced with unamended seawater in all experimental units.

The same bowls used for the salinity experiment were placed in an array on a table in the greenhouse and treatments were randomly assigned. Seawater (200 ml, ~4.0 cm depth) of the proper N concentration and 2 or 4 g (wet weight) subsamples of algae were placed in each experimental unit as in the salinity experiment. The experiment lasted 4 wk (16 May-13 June) to allow time for N pulses in week 1 and 2 and no external N supplied in weeks 3 and 4. In this experiment, temperature and humidity in the greenhouse were regulated by a misting system; this was helpful in retarding evaporation. Average high temperature was 29°C and the average low was 16°C, with a range of 13-32°C. Thus, at this time of year, both day and night temperatures in the greenhouse were 1-2°C warmer than ambient levels.

Algal growth was estimated weekly by measuring changes in wet weight over time as described for the salinity experiment. Cumulative growth or loss rates were calculated as percentage change in wet weight from initial weight. Tissue and water column N contents were analyzed as in the salinity experiment. In addition, N content of algal tissue was calculated on a per experimental unit basis by multiplying the % N by the dry wt of algae for each bowl. Among-treatment differences in mean algal growth, algal tissue N, and water column N were determined with ANOVA (alpha = 0.05). Standard tests of ANOVA assumptions (normality and homogeneity of variances) were conducted. Mean values of tissue N for *E. intestinalis* and *U. expansa* grown alone and together were compared with *t*-tests as in the salinity experiment.

3. Results

3.1. Variable salinity experiment (N sufficient)

3.1.1. Growth response: Day 0-8

Salinity had an effect on the growth rate of both *E. intestinalis* (p < 0.01) and *U. expansa* (p < 0.01) after 8 days of experimental treatment (Fig. 1a). However, the pattern and magnitude of response varied among the two algae. In general, growth of *U. expansa* was more variable than growth of *E. intestinalis* across all salinity treatments. *U. expansa* grew most rapidly at 35 ppt; mean growth at oceanic salinity was 7.5% day⁻¹. At a salinity of 25 ppt, mean growth was lower (~4.5% day⁻¹), but did not vary significantly from growth at 35 ppt (PLSD p < 0.05). At 15 ppt, growth rate of *U.*



Fig. 1. Mean daily (bars are ± 1 SE) percent change in biomass for *Enteromorpha* and *Ulva* under different salinity treatments after a) 8 days and b) 17 days of growth. The two species of algae were grown together and alone for each treatment, but values were pooled after two-factor ANOVA detected no differences due to the presence of the other species.

expansa was significantly slower than at the higher salinity treatments; mean growth was less than 0.5% day⁻¹.

In contrast, although growth of *E. intestinalis* was affected by salinity treatment, the range of growth rates was relatively narrow (Fig. 1a; 2.6–4.5% day⁻¹). Growth rate of *E. intestinalis* was faster at 35 ppt than at the two lower salinities (PLSD, p < 0.05); there were no significant differences in growth among the 15 and 25 ppt treatments. Mean growth rate exceeded 2.5% day⁻¹ when salinity was less than half of ambient seawater (15 ppt).

The relative growth rates of *U. expansa* and *E. intestinalis* varied across the range of salinity tested in this experiment. Salinities of 35 ppt favored the growth of *U. expansa* (*t*-test, p < 0.01) over *E. intestinalis* (Fig. 1a). At 25 ppt, the growth rates of *U. expansa* and *E. intestinalis* did not differ significantly. In the 15 ppt salinity treatment, growth rate of *E. intestinalis* was 2.5 times faster than *U. expansa* (*t*-test p < 0.01).

3.1.2. Growth response: Day 8 - 17

Between day 8 and 17 growth rate of *U. expansa* was uniformly low across salinities; the salinity treatment effect on *U. expansa* disappeared (p = 0.76; Fig. 1b). In contrast, salinity affected the growth rate of *E. intestinalis* between day 8 and 17 (p < 0.05), with growth significantly higher at 35 ppt than in the other two salinity treatments (PLSD p = < 0.01). In addition, the pattern of growth among salinity and algal species was reversed in this time period. Growth of *E. intestinalis* was faster than *U. expansa* in the 35 ppt treatment (*t*-test, p < 0.01), but no different in the lower salinity treatments. We hypothesized that nutrients became limiting in the experimental units during this period, and were more limiting to *U. expansa* than *E. intestinalis*.

3.2. Variable N experiment (salinity constant)

3.2.1. Growth response

There was an effect of both N treatment and the presence of U. expansa (ANOVA; p < 0.05) on the growth rate of E. intestinalis in all but one time interval. The exception was week 1, where there was a significant effect of the presence of U. expansa (ANOVA p < 0.001), but no effect of nutrient treatment (p = 0.23) on growth. E. intestinalis grow very slowly when alone in no-N addition experimental units (Fig. 2a); after 4 wk, total biomass was only 7% greater than the initial biomass. At all sampling periods, growth of E. intestinalis in no-N addition treatments was greater in the presence of U. expansa than in its absence; biomass increased 26% in week 1, decreased to ~10% in week 2, and then increased to about 20% above initial biomass for the rest of the experiment (Fig. 2a). At the end of the experiment, biomass of E. intestinalis was 2.8 times greater in the presence of U. expansa than in its absence.

The growth rate of *E. intestinalis* in bowls without *U. expansa* was faster with N addition than without added N (compare Fig. 2b and Fig. 2c to Fig. 2a). With medium-N addition, the final cumulative change in biomass was about 14% while at high-N addition levels cumulative change in biomass was over 22%. In both medium- and high-N treatments, the growth of *E. intestinalis* was greater in the presence of *U. expansa* than in its absence, similar to the pattern found in the no-N addition treatment.



Fig. 2. Mean cumulative change from initial biomass (bars are ± 1 SE) for *Enteromorpha* and *Ulva* grown under three levels of N and with or without the other species.

Final change in biomass in the presence of *U. expansa* was $\sim 27\%$ and 51% in the medium and high treatments, respectively. Thus, growth of *E. intestinalis* was twice as high with *U. expansa* than without in both nutrient-addition treatments.

When grown alone, the response of *U. expansa* to N addition was similar to that of *E. intestinalis* for all N treatments. There was a significant effect of N treatment on biomass of *U. expansa* for all but the first week of the experiment (ANOVA p < 0.05 for 3 of 4 tests). After 4 wk, cumulative change in biomass of *U. expansa* alone in the no-N addition treatment was 5.5% (Fig. 2d). Change in biomass increased with N addition; with medium and high addition, biomass was ~13% and 24% of initial values, respectively (Fig. 2e and Fig. 2f). These rates were comparable to the response of *E. intestinalis* grown alone for all N treatments.

In contrast, there was a significant negative effect of *E. intestinalis* on the growth of *U. expansa* in weeks 1 and 2, when N was pulsed into the experimental units (p < 0.05); however this treatment effect did not continue beyond week 3 when the N addition ceased. In the no-N addition treatment, *U. expansa* in the presence of *E. intestinalis* lost biomass in the first week of the experiment and remained low through the 4 wk, ending with a loss of ~7% of the original biomass. In the medium- and high-N treatments initial losses of *U. expansa* in the first week were similar (Fig. 2e and Fig. 2f), but biomass then increased through time for gains of 8 and 24% respectively.

3.2.2. NO_3^- uptake rates

Mean concentration of nitrate in the water column was $< 4 \ \mu$ M l wk after each water change, regardless of NO₃⁻ addition rate (Fig. 3). In the no-N addition treatment initial water column nitrate ranged from 12.3–14.7 μ M with very little variability within or among weeks. In the medium- and high-N addition, initial concentrations of NO₃⁻ were 250 and 840 μ M for the first 2 wk, but the same as the no-N addition for weeks 3 and 4 of the experiment. For all 4 wk, water collected from bowls after 1 wk with algae had very low NO₃⁻ concentration, with no differences among treatments. Thus, algae were taking up N rapidly even in the highest N treatment.

Initial ammonium concentration did not differ among N treatments; initial NH₄ concentrations were very low, ranging from 1–3 μ M (Fig. 4a). In the first 2 wk of the experiment, there was some evidence that greater concentrations of ammonium occurred in treatments, with greater NO₃⁻ addition rates. There was a significant effect of N treatment on the amount of NH₄ in the bowls with *E. intestinalis* alone in the May 16–23 sampling period, and in all bowls (*E. intestinalis, U. expansa* and both) in the May 23–30 interval (ANOVA p < 0.05). However, there was no treatment effect in the next two sampling periods. During this time ammonium concentration became more variable. Despite the treatment effects, mean ammonium concentration never exceeded 6 μ M; this showed that there were no large reserves of ammonium in the medium- and high-N treatments.

3.2.3. Dissolved organic nitrogen (DON)

Concentration of DON in the initial water for all 4 water changes ranged from $4.5-16.5 \mu$ M; these values did not vary much within a single date (Fig. 5a). After a week of treatment and in the presence of algae, DON increased in all treatments



Fig. 3. Water column nitrate concentration for three N treatments (bars are ± 1 SE) immediately after weekly water changes (initial) and after 1 wk of growth with *Enteromorpha*, Ulva or both algae (1 wk).



Fig. 4. Concentration of ammonium in the water column for three N treatments (bars are ± 1 SE) immediately after weekly water changes (a) and after 1 wk of growth with *Enteromorpha*, *Ulva*, or both species (panels b–d).

(compare Fig. 5a to Fig. 5b, Fig. 5c and Fig. 5d). Concentration of DON in the water column depended on both the N treatment and the alga, and it varied through time. At the end of the first week of the experiment (May 16–23), there were no differences among treatments in mean DON in units containing either *E. intestinalis* or *U. expansa* alone. In each treatment, there was the most DON in the bowls with *E. intestinalis* alone, intermediate with mixed species, and the least with *U. expansa* alone.

By the end of the second week of the experiment (May 23–30), this pattern changed (Fig. 5). There was an effect of N addition on DON in treatments with both *U. expansa* and *E. intestinalis* alone (p < 0.05); DON was highest in the high-N treatment. During weeks 3 and 4 there was an effect of N treatment on DON in all experimental units regardless of whether the algae were alone or together (p < 0.05). However, despite the relatively high concentrations of DON in the water column (up to 100 μ M), DON was not the major reservoir of N in the N-enriched treatments (250 and 800 μ M NO₃⁻ added in the medium and high treatments, respectively).

3.2.4. N stored in algal tissue

The N content of algal tissue was related to the N treatment, but was unaffected by the presence of the other alga (Fig. 6). The N content of *U. expansa* and *E. intestinalis* was



Fig. 5. DON concentration for three N treatments (bars are ± 1 SE) immediately after weekly water changes (initial) and after 1 wk of growth with *Enteromorpha*, Ulva, or both species (1 wk).

very low at the beginning of the experiment, 0.94% (SE = 0.03) and 0.99% (SE = 0.02) of dry weight, respectively. After 2 wk of N treatment, N content increased in all treatments, including the no-N addition (Fig. 6a and Fig. 6b). There was an effect of N treatment on the N content of both *U. expansa* and *E. intestinalis* (p < 0.001 for both tests) after 2 wk, but no effect of the presence of the other species. Mean N content of *U. expansa* was similar in the medium- and no-N addition treatments and higher in the high-N treatment (1.2% vs. 1.8%). The pattern was similar for *E. intestinalis*, except that N content was more variable within treatment.

During weeks 3 and 4 of the experiment, water in all units was replaced with low nutrient seawater. After 2 wk in no-N addition seawater, N content in the algal tissue was lower in all treatments (Fig. 6c and Fig. 6d). However, the effect of the earlier N treatment on N content remained for both *E. intestinalis* and *U. expansa* (p < 0.001 for both tests); for both species, N content in the high-N treatment was highest. The N content of algae in all but the high-N treatment was lower than the initial content, even though the ambient seawater (~16 μ M) was higher in N concentration than the culture water (~3 μ M). This suggests that both algae initially respond to N pulses by uptake, and then by growth that utilizes sequestered N.

The total amount of N sequestered in algal tissue within each experimental unit varied among treatment and sampling period (Fig. 7). After 2 wk, there was an effect of N treatment on the total amount of algal N experimental $unit^{-1}$ for both *E. intestinalis* and



Fig. 6. Percentage of the dry weight of algae that was N in *Enteromorpha* and *Ulva* grown under three levels of N and with or without the other species. Tissue N prior to N treatment was 0.94 (SE = 0.03) and 0.99 (SE = 0.02) for *Enteromorpha* and *Ulva*, respectively. Tissue N was analyzed again after two wk of growth with pulsed N treatment and then at the end of the experiment, after two wk of low N for all treatments.

U. expansa (p < 0.001 for both tests) with N sequestered in algal tissue increasing with N treatment. When grown alone, there was no difference between the amount of N experimental unit⁻¹ sequestered by either alga (~11, 13.5, and 17 mg N experimental unit⁻¹ in the no-, medium and high addition treatments). Similarly, there was no difference in the N content of the *E. intestinalis* and *U. expansa* when grown together in the no-addition and medium N treatments (Fig. 7a and Fig. 7b; *t*-test, p > 0.05 for both tests). In contrast, the amount of N sequestered in the tissue of *E. intestinalis* exceeded that in *U. expansa* in the high-N treatment (Fig. 7c; *t*-test p < 0.01). This suggests that starved *E. intestinalis* may be a better competitor for N when N is supplied in pulses.

After the fourth week of the experiment (2 wk in no-N addition seawater), the total amount of N contained in algal tissue had declined in all treatments (Fig. 7d, Fig. 7e and



Fig. 7. Nitrogen contained in the tissue of *Enteromorpha* and *Ulva* calculated on a per experimental unit basis. Tissue N was analyzed after two wk of growth with pulsed N treatment and then at the end of the experiment, after two wk of low N for all treatments. A* indicates significant differences in mean values for *t*-tests. Standard errors are in parentheses.

Fig. 7f). The pattern of N loss from algal tissue varied between species and with the presence of the other alga. In the no-N addition treatment, the N in algal tissue declined to just under 8 mg N experimental unit⁻¹, with no difference between the amount of N in the tissue of *E. intestinalis* and *U. expansa* grown alone (Fig. 7d). In contrast to the results at 2 wk, after 4 wk there was a difference between the amount of N in *E. intestinalis* and *U. expansa* grown together (*t*-test, p < 0.05), suggesting *E. intestinalis* benefits from the presence of *U. expansa* during prolonged low-N supply. In this treatment, *U. expansa* lost biomass (Fig. 2d).

In week 4, the range of tissue N experimental unit⁻¹ in the medium-N treatment was only slightly higher (7.5–9 mg N experimental unit⁻¹) than that found in the no-N addition treatment (Fig. 7e). However, the patterns of N distribution were different. When grown alone, there was more N in the tissue of *U. expansa* than *E. intestinalis* (*t*-test, p < 0.05), despite the similarity in biomass (Fig. 2b and Fig. 2e). When grown together, the algae contained equal amounts of N, despite the much greater biomass of *E. intestinalis* than *U. expansa* (27% vs. 8% change from initial). Thus, *E. intestinalis* was able to sustain growth at much lower tissue N than *U. expansa*.

In the high-N treatment, the range of tissue N experimental unit⁻¹ was higher than in the other two treatments (Fig. 7f; 12–13 mg N experimental unit⁻¹) after 4 wk. There was no difference in the tissue N content of *E. intestinalis* and *U. expansa* grown alone. Both species were still growing on N stored during the earlier N treatment (Fig. 2c and Fig. 2f). There was also no difference between the N experimental unit⁻¹ when the two algae were grown together despite the large difference in biomass (51% vs. 23% change from initial biomass); this result was similar to the pattern found in the medium-N treatment, again suggesting that *E. intestinalis* can grow well with lower N content than *U. expansa*, and that it actually benefits from the presence of *U. expansa*.

4. Discussion

The results of these experiments suggest that differential salinity tolerances and N requirements may determine the pattern of succession of dominant macroalgae in coastal lagoons of southern California. In addition, the presence of *U. expansa* may facilitate succession from *U. expansa* to *E. intestinalis* once environmental conditions are unfavorable for *U. expansa*.

When N is in sufficient supply with N reserves of $\sim 3\%$ in the algal tissue, the two macroalgae have differential tolerances to low salinity. At oceanic salinity both species grow at their maximum rate, and because *U. expansa* grows faster than *E. intestinalis*, it will dominate under these conditions. Both species are negatively affected by low salinity. However, *E. intestinalis* is affected less than *U. expansa*, and thus is favored when salinities drop below 25 ppt. This may explain the dominance by *E. intestinalis* directly after rain events. When nutrients can be remobilized, because of luxury uptake and storage of N in tissue, but salinity returns to oceanic, *U. expansa* should regain dominance. Similarly, a nutrient pulse that is greatly enriched with N should favor *U. expansa* if water remains saline.

When starved for N, as evidenced by low tissue N (< 1%, a condition found in the dry season), *E. intestinalis* and *U. expansa* compete directly for nutrients. When grown

alone, they have similar N uptake and growth rates. Classic competition theory would predict that when grown together, each would sequester N, and the one with the greater ability to exploit the limiting resource, i.e., with a greater ability to take up N when N is scarce, would have a negative effect on the other species (Tilman, 1982). In our experiment, when N was limiting, *E. intestinalis* was the superior competitor, and it had a negative effect on growth of *U. expansa.* However, *E. intestinalis* did not always sequester more N. Rather, it was able to use the same amount of N more efficiently because it grows faster with lower tissue N content.

In the field, U. expansa usually has higher tissue N than E. intestinalis, even when collected within a few meters (Boyer and Fong, unpubl. data). The results of these experiments suggest that E. intestinalis is a better competitor for N when N is in low supply or supplied in rapid pulses, but that U. expansa is able to take up and sequester N and grow more quickly when N is in excess over a longer time and when salinities are near oceanic.

U. expansa facilitates the growth of *E. intestinalis* when N is in short supply; when grown together, there is a positive effect of *U. expansa* on *E. intestinalis*. This is a situation that may occur at the beginning of the dry season, when external N loading ceases. One explanation is that the mechanism of this facilitation may be the release or 'leaking' of DON by *U. expansa* when it no longer has N supplies to grow. Alternatively, phytoplankton, bacteria or other protists may be contributing to dissolved inorganic and organic pools of N. *E. intestinalis* may be able to take up and assimilate DON released to the water column directly, or it may take up inorganic N produced by bacterial decomposition of DON as rapidly as it is released to the water column. Thus, inorganic N concentration in the water column always remains low in the presence of rapidly growing *E. intestinalis*. We hypothesize that *U. expansa* facilitates growth of *E. intestinalis* in the classic definition used by Connell and Slatyer (1977), i.e., *U. expansa* modifies the environment to make it less suitable for itself and more suitable for *E. intestinalis*.

Acknowledgments

This project was funded, in part, by the California Department of Transportation, District II. Partial funding was provided by an award from the Earth Island Institute, with additional assistance from the U.S.D.I. National Biological Service (Cooperative Agreement #14-48-0009-92-932). Nutrient analysis was done by M. Hanna. We gratefully acknowledge members of the Pacific Estuarine Research Laboratory, especially Dr. J. Callaway, G. Williams, and G. Noe, for their constructive criticism of an early manuscript as well as their intellectual contribution throughout the work.

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