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Journal of Experimental Marine Biology and Ecology 336 (2006) 99–109

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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Nitrogen content in the brown alga *Fucus gardneri* and its relation to light, herbivory and wave exposure

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Received 5 January 2006; received in revised form 6 April 2006; accepted 20 April 2006

Abstract

Nitrogen is a necessary element for much of seaweed physiology, and is the most common limiting nutrient for marine macroalgae. Therefore, the content of nitrogen in algal tissue is often considered a useful indicator of algal productivity. However, the significance of algal nitrogen content in the field is understudied. We used a factorial manipulation of light and herbivory at three sites in order to evaluate how three factors (light, herbivory, wave exposure) affect the nitrogen content of the brown alga *Fucus gardneri*. We found that nitrogen content was a function of (1) nitrogen supply via amount of water flow and (2) irradiance, possibly via photoinhibitory effects. This research shows that local effects, by shifting nutrient allocation, can change nitrogen content over spatial scales of centimeters.

Keywords: Algal nitrogen content; Herbivory; Irradiance; Nutrient supply; Phlorotannins; Productivity; Water motion

1. Introduction

Nitrogen is widely recognized as the most common limiting nutrient for marine macroalgae, and the broader significance of this observation has motivated a variety of research. Physiological studies have investigated the relationship between nitrogen availability and growth, and have shown that many algae can store unused nitrogen (reviewed by Lobban and Harrison, 1994). Such stores are usually quantified by measuring tissue nitrogen concentration, which we will refer to as nitrogen content.

Attempts to connect algal nitrogen physiology to local environmental conditions have demonstrated variation of nitrogen content in relation to ambient supply. A common pattern is a relative increase in nitrogen content in high-supply and/or low-light conditions (e.g., Chapman and Craigie, 1977; Hanisak, 1979; Rosenberg and Ramus, 1982; Asare and Harlin, 1983; Germann et al., 1987; Pedersen and Borum, 1996; Brenchley et al., 1998). Increased nitrogen content may reflect storage that buffers plant productivity against periods of nutrient scarcity, and in some species, the lag between ambient nitrogen depletion and growth cessation can be weeks or months (e.g., Chapman and Craigie, 1977; Pedersen and Borum, 1996). In contrast,

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doi:10.1016/j.jembe.2006.04.013

other species have shown a tight correlation between growth rate and ambient nutrient supply, suggesting little storage buffer (e.g., Wheeler and North, 1980). The species we studied, *Fucus gardneri* (Silva), has not been investigated for evidence of nitrogen storage. However, the very similar Atlantic species, *Fucus distichus*, has been shown in laboratory studies to accumulate nitrogen under 'winter' conditions of low temperature and light, and tissue nitrogen can reach at least 6%, well beyond the subsistence quota of 0.6% (Rosenberg et al., 1984).

One implication of previous work is that light conditions can interact with nitrogen supply to determine nitrogen content. Over a time scale of hours, pathways involved in photosynthesis and carbohydrate metabolism interact with pathways involved in the uptake, assimilation, and allocation of nitrogen (reviewed by Huppe and Turpin, 1994). Simultaneous regulation of these pathways is to be expected because photosynthesis provides the energy and carbon skeletons for nitrogen uptake and assimilation, and over 50% of plant nitrogen is allocated to the chloroplasts (Huppe and Turpin, 1994). For the purposes of our study, what is relevant is the long-term effects of the coupling of photosynthesis and nitrogen metabolism. Over a time scale of months, the amount of energy and carbon yielded through photosynthesis determines the rate of nitrogen use and therefore interacts with nitrogen supply to determine relative demand for nitrogen. However, the interaction of light conditions and nitrogen conditions is complicated by photoinhibition. High light levels can cause a reversible or an irreversible decline in photosynthetic capacity (reviewed by Long et al., 1994). Such effects are known to occur under normal light levels in full sunlight for many macroalgae, resulting in diurnal photoinhibition during the early afternoon (reviewed by Hanelt, 1996). Thus, the combined effects of varying irradiance and varying nitrogen availability could be hard to predict; e.g., increased light exposure could cause either an increase or a decrease in nitrogen demand depending upon the reaction of the photosynthetic machinery. Because *F. gardneri* experiences variable light conditions, from self-shading in dense canopies to direct sunlight elsewhere, we wanted to test experimentally how different light levels affect nitrogen content. We expected self-shading to decrease the amount of photosynthesis, resulting in lower nitrogen demand and greater nitrogen storage.

Ambient nitrogen supply and irradiance are therefore two ecological factors that may be important in determining algal nitrogen content. A third potential factor is an alteration in carbon allocation resulting from the loss of photosynthetic surfaces to herbivores or herbivore-induced phlorotannin production. Phlorotannins are a class of carbon-based metabolites that occur in brown algae; their purported functions include being a structural component of cell walls, deterring herbivores, and providing protection from UV radiation (reviewed by Ragan and Glombitza, 1986; Targett and Arnold 1998; Schoenwaelder, 2002). Phlorotannins in *F. gardneri* are known to increase by about 20% following simulated (Van Alstyne, 1988) and actual (Dutton and Van Alstyne, unpublished data) grazing. In kelps, induced increases in phlorotannins tend to persist for 5–7 days after the inducing stimulus is removed (Hammerstrom et al., 1998). The synthesis of phlorotannins should require both nitrogen and carbon, potentially causing a shift in their availability for other functions. Likewise, a relaxation in phlorotannin production due to the removal of an inducer may increase the amounts of carbon and nitrogen available for other functions in a way that is similar to an increase in irradiance. Phlorotannin concentrations and inducibility can be affected by environmental nitrogen concentrations. For example, phlorotannin concentrations in *Fucus vesiculosus* were negatively correlated with ambient nitrogen concentrations in the north Baltic Sea (Ilvessalo and Tuomi, 1987) and in Massachusetts, USA (Yates and Peckol, 1993). Likewise, mechanical damage to *F. vesiculosus* resulted in an increase in tissue phlorotannin concentrations at a low nutrient site but not a high nutrient site (Yates and Peckol, 1993).

Finally, nitrogen content could be altered by the degree of wave exposure. Most directly, waves provide an increase in water velocity and turbulence that can enhance nutrient uptake by reducing or eliminating the diffusion boundary layer that forms around algal fronds (Hurd, 2000). Potential indirect effects include the removal of algae and their herbivores by waves, the slowing of herbivore feeding rates, and an increase in light interception though frond stirring (Leigh et al., 1987). There are thus multiple pathways by which varying wave exposure could alter nitrogen content. The most direct effect would be an increase in nutrient loading under increased wave exposure, but wave exposure could also contribute to the effects of light conditions and herbivory that were outlined above.

In order to understand whether light supply and herbivore density could influence the nitrogen content of *F. gardneri*, we manipulated light and herbivorous snail densities in plots of *F. gardneri* at three sites. The sites differed in their wave exposure and, therefore, possibly nitrogen supply as well. We estimated the

demographic response of *F. gardneri* as well as carbon, nitrogen, and phlorotannin content.

2. Natural history

F. gardneri is a brown alga that is abundant in the mid- to high intertidal zones on the rocky shores of northwestern North America. It forms large beds that can harbor copious herbivorous gastropods and crustaceans (200–5000 grazers m^{-2}). At the experimental sites, the small herbivorous gastropods *Littorina sitkana* (Philippi), *Littorina scutulata* (Gould), and *Littorina subrotundata* (Carpenter) are the most prevalent, and *L. sitkana* are known to cause significant damage to *F. gardneri* (Van Alstyne, 1990). *F. gardneri* grows apically, branching dichotomously, with flattened thalli 10–25 cm tall and 1.5–2.5 cm broad at our study sites. Reproductive organs are housed in apical swellings called receptacles that can constitute one-fourth to one-third the length of the thallus (Abbott and Hollenberg, 1976).

The experiment was originally designed to use two sites of different wave exposure, with identical manipulations at each site. We further used two sub-sites at the 'wave-exposed' site, due to limited *F. gardneri* populations of a size large enough for manipulation. However, measurement of flow at these sub-sites revealed a large difference (Fig. 1a), and other variables differed as well. Therefore, we chose to treat the one 'wave-protected' site and the two 'wave-exposed' sub-sites as three separate sites for analysis. The two wave-exposed sites were on Tatoosh Island, Washington, an island 0.5 km from the tip of Cape Flattery at the northwest corner of the Olympic Peninsula (48°24′N, 124°44′W). The intertidal zone on Tatoosh is exposed to the full force of oceanic swells from the Pacific Ocean on the island's northern, southern, and western shores. The two sites on Tatoosh were the Main Beach, which faces NNE, and Strawberry Draw, which faces SE; these sites experience somewhat lower wave energy than other areas around the island. The sites are separated by ~200 m of shoreline, the Main Beach had visibly more herbivores than Strawberry Draw, and the two sites differ measurably in wave energy influx (Fig. 1a). The third site was at First Beach, a rocky outcropping several km into the Strait of Juan de Fuca (48°23′N, 124°36′W); due to its location in the Strait this site is more sheltered than Tatoosh, and flow at our site was correspondingly lower (Fig. 1a). First Beach also contained a greater density of *Littorina* than Tatoosh.

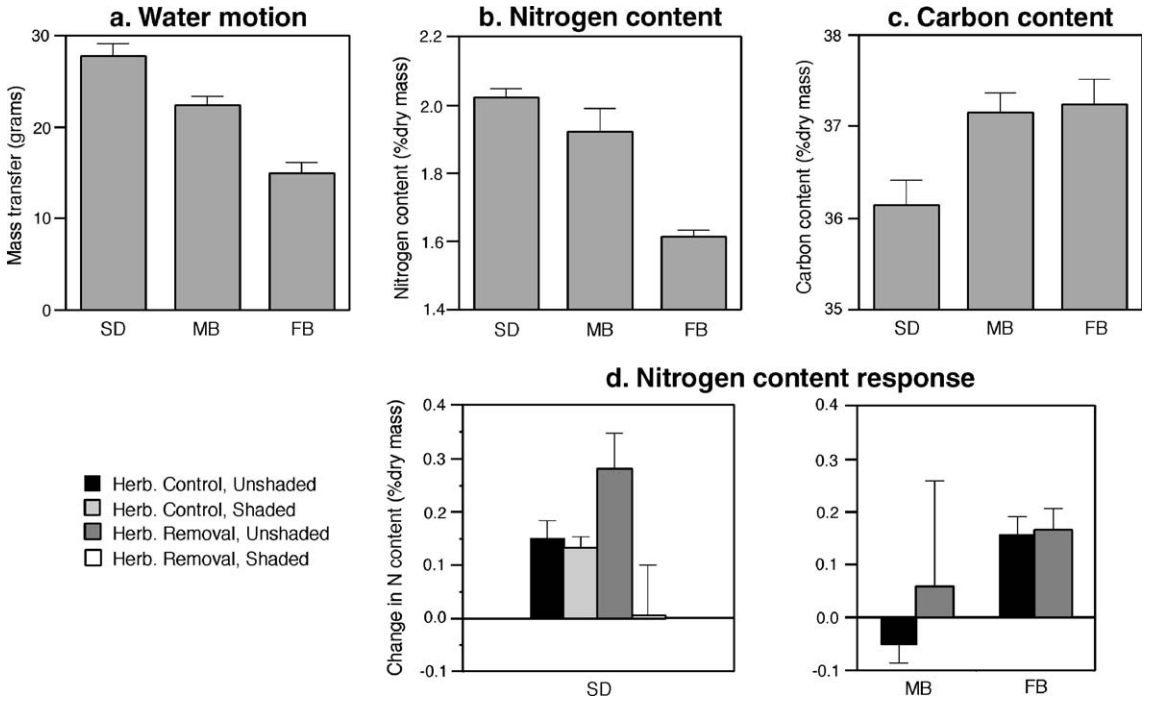

Fig. 1. Site comparisons of (a) water motion (mass transfer, dissolution in g), (b) tissue nitrogen (% dry mass), (c) tissue C (% dry mass) and (d) tissue N as a response to treatment by site (change in % dry mass). SD = Strawberry Draw, MB = Main Beach, FB = First Beach. Data are means ± 1 S.E.

3. Methods

3.1. Treatments

We tested the effects that herbivores and light have on *F. gardneri* growth, reproduction, chemical composition. A light manipulation and an herbivory manipulation were imposed in a factorial design, with twelve treatment blocks partitioned among the three sites (six blocks at First Beach, three at Main Beach, three at Strawberry Draw). In each block, four 30 cm × 30 cm plots of dense *F. gardneri* were thinned by removing primarily the larger/older algae and leaving about twelve smaller algae (mean area 58.6 cm^2). This pruning was performed for two reasons: (1) to eliminate variance in density between sites and (2) to remove any individuals that were large enough to shade others, because our light manipulation was an artificial test of self-shading effects. Of the twelve remaining algae, six were tagged for measurement, and two others had tissue clipped for carbon and nitrogen analysis at the beginning of the experiment. One of four treatment combinations was randomly assigned to each of the four plots: Shaded + Herbivores Reduced, Shaded + Herbivore Control, Unshaded + Herbivores Reduced, and Unshaded + Herbivore Control. All experimental manipulations of *F. gardneri* were performed for approximately 10 weeks, from June 28th to September 7th, 2002.

The light manipulation was designed to isolate the shading effects that large *F. gardneri* can have on other individuals, without altering the moisture-retention effects of these large algae. For the shading treatment, three 7.6 × 30.5 cm strips of 30 mil black vinyl were grommeted at the strip's center and screwed into the rock substrate along the midline of the plot, using stainless steel screws (Fig. 2). The strips were also partially bisected lengthwise. This shading construct was intended to mimic a canopy of larger *F. gardneri*, blocking a significant amount of light during both submergence and exposure. The control for the shading treatment consisted of the same setup, with 10 mil clear plastic replacing the black vinyl.

For the herbivore reduction treatment, all herbivores were removed twice from the plot on the first and fourth, fifth, or sixth day of every two-week tidal cycle. In addition, a 7.6 cm border around the plot was scraped bare, to discourage re-entry by herbivores. The control for herbivore reduction was simply a lack of intrusion upon the herbivores. The number of herbivores present in treatment and control plots at the second removal day of the cycle was counted several times to gauge treatment efficacy. On average, the effect of the herbivore reduction was still visible 3 days after removal, but was not visible 10 days later, as herbivores immigrated from the surrounding environment.

3.2. Physical measurements of the artificial canopy treatments

The temperature and light regimes created by the artificial canopy treatments (black vinyl and clear vinyl) were compared to natural conditions experienced by *F. gardneri*. We selected two representative microenvironments for comparison, a canopy of large *F. gardneri* and an 'open canopy' lacking larger plants.

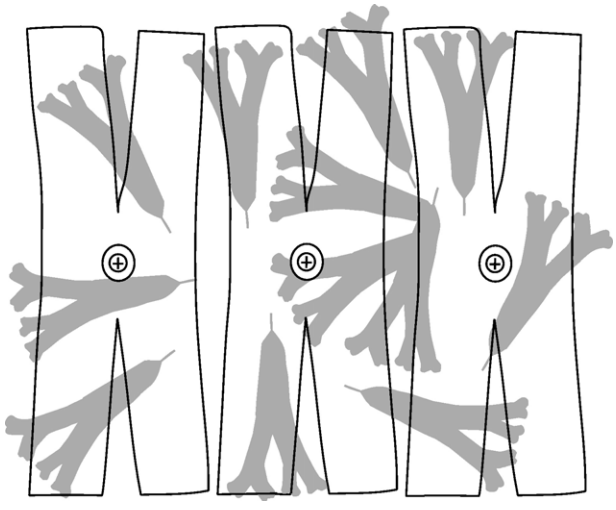

Fig. 2. Schematic drawing of the light manipulation treatment. *Fucus* are in grey, vinyl shade strips are drawn in outline.

Temperature in the field was measured by placing an Ibutton temperature logger (Dallas, TX) in one plot of each of four treatments at Strawberry Draw (open canopy, *F. gardneri* canopy, clear vinyl, black vinyl). The Ibutton loggers were enclosed in a single layer of latex and secured to rocks with Z-spar Splash Zone Epoxy. Temperature data were logged from 4–31 Aug 2005 in 20-min intervals. Light data and additional temperature data were recorded with HOBO Pendant Data Loggers (Onset, Pocasset, MA). These were deployed in the same four plots from 18 Aug–2 Sept 2005, with measurements taken at 10-min intervals. Additional light data were gathered using an outdoor mesocosm setup. An opaque 21-l tub was filled with fresh seawater and placed in full sunlight on 8 Aug 2005. *F. gardneri* and the artificial canopies were suspended in the tub with monofilament, and light levels beneath each treatment were measured with a light probe (LiCor LI-1000 Light Meter, Lincoln NE). For the *F. gardneri* canopy treatment, probe measurements were made under one of four plants collected from Strawberry Draw on 6 Aug 2005.

3.3. Analysis of algal response

The six tagged algae in each plot were measured at the first, fifth, and tenth weeks of the experiment. The number of receptacles present at the end of the experiment was counted as a measure of reproductive output. Two of the algae that were not tagged were collected for carbon and nitrogen analysis at the beginning of the experiment, to get a pre-treatment carbon and nitrogen concentration for each replicate. Two additional tagged algae from each plot were selected randomly at the conclusion of the experiment for carbon and nitrogen analysis.

To create a size estimator that could be used rapidly in the field without damaging the algae; we sampled 25 non-experimental individuals from each site. We measured the maximum length (holdfast to frond apex), the number of apical tips, and the frond area with a LI-COR LI-3100 leaf area meter for each alga. By regressing thallus area on algal length and number of apical tips, we found that the number of apical tips explained 92% of the variation in algal area at Tatoosh (Area = 4.76 * (number of tips) − 5.46), and 83% at First Beach (Area = 3.13 * (number of tips) − 5.3); adding thallus length to the regression only increased R^2 by 1% at each site. Therefore, by counting the number of apical tips, size estimates in cm^2 for each tagged alga were acquired about once a month.

To assess the carbon and nitrogen content of algae in treatments, ~2 cm^2 of vegetative frond was collected from each alga and held on ice until it could be transported to a drying oven. The tissues were dried at 60 °C and sent to the Marine Chemistry Laboratory at the University of Washington for carbon and nitrogen analysis (CEC 440-SHA Elemental Analyzer; Lehman Labs, Inc.). Preliminary data suggested that the shading treatment was ineffective (this was contradicted by the final data), and so only algae from the unshaded treatment were sent for carbon and nitrogen analysis, except for the three Strawberry Draw replicates, for which both shaded and unshaded samples were sent.

Phlorotannins were measured only in the three Strawberry Draw replicates and the Light Control plots of the First Beach replicates. Samples were frozen and shipped by overnight mail to the Shannon Point Marine Center (SPMC) in Anacortes, WA, USA, for analysis. At SPMC, the algal tissues were frozen in liquid nitrogen, lyophilized, and ground with a SPEX mixer/mill to a fine powder. The ground tissues (~0.1 g) were then extracted in 10 mls of 80% aqueous methanol and phlorotannin concentrations were measured using a Folin–Ciocalteu method as described in Van Alstyne (1995). Phloroglucinol-dihydrate was used as a standard.

We estimated the effects of site and herbivore and light treatments on *F. gardneri* growth by measuring the change in size through time. Growth rates can be a function of size in algae (Pfister, 1992; Pfister and Stevens, 2002), and the initial sizes differed greatly among the experimental plots. Estimates of growth rates with the effects of size removed can be made by using the residuals from a linear regression of growth on size (sensu Pfister and Stevens, 2002); however, this method assumes that the size–growth relationship is not altered by the treatments. Therefore, we tested whether the growth–size relationship was equivalent among treatments with a homogeneity-of-slopes ANCOVA (growth rate as response to the treatments, size as covariate). There was no significant effect of treatment on the size-growth slope ($p > 0.05$), so we regressed growth rate on final algal size. Growth rate and size were significantly correlated ($r = 0.777$, $p < 0.001$). The residuals of this regression were used in all analyses of treatment effects and the relationship of growth to nutrients, reproduction, and phlorotannin content.

To distinguish treatment effects among nutrient levels, we first determined whether individual replicates at a given site differed significantly in tissue nutrient content before the experiment with ANOVA. When there was no significant difference, absolute

values could be used to analyze treatment effect. When initial differences among replicates existed, nutrient response was estimated using the change from the initial state as the variable for comparison, i.e., change = (post-treatment value) − (pre-treatment value). Finally, in analyzing reproductive output, the number of receptacles was log-transformed in order to normalize the data.

3.4. Estimating water flow rates

In order to estimate water flow at each site, a dissolution-based method was employed. Empty film canisters were punctured with 2.5-in. stainless steel screws, such that the screw stood along the axis of the cylinder, with its tip extending about 0.25 in. from the opening of the canister. The canister was then filled with wet, dense dental plaster and permitted to dry. At this point the canister was removed, leaving a cylinder of plaster with a screw through its axis. We assumed that the dental plaster would dissolve in relation to water flow (Sutherland, 1990; Yund, 1991; Menge et al., 1995). Ten of the Dentite cylinders were weighed, screwed to the substrate at each site, collected 3 days later, and weighed again to determine the change in mass. For all measurement periods, there was no precipitation during the 3-day period, so all dissolution can be attributed to immersion, wave, and current effects.

3.5. Ambient water nutrient analyses

Ammonium, nitrate and phosphate concentrations were quantified at ten sites on Tatoosh and adjacent to the First Beach site in May, June, and August. Water collected with a 60-ml syringe 2–3 h after low tide was filtered through a GF/C filter into acid-washed HDPE plastic bottles. Samples were immediately put on ice, and then frozen within 4 h of collection. Water nutrient concentrations (PO_4^{3-}, SiO_4, NO_3^-, NO_2^-, NH_4^+) were analyzed by the Marine Chemistry Laboratory at the University of Washington (methods from UNESCO, 1994).

4. Results

4.1. Site characteristics

F. gardneri at the three sites differed significantly in their average nitrogen content, with a descending order of Strawberry Draw, Main Beach, and First Beach (ANOVA $p<0.001$, Table 1, Fig. 1b). Such a large difference in nitrogen content suggests differential nitrogen supply from the surrounding environment. Although we had relatively few simultaneous

Table 1
ANOVAs for response to site and treatment effects

Response factor(s)		*df*	*F*	*p*
(A) Growth	Herb Treat	1	5.3454	0.0266*
	Light Treat	1	0.7304	0.3983
	Site	2	6.2335	0.0047*
	Herb * Light	1	1.6780	0.2034
	Site * Herb	2	2.3235	0.1124
	Site * Light	2	0.0057	0.9942
	Site * Herb * Light	2	1.4618	0.2452
	Error	36		
(B) Reproduction	Herb Treat	1	0.1913	0.6644
	Light Treat	1	7.1832	0.0110*
	Site	2	2.3292	0.1118
	Herb * Light	1	0.0844	0.7730
	Site * Herb	2	0.4958	0.6131
	Site * Light	2	0.3966	0.6755
	Site * Herb * Light	2	1.2800	0.2903
	Error	36		
(C) Size	Herb Treat	1	1.2529	0.2704
	Light Treat	1	0.0000	0.9946
	Site	2	21.973	0.0000**
	Herb * Light	1	0.0014	0.9706
	Site * Herb	2	0.3195	0.7285
	Site * Light	2	0.4487	0.6419
	Site * Herb * Light	2	0.2856	0.7532
	Error	36		
(D) Number of herbs	Herb Treat	1	0.9150	0.3451
	Light Treat	1	0.0136	0.9076
	Site	2	13.342	0.0000**
	Herb * Light	1	0.1329	0.7175
	Site * Herb	2	0.0001	0.9998
	Site * Light	2	0.0750	0.9278
	Site * Herb * Light	2	0.0426	0.9583
	Error	36		
(E) % N	Herb Treat	1	0.5250	0.4758
	Site	2	45.352	0.0000**
	Site * Herb	2	2.3610	0.2754
	Error	24		
(F) % C	Herb Treat	1	0.2000	0.6573
	Site	2	4.9600	0.0157*
	Site * Herb	2	1.2000	0.3187
	Error	24		
(G) Flow	Site	2	21.571	0.0000**
	Error	35		
(H) Phloro.	Herb Treat	1	0.0900	0.7620
	Site	1	25.900	0.0000**
	Site * Herb	1	0.8100	0.3785
	Error	19		

Dependent variables are growth rate (in residuals), reproductive effort (log(1+ number of receptacles), final size (cm^2), number of herbivores (density per plot), nitrogen and carbon content (% dry weight), flow (Δdissolution in g), phlorotannin content (% dry weight). ANOVAs are three-way (Herbivore Treatment * Light Treatment * Site), two-way (Herbivore Treatment * Site), or one-way (Site). *$p<0.05$; **$p<0.001$.

collections at both Tatoosh Island and First Beach ($n=4$), the data did not indicate any strong difference between the two sites in nutrient concentration (Fig. 4). However, water flow was much greater at the Tatoosh sites (Table 1, Fig. 1a), indicating that even if the nutrient concentration does not differ between sites, nutrient supply should be greater at the Tatoosh sites.

The densities of herbivores and growth rates of *F. gardneri* at the three sites both displayed a pattern opposite to the tissue nitrogen concentrations (Fig. 3a and e). There was a descending order of First Beach, Main Beach, Strawberry Draw in each case. *F. gardneri* size, receptacle number, and carbon concentration also differed between sites. Algae at Strawberry Draw were twice as big as algae at the other two sites, and they produced more reproductive tissue (Fig. 3b and c). As expected, algal size and receptacle number were strongly positively correlated at all sites ($p<0.001$ for all sites; FB: $r=0.438$ $n=105$, MB: $r=0.473$ $n=52$, SD: $r=0.784$ $n=32$), presumably because larger algae possess more axial tips on which to form receptacles. Finally, the Strawberry Draw algae, although larger, had a lower carbon content (Fig. 1c).

4.2. Physical measurements of the artificial canopy treatments

Light and temperature conditions created by the two artificial canopy treatments were compared to two types of natural environments, a canopy of large *F. gardneri* and an 'open canopy' lacking any large plants. One of our Ibutton loggers and two of our Onset loggers failed in the field, but the combination of two sets of temperature data and two sets of light data allowed us to compare effects of the four treatments. Although we did not have replication of the data loggers, we used a repeated measures ANOVA on our replicated estimates to test for differences between treatments while accounting for the large diurnal variation in our dependent variables. To test for an effect of tide height, field measurements were coded as 'exposed' if the predicted tide level was less than 2.0 ft above MLLW, and 'submerged' if more than 4.0 ft above MLLW,

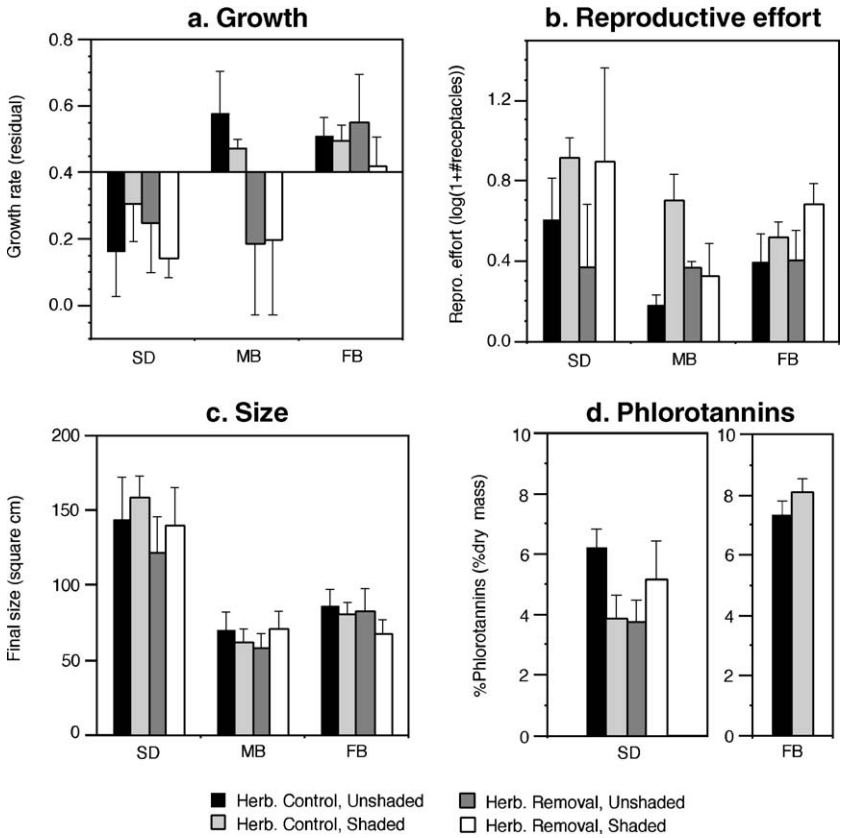

Fig. 3. Site and treatment comparisons of (a) growth rate (in residuals), (b) reproductive effort (log(1+number of receptacles), (c) final size (cm^2), and (d) phlorotannin content (% dry mass). SD=Strawberry Draw, MB=Main Beach, FB=First Beach. Data are means+1 S.E.

relatively conservative designations in the event that there was wave splash.

Ibutton temperature data showed a significant difference between Open Canopy, Clear Vinyl, and Black Vinyl treatments (the *Fucus* Canopy logger failed). The Open Canopy was on average 0.5 °C warmer than the vinyl treatments (respective means and standard errors in °C are 11.785±0.046, 11.313±0.025, 11.281±0.041; one-way repeated-measures $F=566.99$, $p<0.0001$, $n=1919$). Temperature data from the Onset loggers showed that the Black Vinyl treatment was on average 0.2 °C cooler than the *Fucus* Canopy (respective means and standard errors in °C are 11.881±0.043, 12.044±0.046, $F=555.999$, $p<0.0001$, $n=2101$; the Clear Vinyl and Open Canopy loggers failed). Light data from the Onset loggers showed that there was no significant difference between the Black Vinyl and *Fucus* Canopy treatments (respective means in μmol·photons·m^{-2} s^{-1}, 30.448±2.634, 29.994±2.328, $F=0.0622$, $p=0.80$, $n=2101$). However, there was a potential treatment-by-tide height interaction, with the Black Vinyl treatment causing higher light incidence during exposed conditions, and lower light incidence during submerged conditions, though we could not test the significance of this interaction with our data (mean±S.E.: Vinyl Exposed, 39.275±6.538, *Fucus* Exposed, 19.128±1.754, Vinyl Submerged, 11.768±1.095, *Fucus* Submerged, 17.601±2.387). Light data from the mesocosm experiment mirrored that of the field estimates, with the Black Vinyl and *Fucus* Canopy treatments having similar effects (both reduced light by ~90%). The mesocosm data indicated that the Clear Vinyl reduced light by only ~7% (mean± S.E.: Open Canopy, 1389.4±17.3, Black Vinyl, 104.4±49.0, *Fucus* Canopy, 145.0±52.9, Clear Vinyl, 1290.0±44.2, $n=12$). Thus, the physical data indicate that our black vinyl treatment succeeded in lowering the light level greatly and comparably with a natural *Fucus* canopy, while the clear vinyl treatment scarcely reduced light but served as a control for the slight temperature effects caused by the vinyl.

4.3. Light

Shading decreased nitrogen content at the one site where it was measured (Fig. 1d, Table 2), but it had no effect on carbon or phlorotannin content. At Strawberry Draw, all replicates gained nitrogen over the summer, but the shaded replicates gained less (two-way ANOVA, $p=0.039$, Fig. 1d). At all three sites, shading resulted in greater reproductive effort, (three-way ANOVA, $p=0.01$, Fig. 3b).

Table 2
ANOVAs for response to treatments at the Strawberry Draw site

Response Factors		*df*	*F*	*p*
(A) Δ %C	Herb Treat	1	0.18	0.686
	Light Treat	1	0.14	0.719
	Herb*Light	1	0.09	0.767
	Error	8		
(B) Δ %N	Herb Treat	1	0.00	0.980
	Light Treat	1	5.67	0.039*
	Herb*Light	1	4.26	0.073
	Error	8		
(C) C:N	Herb Treat	1	0.22	0.648
	Light Treat	1	0.36	0.567
	Herb*Light	1	4.12	0.077
	Error	8		
(D) Phloro.	Herb Treat	1	0.37	0.56
	Light Treat	1	0.24	0.64
	Herb*Light	1	3.72	0.10
	Error	7		

Effects of light and herbivore manipulations on relative nitrogen/carbon content (% dry weight), carbon/nitrogen ratio (from % dry weight), and phlorotannin content (% dry weight). *$p<0.05$; **$p<0.001$.

4.4. Herbivory

The herbivore removal treatment resulted in a significant density decrease of 28%, when compared to controls 3 days later (Wilcoxon signed-rank test for paired data, all sites pooled, $p<0.001$). However, herbivore density could be on par with controls by the next series of workable low tides, 10–14 days later. Our herbivore removals thus achieved pulses of reduced herbivore density, but not continuous reduced density. There were no instances where the herbivore removal treatment affected nitrogen, carbon, or phlorotannin content (Tables 1 and 2). Herbivore removal effects on growth were opposite to that predicted, as herbivore removal significantly decreased *F. gardneri* growth (three-way ANOVA, $p=0.027$, Table 1, Fig. 3a).

4.5. Correlation analyses

Phlorotannin content was not significantly correlated with nitrogen content at First Beach ($r=0.275$, $p=0.20$, $n=23$) or Strawberry Draw ($r=0.11$, $p=0.68$, $n=16$). Phlorotannin content tended to increase with carbon content at First Beach, but was not significantly correlated ($r=0.406$, $p=0.055$, $n=23$) and was significantly correlated with carbon at Strawberry Draw ($r=0.581$, $p=0.018$, $n=16$). Growth rate was not correlated with phlorotannin content at either site (FB: $r=-0.259$, $p=0.25$, $n=23$; SD: $r=0.276$, $p=0.30$, $n=16$). At Strawberry Draw, reproductive output was negatively

correlated with change in nitrogen content ($r=-0.760$, $p=0.004$, $n=12$; correlation is for plot-averaged values of log(1 + number of receptacles) and Δ%N).

5. Discussion

5.1. Site-scale differences in nitrogen content

Algae at our three sites differed significantly in mean nitrogen content, and this site-scale difference was mirrored by site differences in water motion and herbivore density. Both water motion and nitrogen content decreased in magnitude from Strawberry Draw to Main Beach to First Beach (Fig. 1a and b), and herbivore density varied in the inverse order. In contrast, there was little difference between sites in absolute concentration of ambient nitrogen sources (Fig. 4), suggesting that water motion could account for the large differences in nitrogen content. Leigh et al. (1987) have argued that wave energy can increase nutrient uptake due to a high influx of fresh water to algal fronds. In slowly moving water, a diffusion boundary layer should form around the frond surface. The most rigorous test of this hypothesis would be the quantification of diffusion boundary layer formation in situ, but the difficulty of such measurement has so far precluded such an approach (Hurd, 2000). However, a dissolution-based method such as what we employed has been established as a reliable indicator of mass transfer across surfaces (Porter et al., 2000). Due to the correlation between mass-transfer rate and nitrogen content of *Fucus* among our sites, and the lack of strong site differences in ambient nitrogen sources, water motion may be the cause of between-site variation in algal nitrogen content.

The presence of animals has been shown to influence nitrogen concentrations around seaweeds. Epifaunal animals (Taylor and Rees, 1998) and sessile invertebrates (Williamson and Rees, 1994) can serve as nitrogen sources for some seaweeds, which potentially could result in enhanced tissue nitrogen concentrations. However, nitrogen excretion by herbivores is unlikely to have had a significant effect on *F. gardneri* in this study since among-site tissue nitrogen concentrations were inversely related to herbivore densities.

5.2. The response to light reduction

Our temperature and light measurements demonstrate that the black vinyl treatment successfully reduced light levels to a degree similar to an *F. gardneri* canopy. Compared to an *F. gardneri* canopy the black vinyl

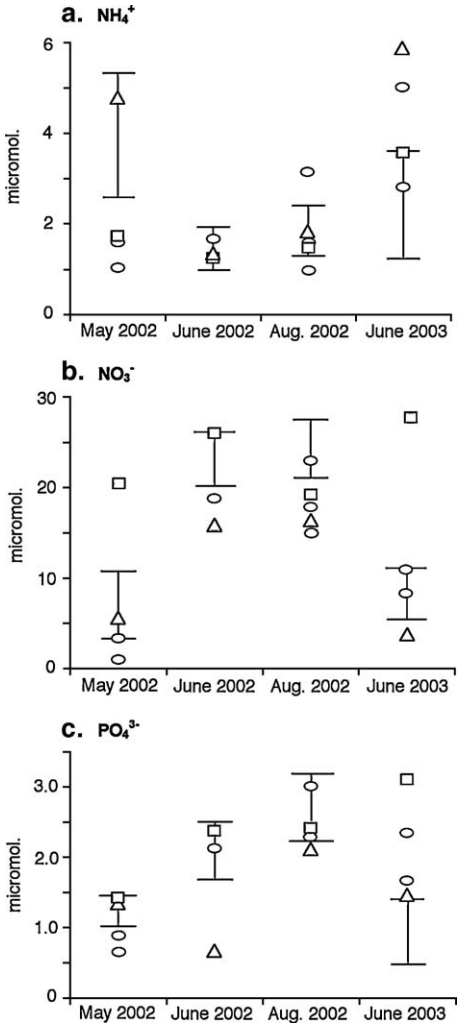

Fig. 4. Ambient nutrient levels (in μM). Intervals are 95% CI for 10 Tatoosh sites. Squares are Main Beach values, triangles are Strawberry Draw values, circles are First Beach values.

caused a small but significant temperature reduction of 0.2 °C. Likewise, our clear vinyl treatment allowed for approximately ambient light levels while maintaining a temperature indistinguishable from that beneath the black vinyl treatment, thus separating irradiance effects from temperature effects.

We expected 'shaded' and 'unshaded' treatments to create conditions for more and less growth, respectively. However, the shaded treatment did not cause relatively higher growth rates, and the unshaded treatment caused a relative increase in reproductive output. A possible explanation for the reduced number of receptacles in the higher light levels is that the unshaded conditions were far enough beyond saturating light levels to result in photoinhibition or photodamage;

decline in photosynthesis under natural highlight conditions has been observed in many algae (Hanelt, 1996). Unfortunately, we did not design our experiment to look at such an effect directly, and cannot test this hypothesis.

An alternative explanation for the greater reproductive output in the shaded treatments is that the decreased light levels caused by the shading treatment may have triggered the formation of receptacles at the apical tips. Reproduction in *F. gardneri* in this region peaks in fall and winter (Ang, 1991) when the amount of light reaching intertidal algae is lowest because of reduced incident light levels and because the lowest spring tides are occurring at night. The cue(s) triggering reproductive activity in this species are not well known; however, the reduced light levels that occur in the fall as the lowest tides switch from day to night and as incident light levels decrease would be a likely candidate. The lack of a change in carbon content in the shaded treatments also suggests that reduced light is not a significant metabolic stress and may be serving as an environmental cue.

Shading caused a reduction in nitrogen content at the Strawberry Draw site and the effect was most dramatic within the herbivore reduction plots. This could have occurred because reproductive tissues, which increased in the shaded treatments, can act as a nitrogen sink (as seen by Brenchley et al., 1998). This interpretation is supported by the negative correlation between reproductive output and nitrogen content. Because increased reproductive output was not associated with decreased growth rate, we do not interpret the increase in reproductive output as a response to potentially unfavorable conditions.

5.3. The response to herbivore reduction

Because our herbivore reduction manipulation only succeeded in reducing herbivore density for bi-weekly pulses of the experiment, we cannot draw strong conclusions about the effects of herbivores on the physiology of these algae. Although herbivore reduction had no effect on nitrogen content, it did result in decreased growth rate.

Phlorotannin concentrations were higher at the First Beach site than the Strawberry Draw site; however, they did not change when herbivores were excluded at either site. The site-specific differences in the phlorotannin concentrations may be due to either localized genetic differences in the algae or to induction by environmental conditions at the two sites. Phlorotannin concentrations can increase with increases in grazing pressures (Van Alstyne, 1988), and grazer numbers were higher at the First Beach site. Phlorotannin concentrations can also be negatively correlated with environmental nitrogen concentrations (Ilvessalo and Tuomi, 1987; Yates and Peckol, 1993), and algae at the Strawberry Draw site had higher nitrogen concentrations than algae at the First Beach site, although nitrogen and phlorotannin concentrations were not correlated among individuals at these sites.

If the between-site differences were a result of herbivore induction then there are several possible reasons why phlorotannin concentrations did not drop in the herbivore reduction treatments. The first is that there may not have been sufficient time for phlorotannin concentrations to relax following the drop in grazing pressures. This is unlikely because our experiments were run for 10 weeks and phlorotannin relaxation following mechanical induction in other algae occurs over a period of several days (Hammerstrom et al., 1998). Phlorotannin concentrations may also not have dropped because grazing was not sufficiently reduced. Induction is likely to occur in response to a threshold level of a stimulus, and, although we reduced grazing pressures by removing herbivores, we did not eliminate it entirely. Therefore, grazing may not have been reduced below the threshold level needed for induction. Also, phlorotannin induction can occur in response to grazing on nearby conspecifics in some algae (Toth and Pavia, 2000). This is an unlikely explanation for the phlorotannin differences in this species because initial evidence suggests that *F. gardneri* phlorotannins do not increase in response to grazing on conspecifics (K. Van Alstyne, unpublished data).

6. Conclusion

Our research aimed to evaluate the importance of several ecological factors potentially tied to nitrogen physiology. Our results show that variance in water motion and light conditions can both affect nitrogen content. Water motion probably affects nutrient supply, and light probably affects the rate of nitrogen use via shifts in the timing or extent of reproductive activity. Variance in herbivory was not supported as a determinant of nitrogen content. The small temporal and spatial scale of our experiment restricts our view to a relatively local scale of factors, but the significant differences in nitrogen content produced by the interaction of two locally varying factors (water motion and light) argue that nitrogen content be considered as much a phenomenon of local ecology as of broader environmental gradients.

Acknowledgements

We thank the Howard Hughes Foundation and the Richter Fund of the University of Chicago (K. F. E.), NSF OCE 97-11802, NSF OCE 01-17801 and the Mellon Foundation (C. A. P.), and an NSF grant (IBN-0090825) to KLV. Comments by M. Liebold and J. Bergelson improved the manuscript. We are grateful to the Makah Tribal Nation for access to the study sites. **[SS]**

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