Effects of light and nutrient availability on the growth, secondary chemistry, and resistance to herbivory of two brown seaweeds

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The separate and interactive effects of light intensity and nutrient availability on the growth, protein content, concentration of C-based secondary metabolites, and susceptibility to herbivory of two brown seaweeds, *Dictyota ciliolata* (Dictyotales) and *Sargassum filipendula* (Fucales), were assessed in the context of the carbon/nutrient balance (CNB) hypothesis. Responses of *Dictyota* and its terpenoid secondary metabolites were often at variance with predictions of the CNB hypothesis while several responses of *Sargassum* and its phenolic secondary metabolites were often predicted by the CNB hypothesis. Findings for these, and other, seaweeds parallel recent terrestrial studies suggesting that the CNB hypothesis rarely predicts responses of terpene producing plants, but more commonly predicts responses of phenolic producing plants.

In a microcosm experiment, nutrient addition significantly increased *Dictyota* growth but had no effect on concentrations of sterols, proteins, terpenes, or susceptibility to herbivores. In a field experiment, nutrient addition did not affect growth or protein levels, generally increased terpenoid metabolites, but did not affect the susceptibility of *Dictyota* to herbivores. Increased light in outdoor microcosms resulted in more growth, reduced terpene concentrations, and increased protein content in *Dictyota*. The high-light plants with more protein and less terpenes were preferentially consumed by the sea urchin *Arbacia punctulata*, but were eaten significantly less readily by the amphipod *Ampithoe longimana*. In a field experiment, higher light intensity increased growth but had no effect on secondary metabolites, protein content, or the alga's susceptibility to herbivores. For *Sargassum filipendula*, plants that settled from spores and grew naturally in an experimentally shaded environment had lower concentrations of phlorotannins than less-shaded plants, but this difference did not affect the plants' susceptibility to amphipod grazing. Branches of *S. filipendula* transplanted to a heavily shaded environment grew less than branches transplanted to a less shaded environment (i.e., light limited growth); however, the concentration of protein and phlorotannins, and the susceptibility of plants to amphipod grazing did not differ. Nutrient addition had no effect on protein or phlorotannin concentrations, but significantly increased the palatability of plants in the higher light treatment. Although the CNB hypothesis has been influential and provides a theoretical template for predicting how various stresses will affect plant defenses and susceptibility to herbivory, ecologists need to consider additional factors in predicting the responses of plants to environmental variation.

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To predict plant responses to environmental changes and how these changes may affect plant resistance to herbivory, ecologists must learn which genetic, physical, and biological factors are important in generating phenotypic variation in plant defenses (Denno and McClure 1983). Variations in resources needed for plant growth have been invoked to explain interspecific variation in the types and concentrations of chemical defenses on evolutionary time scales (Bryant et al. 1983, Coley et al. 1985) as well as intraspecific variation in the concentrations of secondary metabolites on ecological time scales (Bryant et al. 1983). The carbon/nutrient balance (CNB) hypothesis (Bryant et al. 1983) is a prominent theory that predicts phenotypic variation of plant secondary metabolites based on the relative availability of carbon and essential nutrients.

The CNB hypothesis states that when growth is not limited by nutrients, plants will allocate photosynthate to growth rather than to C-based secondary metabolites (Bryant et al. 1983). However, low nutrient availability often limits plant growth more than photosynthesis (Chapin 1980); this results in excess fixed carbon that can be utilized as substrate for C-based secondary metabolites. In so far as only excess photosynthate is used for chemical defenses, this theory assumes a minimal cost of defenses. Environmental conditions that theoretically result in a high C/N ratio, and hence, higher concentrations of C-based secondary metabolites, include high light, high inorganic carbon availability, and low nutrient availability. Low light or high nutrient situations result in a lower C/N ratio; under these conditions, plants should allocate most of their photosynthate to growth processes and concentrations of C-based secondary metabolites should decrease. The CNB hypothesis also states that the concentrations of nitrogen-based secondary metabolites (e.g., alkaloids, peptides, and cyanogenic metabolites) should increase under low C/N ratios (Bryant et al. 1983); however, the logic regarding N-based defenses is less compelling given "nitrogen-based" secondary metabolites actually contain much more carbon than nitrogen.

The broader applicability of chemical defense theories developed by terrestrial workers can be assessed in an independent environment using seaweeds. Seaweeds are good organisms to examine the CNB hypothesis because they are fast growing relative to terrestrial plants and produce several classes of C-based secondary metabolites (Faulkner 1993 and earlier reviews cited therein). Despite these advantages, only one published investigation of the CNB hypothesis has focused on seaweeds (Yates and Peckol 1993). Here we study the response of two species of brown seaweeds to altered light intensity and nutrient availability in the context of the CNB hypothesis. We measure the response of phlorotannins produced by *Sargassum filipendula* C. Agardh (a brown alga in the Fucales) and of diterpenes produced by *Dictyota ciliolata* Kützing (a brown alga in the Dictyotales) to various light and nutrient levels. We also assessed how these responses influenced the susceptibility of the seaweeds to two sympatric herbivores, the sea urchin *Arbacia punctulata* (Lamarck) Philippi and the amphipod *Ampithoe longimana* Smith. Additionally, we assessed the effects of the various light and nutrient treatments on a group of C-based primary metabolites, the sterols, in *D. ciliolata* for comparison of C-based primary vs secondary metabolites.

Methods

Study site and organisms

Radio Island Jetty, North Carolina, USA (34°34'N, 76°40'W) is located in Bogue Sound near an inlet that keeps the site flushed with full salinity seawater. Nutrient availability in these waters is highly variable during the summer as dissolved inorganic nitrogen can fluctuate from nearly zero to about 6 μM within 0.5–2 d (Litaker et al. 1987). Like other hard substrate habitats in coastal North Carolina, this site is characterized by turbid water, high algal biomass, and high numbers of omnivorous urchins and fishes (Hay 1986, Pfister and Hay 1988). Because light is rapidly attenuated by the turbid water, algae are found only above a depth of about 4 m. Despite turbid water and high herbivore densities, *Dictyota ciliolata* and *Sargassum filipendula* thrive at shallow depths on Radio Island Jetty and other inshore hard substrate habitats. We have observed *D. ciliolata* from mean low tide (MLT) to about 3 m below MLT from May to November and *S. filipendula* at a depth of 0–3 m year-round at Radio Island Jetty.

The brown seaweeds *Dictyota ciliolata* and *Sargassum filipendula* and the sea urchin *Arbacia punctulata* that were used in this study came from Radio Island Jetty. Because densities of the amphipod *Ampithoe longimana* are highly variable in the field (Duffy and Hay 1991, Cronin and Hay 1996a), they were initially collected from *Dictyota menstrualis* (Hoyt) Schnetter in grass beds in Bogue Sound, North Carolina and then cultured in six 20-l flow-through tanks on a mixed diet of freshly collected *S. filipendula*, *Padina gymnospora* (Kützing) Sonder, *D. ciliolata*, *D. menstrualis*, *Ulva* sp., *Enteromorpha* sp., and *Hypnea musciformis* (Wulfen) Lamouroux. This allowed a predictably accessible supply of amphipods. Because starvation stress can influence feeding behavior (Cronin and Hay 1996b), herbivores used in feeding assays had excess algal food available to them until just before feeding experiments.

Both *Dictyota ciliolata* and *Sargassum filipendula* are low preference foods for the sea urchin *Arbacia punctulata* (Hay et al. 1986, 1987, Cronin and Hay 1996b, c).

The amphipod *Ampithoe longimana* treats *D. ciliolata* as a high preference food (Cronin and Hay 1996b, c) and *S. filipendula* as a low to intermediate preference resource (Hay et al. 1987, Duffy and Hay 1991). *Dictyota ciliolata* produces three diterpenoid secondary metabolites: pachydictyol A, dictyol B acetate, and dictyodial (Cronin et al. 1995). Pachydictyol A typically occurs at about 0.01–0.02% of plant wet mass (=WM) and dictyol B acetate ranges from about 0.05–0.09% WM; true concentrations of dictyodial are not known because it is relatively unstable (Cronin et al. 1995, Cronin and Hay 1996b, c). Feeding by *Arbacia punctulata* is deterred by pachydictyol A at 0.013% WM (roughly equal to natural concentrations) and by dictyol B acetate at 0.0029% WM (=3–6% of natural concentration, Cronin and Hay 1996b). These compounds each deter *A. longimana* at 0.058% WM, which is about 3–6 × natural concentration for pachydictyol A but 64–100% of natural concentration for dictyol B acetate (Cronin and Hay 1996b). Because of its instability, effects of dictyodial on herbivore feeding behavior are difficult to determine. Available tests, however, suggest that dictyodial might deter urchin feeding at concentrations similar to those found in *D. ciliolata*, but this level of dictyodial does not appear to affect feeding by the amphipod *A. longimana* (Cronin and Hay 1996b). Although phlorotannins occur in *S. filipendula* from Radio Island Jetty (V. J. Paul pers. comm., this study), nothing is known about how *Sargassum* phlorotannins affect interactions with these herbivores. Phlorotannins are common in fucoids and can deter feeding by some species of herbivorous fishes and invertebrates (Steinberg 1985, 1988, Ragan and Glombitza 1986, Van Alstyne 1988, Van Alstyne and Paul 1990, Boettcher and Targett 1993): they are, however, ineffective against others (Steinberg et al. 1991, Steinberg and van Altena 1992, Targett et al. 1995).

Chemical analyses

Tissue to be used for chemical analyses was collected from the same portion of the seaweeds that were used for feeding assays and was collected as feeding assays were started because we wanted to measure what was available to the herbivores. *Dictyota* tissue used for chemical analyses and feeding assays came from the upper 5–7 cm of an alga. *Dictyota* samples used for quantifying secondary metabolites were spun in a salad spinner to remove excess seawater, weighed to the nearest mg, submersed in 2:1 dichloromethane: methanol (DCM:MeOH), and stored at −25°C until they were extracted twice in 6 ml of 2:1 DCM:MeOH and analyzed with silica high performance liquid chromatography (HPLC) following the methods outlined by Cronin et al. (1995). This method also allowed us to quantify sterols, which eluted as a large, single peak after dictyol B acetate.

For *Sargassum*, leaflets 4–7 nodes from the apex were used for feeding assays and chemical analysis because they were relatively large and free of epibionts. The tissue used for phlorotannin measurement was freeze-dried and ground into small pieces. Phlorotannins from these samples were quantified using a modified Folin-Denis method (Ragan and Craigie 1978, Yates and Peckol 1993). About 7 mg of ground, freeze-dried tissue (weighed to the nearest 0.1 mg), were extracted with 1.00 ml of 50% aqueous methanol for 1 d at 1°C with occasional stirring. Two identical 100 µl aliquots of the extraction solution were placed in separate test-tubes. Both aliquots were diluted to 8.4 ml with acidic 10% methanol (pH = 3.3). Polyvinylpolypyrrolidone (PVPP; Sigma) was added to one of the test-tubes and the other test-tube received no PVPP (−PVPP). The PVPP was added to one of the test-tubes because the Folin-Denis reagent reacts with several chemical constituents, including polyphenolics, proteins, amino acids, and ascorbic acid (Andersen and Todd 1968). In contrast, PVPP is reported to bind specifically with polyphenolics, preventing them from reacting with the Folin-Denis reagent. The −PVPP test tube provided the traditional Folin-Denis reading that included polyphenolics, protein, amino acids, etc., while the +PVPP test tube provided a Folin-Denis reading for everything except polyphenolics. The concentration of phlorotannins was calculated from the difference in the two readings, using phloroglucinol as a standard.

Protein analysis was performed on freeze-dried tissue from *Dictyota* and *Sargassum* using the Bradford (1976) assay as modified by Duffy and Hay (1991) with bovine serum albumin as a standard. Although this method is unsatisfactory for determining absolute quantities of protein, it is generally considered reliable for determining relative protein concentration among parts of individual plants (Davis 1988).

Effects of nutrient availability on *Dictyota ciliolata*

The effects of nutrient availability on *Dictyota ciliolata* were assessed by growing plants at different nutrient levels in outdoor microcosms. Twenty-five *D. ciliolata* plants collected from Radio Island Jetty were divided into halves, spun in a salad spinner to remove excess seawater, weighed to the nearest mg, and immediately returned to seawater. The two halves from each plant were randomly assigned to one of two nutrient treatments (ambient and enhanced nutrients). Each half was entwined in a weighted 3-strand polypropylene rope, and placed in a separate 11-l outdoor tank. Each of the 50 tanks had a continuous flow of seawater pumped from the adjacent sound (~4 l/min) and the water was circulated by oil-free air pumped into the bottom of each tank through a diffuser. To better approximate field conditions, light intensity was reduced to 25% of

surface irradiance with opaque plastic mesh. On each of 14 consecutive days the enhanced nutrient halves received a 25 μM N spike; the water flow to all (i.e., ambient and enhanced-nutrient) tanks was stopped and 25 μM N (in the form of Peters 20-20-20 plant food) were added to the "enhanced nutrients" halves. After one hour the water was turned back on.

At the end of the 2-week treatment, growth rate, protein content, concentrations of pachydictyol A, dictyol B acetate, dictyodial, and sterols, and the palatability to the amphipod *Ampithoe longimana* and the urchin *Arbacia punctulata* were determined for the unfertilized (i.e., ambient nutrients) and fertilized (i.e., enhanced nutrients) paired halves using methods modified from Cronin et al. (1995) and Cronin and Hay (1996b). Relative growth was calculated for each half of the 13 plants for which no apparent breakage occurred in either half during the 2-week period of nutrient treatments.

Because urchins often shred algae during our feeding assays and because the unfertilized and fertilized plant halves were morphologically identical, a no-choice assay was performed to avoid placing the different plant halves with the same urchin. Portions of each half (~ 300 mg) were spun in a salad spinner to remove excess seawater, weighed to the nearest mg, placed in a 1.8-l plastic tub with a single urchin, and urchins were allowed to feed for 2.7 d. Another 300-mg portion of each half, weighed to the nearest mg, was placed in a similar tub with no urchin to control for autogenic changes in mass (Peterson and Renaud 1989, Renaud et al. 1990). The amount of each half consumed by an urchin was calculated with the equation $[(U_0 \times C_f/ C_0) - U_f]$; where U_0 and U_f were the masses of the algal pieces exposed to urchin grazing before and after the assay and C_0 and C_f were the masses of the controls for autogenic changes. The sample size for the feeding assays and other measurements were less than 25 (the number of plants initially used for this experiment) because adequate healthy tissue was unavailable for some assays due to mortality, breakage, or fouling.

Ampithoe longimana ($N = 19$) were simultaneously offered a choice of about 40 mg each of unfertilized vs fertilized halves. White threads of slightly different lengths were sewn through the unfertilized and fertilized pieces to distinguish them before they were placed in a plastic cup with about 100 ml of seawater and one amphipod for 2.3 d. Controls for autogenic changes in mass, paired by individual plant halves, were set up in the same manner, but without amphipods. Because weighing errors could account for a substantial portion of the actual mass of tissue consumed by a single amphipod, we measured the surface area of assay algae before and after amphipod feeding. Pieces of algae were quickly photocopied on acetate sheets and returned to seawater. The surface area of images was measured with an area meter (Li-Cor Model 3100). For each half

of each plant, the wet mass:surface area ratio was determined and used to calculate the amount of tissue consumed by amphipods. Tissue consumption, expressed as wet mass, was calculated with the equation $[((A_0 \times C_f/C_0) - A_f) \times WM/SA]$; where A_0 and A_f were pre-assay and post-assay surface areas of tissue exposed to amphipod grazing, C_0 and C_f were pre-assay and post-assay surface areas of autogenic controls, and WM/SA was the wet mass/surface area ratio of the paired control branch. Tissue samples for analyses of protein ($N = 11$), secondary metabolites ($N = 11$), and sterols ($N = 11$) were collected as feeding assays were being set up.

Data for the characteristics of unfertilized vs fertilized plant halves were analyzed with paired-sample *t*-tests. We predicted that fertilization would increase growth and protein concentrations, but no other a priori predictions were made about the effect of nutrient additions on plant traits. Because there were no significant differences in plant nutritive value or concentrations of secondary metabolites (see Results), the palatability of the plant halves to herbivores was not predicted. Two-tailed *P*-values were used when no a priori predictions were made. Directed *P*-values (P_{dir}), with $\gamma/\alpha = 0.8$ as suggested by Rice and Gaines (1994a), were calculated when a priori predictions were made (e.g., growth rate and protein content). Directed *P*-values can be used when the direction of an effect can be predicted but when the experimenter still wants to be able to test for an effect that is in the unanticipated direction (Rice and Gaines 1994b).

Effect of light intensity on *Dictyota ciliolata*

The effects of 4 levels of light intensity on *Dictyota ciliolata* at ambient nutrient levels were assessed in outdoor flow-through microcosms. Twenty-five plants were divided into quarters, weighed, entwined in weighted 3-strand ropes, and placed in 100 outdoor tanks as described above. Each quarter of a plant was placed in one of the following 4 light treatment; 1, 6, 25, or 100% of ambient surface irradiance. The different levels of light intensity were achieved by using different numbers of black plastic mesh as shading layers.

During the 11 d the plant quarters were grown under the different light treatments, we attempted to maintain similar amounts of algal biomass in each microcosm so the water flow, and hence nutrient supply, per mass of seaweed would be similar among the light treatments. This was done by weighing and removing the appropriate number of whole branches from faster growing quarters. Whole branches were removed instead of small pieces from several branches to avoid simulating herbivory. Although this made the mass of algae among the light treatments more similar during the 11 d of growth, the mass of plant quarters at the end of 11

d was still positively correlated with light intensity. At the end of the 11-d growing period, we determined growth rate, the palatability to *Ampithoe longimana* and *Arbacia punctulata*, protein content, and concentrations of pachydictyol A, dictyol B acetate, dictyodial, and sterols using the same methods employed with the unfertilized and fertilized plant halves, except where noted. Because we observed obvious differences in pigmentation among the various plant quarters, the concentration of chlorophyll *a* was also measured using a spectrophotometer.

The final mass of plant quarters used to calculate growth ($N = 20-25$ for each light level) was defined as the mass of tissue removed during the growing period plus the mass of plant quarters at the end of the growing period. Because the potential productivity of tissue removed during the growing period is not included in this calculation, growth rates of fast growing plants are underestimated. Sample sizes were often lower for the high light treatments because fouling was greater under these conditions. Although epiphytes were removed before weighing the tissues for growth determination, less mass of healthy tissue was available for plant quarters grown under higher light despite the higher growth rates.

Because treatments are not independent and standard ANOVA cannot be properly applied when three or more food-types are simultaneously offered to the same individual consumers (Peterson and Renaud 1989), we used no-choice feeding assays with the urchin and the amphipod to determine the palatability of the plant quarters grown at different light levels. About 60 mg of each plant quarter were offered to single amphipods in about 100 ml of seawater for 1.8 d ($N = 19-22$). For each plant exposed to grazing, a paired piece of that plant was set up in an identical manner except without an amphipod; this controlled for changes in mass not due to herbivory. About 200 mg of quarters grown under 1% ($N = 9$), 6% ($N = 6$), or 25% ($N = 8$) of surface irradiance were offered to individual sea urchins in 1.8-l tubs for 3.1 d. Another set of seaweeds was set up in an identical manner but without urchins; this controlled for autogenic changes in mass. Plants grown at full surface irradiance were excluded from this assay because there was not enough non-fouled tissue to run assays with urchins.

The concentrations of soluble protein, pachydictyol A, dictyol B acetate, dictyodial, and sterols were determined as described above ($N = 15-21$). Chlorophylls were eluted from the Florisil columns that were used to prepare extracts for HPLC analysis (Cronin et al. 1995) with 2.5 ml of methanol after the secondary metabolites had been isolated. The methanol solution with chlorophylls was diluted to 5 ml with MeOH and the concentration of chlorophyll *a* was determined with a spectrophotometer.

High light intensity was predicted to increase growth, but no other a priori predictions about the effects of light intensity were made. However, because plant protein content increased significantly with light intensity and the concentrations of two secondary metabolites decreased significantly with light intensity, we predicted the palatability of the seaweeds to herbivores would increase as light intensity increased. Therefore, directed P-values, again with $\gamma/\alpha = 0.8$ as recommended by Rice and Gaines (1994a), were calculated using ordered heterogeneity tests (Rice and Gaines 1994b) following two-way ANOVA, with treatments blocked by individual plants for growth rates and feeding assays. Differences among means were determined with Tukey's HSD multiple comparisons test.

Effects of light and nutrients on *Dictyota ciliolata*

Direct and interactive effects of light and nutrient availability on *Dictyota ciliolata* were assessed with a 2×2 factorial experiment with a split-plot design. Each block had a fertilized and an unfertilized plot (i.e., 2 levels of nutrients) and each plot was split into a more-shaded and less-shaded half (i.e., 2 levels of light). Twenty pairs of 50×60 cm concrete slabs were placed along the shallow west side of Radio Island Jetty in 1–2 m of water. To produce two levels of nutrient availability, one slab (i.e., plot) in each pair (i.e., block) received a plaster of Paris brick laced with fertilizer while the other slab received a plaster of Paris brick without fertilizer. Each fertilizer brick consisted of 42 ml of water, 8.4 g of Peters 20-20-20 plant food, 4.2 g of ammonium chloride, and enough plaster of Paris to solidify this mixture. Control bricks were made with water and plaster of Paris only. The plaster of Paris bricks slowly dissolved and were replaced every 4–6 d. In addition to the slow, steady release of fertilizer from dissolving plaster of Paris bricks, about 1 g of Peters plant food dissolved in seawater was squirted into the center of the fertilized plots every 1–4 d during the experiment. To produce shaded and nonshaded treatments, each of the forty slabs (i.e., plots) had a 40-cm tall steel frame attached to it that supported shading material. Thick plastic mesh was supported over one half of each plot to shade light; the other half had thin plastic mesh to control for caging effects while providing minimal shade. The thick and thin plastic meshes allowed 19% and 72% (average of 8 plots measured with a submersible 2π light meter) of ambient light to reach the transplanted seaweeds, respectively.

Dictyota ciliolata plants were collected from the jetty adjacent to the concrete slabs, divided into quarters, each quarter was entwined in 3-strand rope and placed in one of the 4 light × nutrient treatments. The ropes were attached to 10 cm posts centered on the top surface of each half of the slab. This procedure was

done underwater by SCUBA divers to minimize stress to the delicate seaweeds. One set of plants placed in the various treatments on 21 August, 1993 was retrieved from the field on 30 August because Hurricane Emily was heading towards the site. A second set of plants was placed in the various treatments on 17 September, 1993, and retrieved from the site on 5 October.

Because experimental plants were never removed from the water before placing them in the treatments, their wet mass, and hence growth, could not be measured. Instead, weighed non-experimental plants were placed in the different treatments for 6–8 d and then reweighed in order to calculate growth rates for the various treatments. The concentrations of protein, pachydictyol A, dictyol B acetate, dictyodial, and sterols of experimental plants were determined using methods described previously. Data from the plants deployed on 21 August and 17 September were pooled and analyzed with split-plot ANOVA (plots were blocked by location along the jetty and time period). Directed P-values were used for plant growth data as growth was predicted to be stimulated with increasing light and nutrient availability and for protein, which was predicted to increase with fertilization.

The set of *Dictyota ciliolata* grown under the 4 light × nutrient treatments from 21 August to 30 August was used to test palatability of plants to *Arbacia punctulata*. The no-choice assay was performed using the methods described previously with the exception that the assay lasted 1.8 d. The mean amount of seaweed consumed from the 4 light × nutrient treatments was compared with a two-way ANOVA (i.e., treatments blocked by individual plant and location along jetty).

The set of *Dictyota ciliolata* grown under the 4 light × nutrient treatments from 17 September to 5 October was used to assess the effect of the treatments on the susceptibility of the seaweed to *Ampithoe longimana*. Groups of 4–6 amphipods ($N = 6$–10 groups) were offered the 6 possible combinations of paired choices from the four treatments. Because groups of amphipods, as opposed to individual amphipods, were used, plant masses instead of surface area were used to measure consumption. For each paired comparison among the 4 treatments, about 80 mg of tissue from each treatment, weighed to the nearest mg after removing excess seawater with a salad spinner, were offered to each group of amphipods. Similar-size pieces from each plant were placed in seawater without amphipods to control for autogenic changes in mass. Amphipods were allowed to feed for 2.2 d. Data from each of the 6 separate feeding assays were analyzed with a paired-sample t-test. Directed P-values were used for comparisons of fertilized vs unfertilized plants within each light level because nutrient addition increased some secondary metabolites. Two-tailed P-values are used for the other comparisons as no a priori predictions were made.

Effect of depth on *Dictyota ciliolata*

Traits of *Dictyota ciliolata* growing at 0–1 m and 3 m were compared using naturally growing plants collected from the two depths. Although this is a narrow depth range, it represents the limits of the alga's vertical distribution at the turbid Radio Island site. The palatability of these plants to *Ampithoe longimana* and *Arbacia punctulata* and the alga's concentrations of pachydictyol A, dictyol B acetate, dictyodial, and sterols were determined using methods described previously except that the choice amphipod assay (1 amphipod per replicate) and the no-choice urchin feeding assays lasted 2.4 and 2.8 d, respectively. These data were analyzed with 2-sample t-tests except for the amphipod feeding assay, which was analyzed with a paired-sample t-test (paired by herbivore). No a priori predictions were made, so 2-tailed P-values were used in statistical tests.

Effects of light and nutrients on *Sargassum filipendula*

The experimental procedures used to test direct and interactive effects of light and nutrient availability on *Dictyota ciliolata* were modified slightly to test the effects of light and nutrient availability on *Sargassum filipendula*. *Sargassum* plants were collected from Radio Island Jetty and returned to the Institute of Marine Sciences in seawater. Four similar branches from each of 20 plants were weighed after excess seawater was removed with a salad spinner, entwined in 3-strand rope, returned to Radio Island Jetty, and one branch haphazardly placed in each of the 4 light × nutrient treatments from 15 October to 2 November, 1993. After the branches had grown under the different light × nutrient regimes they were returned to the lab, cleaned of epiphytes, reweighed to calculate growth rate, and analyzed for phlorotannin and soluble protein content. Data from these measurements were analyzed with split-plot ANOVA. Directed P-values were used as above when a priori predictions were made and 2-tailed P-values were used when no prediction was made.

The palatability of *Sargassum* from the 4 light × nutrient treatments was compared by offering *Ampithoe longimana* the 6 paired choices possible from the 4 treatments as described previously. Each group of 4–6 amphipods received a choice of about 40 mg of blades, weighed to the nearest mg, from each of the two treatments and was allowed to feed for 1.9 d. Data from each assay were analyzed with a paired-sample t-test. Two-tailed P-values were used because protein and phlorotannin contents did not differ among treatments. Thus, we did not predict a change in susceptibility to herbivores.

Some *Sargassum* had recruited to the concrete slabs during the summer of 1993. These naturally settled plants were collected from both the more shaded and less shaded sides of each unfertilized slab and assayed for protein and phlorotannin concentrations and their palatability to *Ampithoe longimana* as described in the previous two paragraphs. Plants from the fertilized slabs were not used because the fertilizer appeared to stimulate the growth of epibionts which could potentially confound light intensity effects. These data were analyzed with paired-sample *t*-tests (paired by slab). Two-tailed *P*-values were used for protein and phlorotannin measurement data and a directed *P*-value was used to test the statistical significance of the feeding assay data because a difference in phlorotannin concentration was detected. allowing a prediction of palatability.

Results

Nutrient availability and *Dictyota ciliolata*

Dictyota ciliolata portions in outdoor tanks that experienced a daily. nutrient spike grew 46% more than control plant portions that experienced ambient nutrient availability ($P_{dir} = 0.029$. Fig. 1A). Thus. nutrients at ambient levels were limiting plant growth. *Dictyota ciliolata* did not produce more nutritious tissue when nutrients were more available. as indicated by similar soluble protein concentrations of plants from the two treatments ($P_{dir} = 0.77$. Fig. 1B). Analysis of lipophilic compounds revealed no differences in the concentration of either C-based secondary (i.e.. diterpenes: $P > 0.3$) or primary (i.e.. sterols: $P = 0.32$) metabolites (Fig. 1C). We would liked to have measured the "total secondary metabolites" (i.e.. added concentration of pachydictyol A. dictyol B acetate. and dictyodial) in the two tissue types but the instability of dictyodial prevented the construction of a concentration curve and determination of absolute concentrations. Thus. we are only able to determine relative concentrations of this compound and cannot calculate "total secondary metabolites".

Fertilization did not influence tissue protein or chemical defenses. so we did not expect herbivores to prefer one plant half over the other. As predicted. neither the amphipod *Ampithoe longimana* ($P_{2\text{-tailed}} = 0.9$. Fig. 1D) nor the sea urchin *Arbacia punctulata* ($P_{2\text{-tailed}} = 0.54$. Fig. 1E) fed preferentially between plant halves grown at the different nutrient levels.

Light availability and *Dictyota ciliolata*

Plant growth and protein content both changed significantly with increasing light levels. *Dictyota ciliolata* grown at 1. 6. 25. and 100 % of ambient light grew −4.

+40. +105. and +189% in 11 d. respectively. showing that light was limiting at and below 25% of ambient (Fig. 2A). It appeared that the alga acclimated its photosynthetic apparatus to a light intensity up to a point and then leveled off. The concentrations of chlorophyll *a* became increasingly higher as light intensity increased from 1% to 25% of ambient. but the concentration of chlorophyll a did not differ between plants grown at 25% and 100% of ambient light (Fig. 2B). Soluble protein concentrations did not differ between plant portions grown at 1% and 6% of ambient light. but protein was significantly higher in plants grown at 25% than in plants grown at 1 or 6% of surface irradiance. Plants grown at 100% had significantly more protein than plants grown at 1. 6. or 25% of surface irradiance.

The concentration of 2 of 3 C-based secondary metabolites differed significantly among the 4 light intensities. The concentrations of dictyol B acetate and dictyodial tended to decrease with increasing light (Fig. 2B). Dictyol B acetate was significantly higher in plant portions grown at 1% of ambient light than in plant
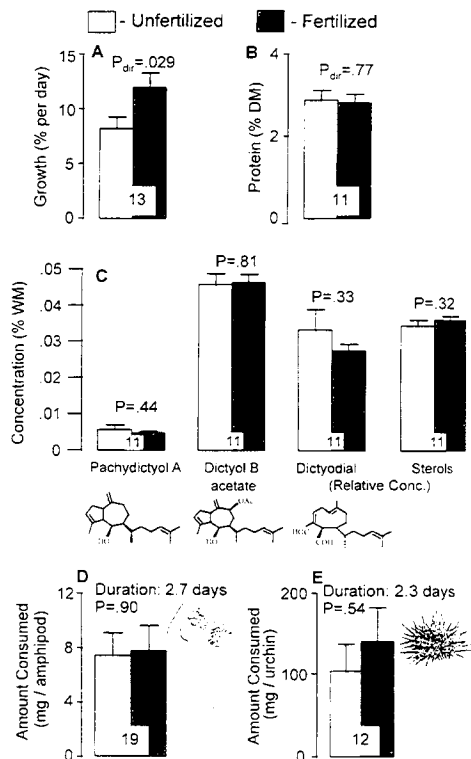

Fig. 1. The effect of nutrient addition on (A) plant growth. (B) soluble protein. (C) C-based secondary (pachydictyol A. dictyol B acetate. and dictyodial) and primary (sterols) metabolites. and susceptibility of *Dictyota ciliolata* to grazing by (D) amphipods and (E) urchins. Open (unfertilized) and stippled (fertilized) bars and error bars represent means − 1 S.E. *P*-values are from paired-sample *t*-tests and sample sizes are given at the base of each pair of bars. The chemical structures of the secondary metabolites are shown below their name.
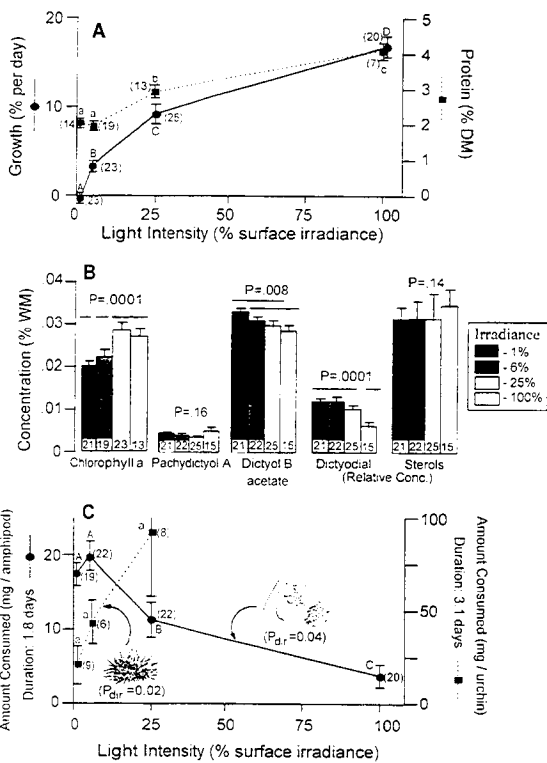
Fig. 2. The effect of light intensity on the (A) growth rates and soluble protein, (B) concentrations of chlorophyll a, secondary metabolites, and sterols, and (C) susceptibility to amphipod and urchin grazing in *Dictyota ciliolata*. Light intensity increases from left to right and sample sizes are given in parentheses or at the base of bars. Letters next to each mean (± 1 S.E.) or lines above the bars correspond to statistical groupings based on Tukey's HSD multiple comparisons test at $\alpha = 0.05$. *P*-value are for the treatment effect from ANOVA blocked by individual plants. Other symbols are as in Fig. 1.

portions grown in full sunlight, neither of which differed significantly from plants grown at 6% or 25% of ambient light. The concentration of dictyodial in plant parts grown at 1%, 6%, and 25% did not differ significantly, but these levels were all significantly higher than levels found in plants grown in full sunlight. Pachydictyol A and the primary metabolite sterols did not differ significantly as a function of light intensity ($P > 0.14$, Fig. 2B).

Because protein content increased and the concentration of some chemical defenses decreased as plants were grown at higher light levels, we predicted the palatability of plants would increase as light intensity increased. The sea urchin *Arbacia punctulata* followed the predicted pattern: they consumed 2–3.6 times as much of the *Dictyota ciliolata* grown at 25% of ambient light as the that grown at 1% or 6% of ambient light (Fig. 2C). Although the Tukey HSD multiple comparison test did not detect any significant differences among the 3 means at $\alpha = 0.05$ (the P_{dir}-value for 1% vs 25% of ambient light was 0.056), the ANOVA did detect a

significant difference among means ($P_{dir} = 0.020$). Surprisingly, the amphipod *Ampithoe longimana* demonstrated the opposite pattern. The dictyol-rich, protein-poor plant portions grown at 1 or 6% of surface irradiance were eaten 3.8–4.5 times more than the protein-rich, dictyol-poor plants grown at full sunlight ($P_{dir} = 0.045$, Fig. 2C).

Effects of light and nutrient availability on *Dictyota ciliolata*

Growth of *Dictyota ciliolata* in a 2×2 factorial experiment with 2 levels each of nutrient availability (ambient and enhanced) and light intensity (19% and 72% of ambient) was influenced by light intensity ($P_{dir} = 0.0004$) but not nutrient addition ($P_{dir} = 0.34$). Plants in the less-shaded treatment grew about 80% more than plants in the more shade treatment. The amount of nutrient enhancement was not determined because it depended on integrated measurements that are impractical to accurately measure (e.g., volume of water diluting nutrients, algal boundary layers in varying flows, and algal nutrient uptake capacities). However, nutrient
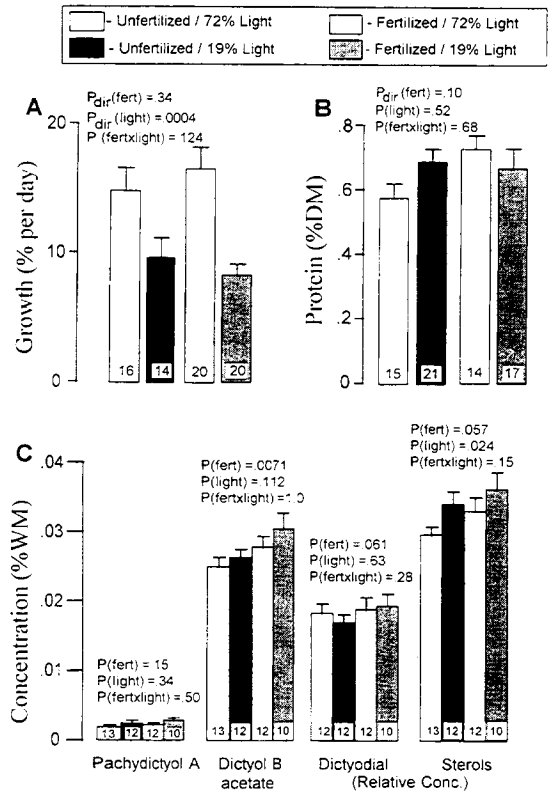

Fig. 3. The direct and interactive effects of light and nutrient availability on the (A) growth, (B) soluble protein, and (C) concentration of C-based metabolites of *Dictyota ciliolata*. *P*-value are from split-plot ANOVA. Other symbols are as in Fig. 1.
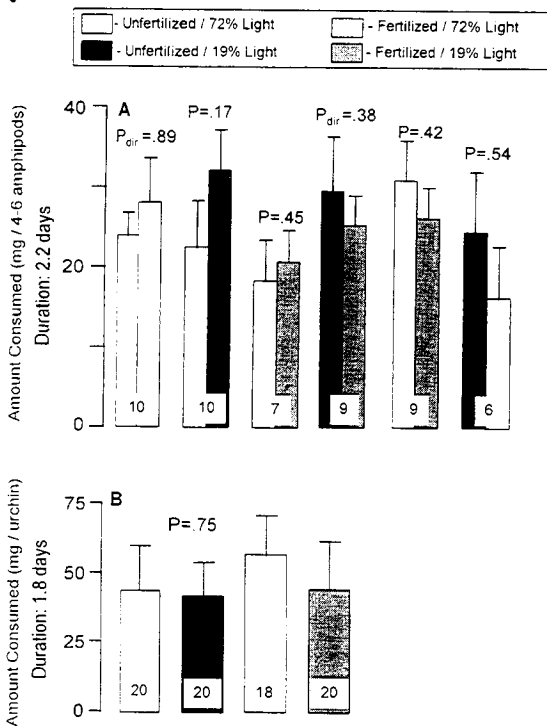
Fig. 4. The amount of tissue from the 4 light × nutrient treatments consumed by (A) *Ampithoe longimana* during choice assays and by (B) *Arbacia punctulata* in a no-choice assay. *P*-values are from paired-sample *t*-tests for the amphipod assays and from the treatment effect of an ANOVA blocked by individual plant for the urchin assay. Other symbols are as in Fig. 1.

availability was enhanced enough to influence some algal metabolites (Fig. 3C) even though neither plant growth nor protein content were affected significantly (Fig. 3A, B). No interactive effects of light intensity and nutrient availability on growth, protein content, or metabolite concentrations were detected (Fig. 3).

The concentrations of pachydictyol A and dictyodial did not differ significantly among the various treatments, although the *P*-value for the fertilizer term in the split-plot ANOVA suggested a possible trend for dictyodial (*P* = 0.061, Fig. 3C). Concentrations of dictyol B acetate were significantly increased by the nutrient manipulation (*P* = 0.0071). Sterols tended to be higher in fertilized plants than in unfertilized plants (*P* = 0.057) and were significantly higher in plants grown in the lower light level (*P* = 0.024). Although fertilization affected concentrations of dictyol B acetate (*P* = 0.007) and nearly affected dictyodial (*P* = 0.06), none of the treatments affected the susceptibility of plants to either amphipods or sea urchins (Fig. 4). Similarly, *Ampithoe longimana* (Fig. 5B) and *Arbacia punctulata* (Fig. 5C) consumed similar amounts of *Dictyota ciliolata* collected from the top (~1 m) and bottom (~3 m) of its vertical range at Radio Island Jetty. Plants from these two depths contained similar

concentrations of metabolites with the exception that dictyodial was 27% lower in deeper plants than in shallower plants (Fig. 5A).

Effects of light and nutrient availability on *Sargassum filipendula*

As with *Dictyota ciliolata*, *Sargassum filipendula* grew more at higher than lower light levels (*P*$_{dir}$ = 0.0009, Fig. 6A). Fertilizer had no significant effect on *Sargassum* growth (*P*$_{dir}$ = 0.13) even though growth of fertilized plants was decreased by 38 to 55% in the fertilized treatment. While cleaning the seaweeds of epiphytes before weighing at the end of the experiment, we noted that fertilized plants appeared to support more encrusting bryozoans than unfertilized plants. Although epibionts were removed before feeding assays, and before protein and phlorotannin measurements, the presence of epibionts could have had indirect effects on these parameters and possibly confounded the effects of fertilizer. Neither protein (Fig. 6B) nor phlorotannin (Fig. 6C) content of *Sargassum* was significantly affected by our light or nutrient manipulations.

Amphipods were offered every possible paired choice among the 4 light × nutrient treatments, but they only
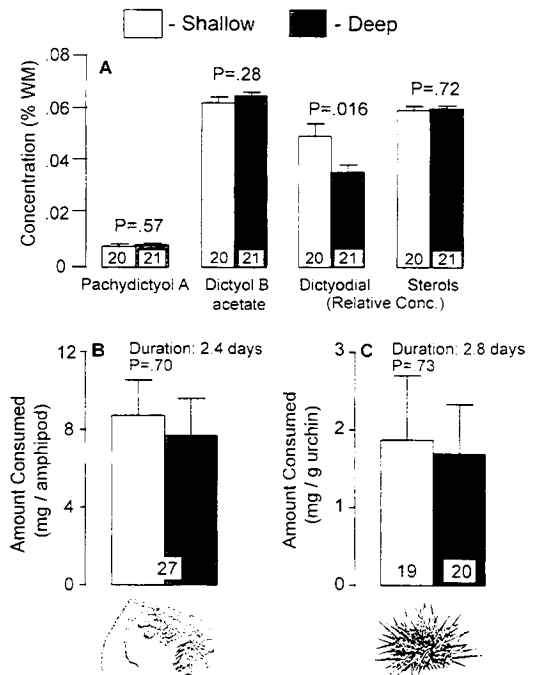

Fig. 5. The (A) concentrations of C-based secondary metabolites and sterols and the palatability to (B) *Ampithoe longimana* and (C) *Arbacia punctulata* of *Dictyota ciliolata* collected from the shallow and deep limits of its vertical range at Radio Island Jetty. *P*-values reported in graphs A and C are from 2-sample *t*-tests and the *P*-value in graph B is from a paired-sample *t*-test (paired by herbivore). Other symbols are as in Fig. 1.
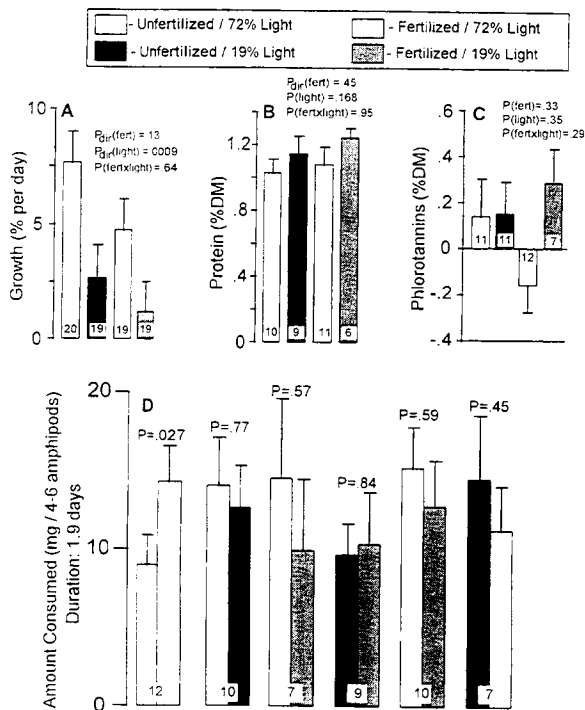
Fig. 6. The (A) plant growth, (B) soluble protein and (C) phlorotannin concentrations, and (D) palatability to *Ampithoe longimana* of *Sargassum filipendula* transplanted to the various light × nutrient treatments shown in the key. *P*-value are from split-plot ANOVA in graphs A–C and from paired-sample *t*-tests in graph D. Other symbols are as in Fig. 1.

variation that occurs over evolutionary, as opposed to ecological, time scales. Although the CNB hypothesis predicts changes in concentrations in all C-based secondary metabolites in relation to the plant C/N status, different classes of these metabolites may respond differently to environmental variation (Waterman and Mole 1989, Muzika 1993), as they are derived from different biosynthetic pathways, represent different proportions of a plant's carbon budget, and may have different turnover rates (Reichardt et al. 1991, Herms and Mattson 1992, Muzica 1993). Early successional herbs and woody plants that produce phenolic compounds appear to conform to the CNB hypothesis (Fajer et al. 1992) more often than plants that produce other classes of C-based secondary metabolites like terpenes (Lincoln and Mooney 1984, Muzika et al. 1989) and furanocoumarins (Zangerl and Berenbaum 1987). As an example, *Abies grandis* (grand fir) offers the opportunity to compare the responses of foliar terpene and phenolic secondary metabolites to nitrogen fertilization in the same species (Muzica 1993). As predicted by the CNB hypothesis, total phenolics decreased as nitrogen fertilization increased growth rates; however, total terpenes did not change in response to fertilization. Phenolics represented a larger portion of the carbon budget than terpenes, being about 7 times more concentrated than terpenes in these plants. Although total phenolics declined with fertilization, only 2 of 13 specific phenolic compounds changed significantly with nitrogen addition. Surprisingly, these two phenolics increased with fertilization.
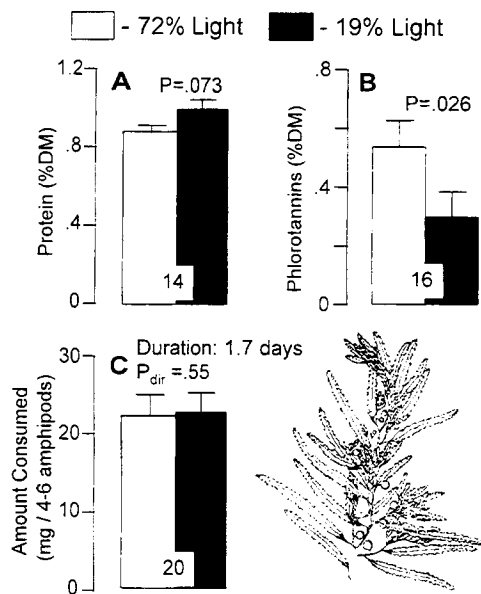
distinguished between the unfertilized vs fertilized plants grown in high light, consuming 50% more of the fertilized plants ($P = 0.027$, Fig. 6D). Amphipods did not distinguish between the other 5 paired comparisons ($P > 0.4$, Fig. 6D).

Protein levels were not significantly different in *Sargassum* that naturally settled and grew in the different light treatments of unfertilized slabs, but levels tended to be slightly higher in the plants from the more-shaded treatment ($P = 0.073$, Fig. 7A). Plants on the less shaded half of unfertilized slabs had 80% more phlorotannins than plants on the adjacent more shaded half ($P = 0.026$, Fig. 7B), but the absolute level of phlorotannins was low in plants from both treatments and *A. longimana* did not distinguish between these plants as food sources ($P = 0.55$, Fig. 7C).

Discussion

The CNB hypothesis was proposed by Bryant et al. (1983) as a framework to explain the response of boreal forest plants to spatial and temporal variation in their environment. This hypothesis focused on intraspecific variation in allocation to defenses that occurs over ecological time scales. This theory can be contrasted to that of Coley et al. (1985) who focused on interspecific

Fig. 7. The (A) soluble protein and (B) phlorotannin content and (C) palatability to *Ampithoe longimana* of *Sargassum filipendula* that recruited to the less shaded and more shaded halves of unfertilized slabs. *P*-values are from paired-sample *t*-tests and other symbols are as in Fig. 1.

The concentrations of C-based secondary metabolites in *Dictyota ciliolata* generally did not respond to nutrient additions in a manner consistent with the CNB hypothesis. When nutrient additions increased growth, which the CNB hypothesis predicts will decrease the concentrations of secondary metabolites as photosynthate is shunted to growth processes, the concentration of pachydictyol A, dictyol B acetate, and dictyodial remained unchanged (Fig. 1C). The CNB hypothesis predicts that because nutrient addition decreases the plant C/N ratio, C-based secondary metabolites should usually decrease when plants are fertilized, yet fertilization of *D. ciliolata* caused a significant increase in the concentration of dictyol B acetate and a non-significant, but suggestive ($P = 0.061$), increase in dictyodial (Fig. 3C). Because the production of C-based secondary metabolites is affected by nutrients, the potential exists that nutrient addition can increase the concentrations of C-based secondary metabolites if their production is more limited by a nutrient-dependent process (e.g., enzyme systems, transcription/translation) than substrates. Similarly, the concentration of C-based resin acids in Scots pines (*Pinus sylvestris*) were increased when these plants experienced increased nitrogen availability (Björkman et al. 1991). In these plants, it was thought that resin acid production was more limited by the size and numbers of resin ducts than by the availability of substrate for resin acid synthesis and that resin duct formation was limited by nitrogen availability.

Dictyota ciliolata responded to differences in light intensity in a manner that was also inconsistent with the CNB hypothesis. In experiments that manipulated light availability, growth of *Dictyota ciliolata* was always positively related to light intensity (Figs 2A and 3A). Thus, we did not have an unambiguous situation where light was available in excess of growth requirements to allow us to distinguish between the following two potential CNB predictions: (1) that concentrations of the secondary metabolites should remain unchanged if light was not available at levels that exceed maximum growth rates or (2) secondary metabolites should increase when higher light intensities afford the alga excess carbon to allocate to defenses (i.e., when nutrients are limiting). Our findings of decreasing C-based secondary metabolites with increasing light (Fig. 2B) are contrary to either prediction of the CNB hypothesis, regardless of the growth potential of the plant.

High light intensity may have decreased the concentration of secondary metabolites if the plants were stressed by the high irradiance. The plants grown at 100% of surface irradiance probably received high doses of ultraviolet radiation, which can decrease concentrations of some secondary metabolites in *Dictyota ciliolata* (Cronin and Hay 1996b). However, *Dictyota ciliolata* collected in the field from two different depths, and hence light environments (although light is not the only variable that differed), did not display a decrease in

secondary metabolite concentration with increased light. Plants growing shallow receive more light and had 38% more dictyodial than plants growing deeper while the concentrations of pachydictyol A and dictyol B acetate did not differ between depths (Fig. 5).

Another potential explanation for the observed decrease in the concentrations of some secondary metabolites in *Dictyota* grown in high-light environments is that fast growing plants have a higher ratio of young to old tissue. Young tissue of *Dictyota ciliolata* has lower concentrations of secondary metabolites than older tissue (Cronin and Hay 1996c), as predicted by the growth-differentiation balance (GDB) hypothesis (Herms and Mattson 1992). The GDB hypothesis stresses that resources must be allocated between growth processes (e.g., cell division and enlargement) and differentiation processes (e.g., cellular specialization, production of defensive chemicals), with growth processes generally preceding differentiation processes (Tuomi et al. 1990, Herms and Mattson 1992). By selecting the top 5–7 cm of *Dictyota ciliolata* individuals for feeding assays and chemical analyses, we used tissue that was phenologically similar among treatments and that was the most recent tissue produced by the alga (thus, much of this tissue could have been produced under our experimental conditions). However, the top 5–7 cm of a fast growing plant would be younger than the same portion of a slow growing plant because *Dictyota* grows via apical meristems. Thus, that the concentrations of some diterpenes were lower in high light than low light treatments (Fig. 2) is consistent with the GDB hypothesis, but contrary to the CNB hypothesis.

The secondary metabolites produced by *Dictyota ciliolata* can significantly affect feeding by co-occurring fishes, urchins, and amphipods in North Carolina (Hay et al. 1987, 1988, Duffy and Hay 1991, 1994, Cronin and Hay 1996a, b, c), but effects of secondary metabolites on consumer feeding can vary with the nutritional quality of the food (Duffy and Paul 1992, Hay et al. 1994). As an example, when our treatments caused small but significant changes in secondary metabolites, but no changes in protein content of *Dictyota* (Fig. 3), there was no significant effect of the treatments on either amphipod or urchin feeding (Fig. 4). In contrast to this, plants with small but significant changes in secondary metabolites coupled with significant changes in protein were treated very differently by both amphipods and urchins (Fig. 2). Surprisingly, *Arbacia punctulata* and *Ampithoe longimana* displayed opposite feeding preferences in this assay. Consumption by urchins was high when protein content was high and chemical defenses were low, as would be expected, but amphipods consumed more tissue that was lower in protein and higher in secondary metabolites. These amphipods are considerably less sensitive to the chemical defenses of *D. ciliolata* than urchins (Hay et al. 1987, Duffy and Hay 1991, 1994, Cronin and Hay

1996b), but they will distinguish between plants with different levels of secondary metabolites when plants are offered as a simultaneous choice (Cronin and Hay 1996a, b, c). Amphipods were not offered a choice during the assays performed with plants from the 4 different light intensities (Fig. 2), thus results demonstrate a willingness to eat the different plants and not feeding preference per se. It is possible that amphipods ate more tissue that was high in secondary metabolites and low in protein because they are very tolerant of secondary metabolites from *D. ciliolata* and they had to eat more tissue to obtain a certain level of nutrients (i.e., they could be compensatory feeders).

Patterns of phlorotannin concentration in *Sargassum filipendula* subjected to various light and nutrient treatments were consistent with some expectations of the CNB hypothesis, but not with the notion that the phlorotannins in this *Sargassum* species affect its susceptibility to herbivores. The concentrations of phlorotannins in *S. filipendula* did not differ when *S. filipendula* branches were transplanted to different light and nutrient environments (Fig. 6) even though the light treatment strongly affected growth; however, phlorotannin concentration was twice as high in plants that recruited and grew in the less shaded half of unfertilized slabs than plants that recruited just centimeters away under the heavily shaded half (Fig. 7B). However, plants that differed in phlorotannin concentration did not differ in palatability to the amphipod *Ampithoe longimana*. Although phlorotannins, as a group, can reduce herbivory by several marine herbivores (Steinberg 1985, 1988, Van Alstyne 1988, Van Alstyne and Paul 1990), more recent studies have found them to be completely ineffective against several generalist herbivores that are common in marine systems (Steinberg and van Altena 1992, Targett et al. 1995).

The brown alga *Fucus vesiculosus* (in the same order as *Sargassum*) is the only other phlorotannin-producing seaweed in which the effects of nutrients on secondary metabolites has been assessed (Ilvessalo and Tuomi 1989, Yates and Peckol 1993). Yates and Peckol (1993) found that the concentrations of phlorotannin in *Fucus* varied with nutrient availability in a pattern that is consistent with the CNB hypothesis. Phlorotannins were higher at a low nutrient site than a high nutrient site. At the low nutrient site, *F. vesiculosus* generally responded to fertilizer by growing more rapidly and by decreasing concentrations of phlorotannins. At the high nutrient site, the alga responded to fertilization to a lesser degree than it did at the low nutrient site: growth rates generally increased but phlorotannin concentrations did not change significantly. Phlorotannins in *Sargassum filipendula* may have responded little to fertilizer because nutrients were not limiting the growth of *Sargassum* at Radio Island Jetty, given that growth of neither *Dictyota ciliolata* nor *S. filipendula* was increased by fertilizer (see also Miller and Hay 1996) but

was increased significantly by higher light levels (Figs 3A and 6A).

Absolute phlorotannin concentrations of the *Sargassum filipendula* used in this study were low (Fig. 7B) compared to values reported for other species of temperate *Sargassum* (Van Alstyne and Paul 1990, Steinberg et al. 1991, Targett et al. 1995). However, these differences among *Sargassum* spp. could be more artifactual than real. The other authors did not perform the polyvinylpolypyrrolidone (PVPP) treatment on phenolic extract which could have led to an overestimation of phenolic content. For example, using the Folin-Denis analysis without the PVPP treatment, Van Alstyne and Paul (1990) found that 1.24% of the dry mass of *Sargassum cristaefolium* from Guam was polyphenolic, yet they were unable to detect the presence of any polyphenolic compounds using the Lindt's reagent, a test specific for phlorotannins. Had we not performed the PVPP treatment on phenolic extracts, our estimates of polyphenolics would have been 9–11 times (Fig. 7B) or 35 times (pooled plants from Fig. 6C) greater than the concentrations reported. As a comparison, the concentration of polyphenolics in *Fucus vesiculosus* was overestimated by about 70% when the Folin-Denis analysis was performed without the PVPP treatment (Yates and Peckol 1993).

Interactions among plants, the environment, plant secondary metabolites, and herbivores are complex and diverse. It is therefore unlikely that a single theoretical framework like the CNB hypothesis will correctly predict the phenotypic responses of all plants to environmental variability. The CNB hypothesis did not correctly predict the response of C-based diterpenes in *Dictyota ciliolata* to altered light or nutrient environments (Figs 1, 2, 3, and 5) nor did it predict the response of monoterpenes in the red alga *Portieria hornemannii* to nitrogen or phosphorus fertilization (Puglisi and Paul in press). In fact, the responses observed in *D. ciliolata* were sometimes opposite of CNB predictions.

The response of C-based phlorotannins in *Sargassum filipendula* to altered light and nutrient levels were not as well documented as for diterpenes in *D. ciliolata*, but changes in phlorotannin concentration were more consistent with predictions of the CNB hypothesis (Figs 6C and 7B). Similarly, the concentrations of phlorotannins in *Fucus vesiculosus* conformed reasonably well to the CNB hypothesis (Ilvessalo and Tuomi 1989, Yates and Peckol 1993). From these limited data on 1 red and 3 brown seaweeds, it appears that the CNB hypothesis may be useful in predicting responses of phlorotannins (in 2 of 2 species) but not of terpenes (in 0 of 2 species). Similarly, terrestrial ecologists have also found that the CNB hypothesis appears to predict the responses of phenolic compounds better than for other C-based secondary metabolites (Reichardt et al. 1991, Muzica 1993). More sophisticated models to predict phenotypic responses of plants to altered environmental conditions

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will need to integrate other factors that may influence variation in chemical defenses including the successional stage of the plant (Bryant et al. 1987), types of chemical defenses (Muzica 1993), turnover rates of compounds (Reichardt et al. 1991), ambient nutrient levels (Bryant et al. 1987, Yates and Peckol 1993), physical, chemical, or biological triggers for metabolite production or degradation (Renaud et al. 1990, Fajer et al. 1992, Cronin and Hay 1996a, c), and nutrient-dependent structures necessary to synthesize, transport, and sequester metabolites (Björkman et al. 1991).

References

Andersen, R. A. and Todd, J. R. 1968. Estimation of total tobacco phenols by their bonding to polyvinylpyrrolidone. – Tobacco Science 12: 107–111.

Björkman, C., Larsson, S. and Rolf, G. 1991. Effects of nitrogen fertilization on pine needle chemistry and sawfly performance. – Oecologia 86: 202–209.

Boettcher, A. A. and Targett, N. M. 1993. Role of polyphenolic molecular size in reduction of assimilation efficiency in *Xiphister mucosus*. – Ecology 74: 891–903.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. – Anal. Biochem. 72: 248–254.

Bryant, J. P., Chapin, F. S. and Klein, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. – Oikos 40: 357–368.

– , Chapin, F. S., Reichardt, P. B. and Clausen, T. P. 1987. Response of winter chemical defense in Alaska paper birch and green alder to manipulation of plant carbon/nitrogen balance. – Oecologia 72: 510–514.

Chapin, F. S. 1980. The mineral nutrition of wild plants. – Annu. Rev. Ecol. Syst. 11: 233–260.

Coley, P. D., Bryant, J. P. and Chapin, F. S. 1985. Resource availability and plant antiherbivore defense. – Science 230: 895–899.

Cronin, G. and Hay, M. E. 1996a. Amphipod grazing induces chemical defenses in the brown alga *Dictyota menstrualis*. – Ecology (in press).

– , and Hay, M. E. 1996b. Susceptibility to herbivores depends on recent history of both the plant and animal. – Ecology (in press).

– , and Hay, M. E. 1996c. Within-plant variation in seaweed chemical defenses: Optimal defense theory versus the growth-differentiation balance hypothesis. – Oecologia 105: 361–368.

– , Lindquist, N., Hay, M. E. and Fenical, W. 1995. Effects of storage and extraction procedures on yields of lipophilic metabolites from the brown seaweeds *Dictyota ciliolata* and *Dictyota menstrualis*. – Mar. Ecol. Prog. Ser. 119: 265–273.

Davis, E. M. 1988. Protein assays: a review of common techniques. – American Biotechnology Laboratory 6: 28–37.

Denno, R. F. and McClure, M. S. 1983. Variable plants and herbivores in natural and managed systems. – Academic Press, New York.

Duffy, J. E. and Hay, M. E. 1991. Food and shelter as determinants of food choice by an herbivorous marine amphipod. – Ecology 72: 1286–1298.

– , and Paul, V. J. 1992. Prey nutritional quality and the effectiveness of chemical defenses against tropical reef fishes. – Oecologia 90: 333–339.

– , and Hay, M. E. 1994. Herbivore resistance to seaweed chemical defense: the roles of mobility and predation risk. – Ecology 75: 1304–1319.

Fajer, E. D., Bowers, M. D. and Bazzaz, F. A. 1992. The effect of nutrients and enriched CO_2-environments on production of C-based allelochemicals in *Platago*: A test of the carbon/nutrient balance hypothesis. – Am. Nat. 140: 707–723.

Faulkner, D. J. 1993. Marine natural products. – Natural Products Reports 10: 497–539.

Hay, M. E. 1986. Associational plant defenses and the maintenance of species diversity: turning competitors into accomplices. – Am. Nat. 128: 617–641.

– , Lee, R. R. Jr. and Guieb, R. A. 1986. Food preference and chemotaxis in the sea urchin *Arbacia punctulata* (Lamarck) Philippi. – J. Exp. Mar. Biol. Ecol. 96: 147–153.

– , Duffy, J. E., Pfister, C. A. and Fenical, W. 1987. Chemical defense against different marine herbivores: are amphipods insect equivalents? – Ecology 68: 1567–1580.

– , Renaud, P. E. and Fenical, W. 1988. Large mobile versus small sedentary herbivores and their resistance to seaweed chemical defenses. – Oecologia 75: 246–252.

– , Kappel, Q. E. and Fenical, W. 1994. Synergisms in plant defenses against herbivores: interactions of chemistry, calcification, and plant quality. – Ecology 75: 1714–1726.

Herms, D. A. and Mattson, W. J. 1992. The dilemma of plants: To grow or defend. – Q. Rev. Biol. 67: 283–335.

Ilvessalo, H. and Tuomi, J. 1989. Nutrient availability and accumulation of phenolic compounds in the brown alga *Fucus vesiculosus*. – Mar. Biol. 101: 115–119.

Lincoln, D. E. and Mooney, H. A. 1984. Herbivory on *Diplacus aurantiacus* shrubs in sun and shade. – Oecologia 64: 173–176.

Litaker, W., Duke, C. S., Kenney, B. E. and Ramus, J. 1987. Short-term environmental variability and phytoplankton abundance in a shallow tidal estuary. – Mar. Biol. 96: 115–121.

Miller, M. W. and Hay, M. E. 1996. Coral-seaweed-grazer-nutrient interactions on temperate reefs. – Ecology (in press).

Muzika, R. M. 1993. Terpenes and phenolics in response to nitrogen fertilization: a test of the carbon/nutrient balance hypothesis. – Chemoecology 4: 3–7.

– , Pregitzer, K. S. and Hanover, J. W. 1989. Changes in terpene production with fertilization of grand fir (*Abies grandis*) seedlings. – Oecologia 80: 485–489.

Peterson, C. H. and Renaud, P. E. 1989. Analysis of feeding preference experiments. – Oecologia 80: 82–86.

Pfister, C. A. and Hay, M. E. 1988. Associational plant refuges: convergent patterns in marine and terrestrial communities result from differing mechanisms. – Oecologia 77: 118–129.

Puglisi, M. P. and Paul, V. J. 1996. Intraspecific chemical variation in the red alga *Portieria hornemannii*: monoterpene concentrations are not influenced by nitrogen or phosphorus enrichment. – Mar. Biol. (in press).

Ragan, M. A. and Craigie, J. S. 1978. Phenolic compounds in brown and red algae. – In: Hellebust, J. A. and Craigie, J. S. (eds), Handbook of phycological methods: physiological and biochemical methods. Cambridge Univ. Press, New York. pp. 157–179.

– and Glombitza, K. W. 1986. Phlorotannins, brown algal polyphenols. – In: Round, F. E. and Chapman, D. J. (eds.) Progress in phycological research, vol. 4. Biopress, Bristol, pp. 129–241.

Reichardt, P. B., Chapin, F. S., Bryant, J. P., Mattes, B. R. and Clausen, T. P. 1991. Carbon-nutrient balance as a predictor of plant defense in Alaskan balsam poplar: Potential importance of metabolite turnover. – Oecologia 88: 401–406.

Renaud. P. E.. Hay. M. E. and Schmitt. T. M. 1990. Interactions of plant stress and herbivory: intraspecific variation in the susceptibility of a palatable versus an unpalatable seaweed to sea urchin grazing. – Oecologia 82: 217–226.

Rice, W. R. and Gaines, S. D. 1994a. 'Heads I win, tails you lose': testing directional alternative hypotheses in ecological and evolutionary research. – Trends Ecol. Evol. 9: 235–237.

– and Gaines. S. D. 1994b. Extending nondirectional heterogeneity tests to evaluate simply ordered alternative hypotheses. – Proc. Natl. Acad. Sci. USA 91: 225–226.

Steinberg. P. D. 1985. Feeding preferences of *Tegula funebralis* and chemical defenses of marine brown algae. – Ecol. Monogr. 55: 333–349.

– 1988. The effects of quantitative and qualitative variation in phenolic compounds on feeding in three species of marine invertebrate herbivores. – J. Exp. Mar. Biol. Ecol. 120: 221–382.

– , and Van Altena. I. A. 1992. Tolerance of marine invertebrate herbivores to brown algal phlorotannins in temperate Australasia. – Ecol. Monogr. 62: 189–222.

– . Edyvane. K.. de Nys. R.. Birdsey. R. and Van Altena. I. A. 1991. Lack of avoidance of phenolic-rich brown algae by tropical herbivorous fishes. – Mar. Biol. 109: 335–343.

Targett. N. M.. Boettcher. A. A.. Targett. T. E. and Vrolijk. N. H. 1995. Tropical marine herbivore assimilation of phenolic rich plants. – Oecologia 103: 170–179.

Tuomi. J.. Niemelä. P. and Sirén. S. 1990. The Panglossian paradigm and delayed inducible accumulation of foliar phenolics in mountain birch. – Oikos 59: 399–410.

Van Alstyne, K. L. 1988. Herbivore grazing increases polyphenolic defenses in the intertidal brown alga *Fucus distichus*. – Ecology 69: 655–663.

– and Paul. V. J. 1990. The biogeography of polyphenolic compounds in marine macroalgae: temperate brown algal defenses deter feeding by tropical herbivorous fishes. – Oecologia 84: 158–163.

Waterman. P. G. and Mole. S. 1989. Extrinsic factors influencing the production of secondary metabolites in plants. – In: Bernays. E. A. (ed.). Insect-plant interactions. Vol. 1. CRC, Boca Raton, FL, pp. 107–134.

Yates. J. L. and Peckol. P. 1993. Effects of nutrient availability and herbivory on polyphenolics in the seaweed *Fucus vesiculosus*. – Ecology 74: 1757–1766.

Zangerl. A. R. and Berenbaum, M. R. 1987. Furanocoumarins in wild parsnip: effects of photosynthetically active radiation. ultraviolet light, and nutrients. – Ecology 68: 516–520.