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Effects of nutrient enrichment on growth and phlorotannin production in *Fucus gardneri* embryos

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ABSTRACT: Resource-allocation models predict trade-offs between growth and chemical defense. The carbon/nutrient balance hypothesis (CNBH) predicts that plants will allocate carbon to growth when nutrients are abundant and allocate it to carbon-based antiherbivore defenses when nutrients are limiting. In marine systems, field and laboratory tests of the CNBH with phlorotannin-producing algae have generally supported the predictions of the model. However, these tests have all measured phlorotannin concentrations in adult algae rather than juveniles, which are susceptible to higher grazing pressures. We experimentally tested some of the predictions of the CNBH in early post-settlement stages of a common intertidal macroalga, *Fucus gardneri*, by growing *F. gardneri* embryos in media enriched with 3 nutrients: nitrogen, phosphorus, and iron. Phlorotannin concentrations across all treatments were correlated with embryo size but not with growth rates. As predicted by the model, nitrogen enrichment significantly enhanced embryo growth rates and decreased phlorotannin concentrations. Iron enrichment alone had no effect on phlorotannin concentrations, but did affect growth. The effects on growth were primarily in altering morphology rather than changing the overall size of the embryos. Phosphorus enrichment had no effect on growth, but did significantly lower phlorotannin concentrations. Surprisingly, there was a significant iron–phosphorus interaction effect on both growth and phlorotannin concentrations. Enrichment with a combination of iron and phosphorus had a stronger negative effect on growth and phlorotannin concentrations than would have been predicted based on the individual effects of these 2 nutrients. The combination of iron and phosphorus enrichment may have physiologically stressed the embryos, resulting in decreased phlorotannin production. Our results suggest that ontogeny plays a strong role in determining secondary metabolite levels and that nutrient addition can affect secondary metabolite production in embryos by (1) altering resource allocation patterns, or (2) providing a physiological stress that results in reduced secondary metabolite production.

KEY WORDS: Phlorotannins · Nutrients · Chemical defense · Resource allocation · *Fucus*

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INTRODUCTION

Nutrients, such as nitrogen, phosphorus, and iron are thought to limit the growth of algae in many marine systems. A recent meta-analysis of the effects of nutrient addition on phytoplankton has emphasized that there is no one limiting nutrient in the oceans (Downing et al. 1999). Instead, the ability of enhanced concentrations of a nutrient to increase phytoplankton

growth will depend on location. For example, nitrogen enhances algal growth in all but pristine waters. In pristine oceanic waters, iron and phosphorus tend to be limiting (Martin & Fitzwater 1988, Martin et al. 1994, Coale et al. 1996, Downing et al. 1999). Phosphorus limitation also tends to be more important in low latitude environments (Short et al. 1985). The ability of nutrients to limit the growth of macroalgae is not as well understood. Nitrogen is considered the major nutrient limiting growth of many coastal macroalgal species during at least some portions of the year (Fujita

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et al. 1989, Wheeler & Björnsäter 1992, Pedersen & Borum 1996, 1997, Stimson et al. 1996, Larned 1998, but see Wootton et al. 1996, Nielsen 1998). However, both phosphorus and iron also have the potential to limit macrophyte growth (Delgado & LaPointe 1994, LaPointe 1995, 1997, Suzuki et al. 1995).

Nutrients may also affect other aspects of resource allocation in plants. Plants have limited resources to partition among essential functions, including growth, reproduction, and defense (Bazzaz et al. 1987). The carbon/nutrient balance hypothesis (CNBH) predicts that nutrient concentrations regulate resource allocation to growth and defense in plants (Bryant et al. 1983). Plants that are not light- or carbon-limited are predicted to allocate carbon to growth when nutrients are abundant and to carbon-based defensive compounds when nutrients are limiting.

In marine systems, tests of the CNBH hypothesis with phlorotannin-producing algae have generally supported the predictions of the model. Phlorotannins have concentration-dependent (Geiselman & McConnell 1981, Steinberg 1985) antifeeding activity towards many (Ragan & Glombitza 1986, Steinberg 1988, Steinberg & van Altena 1992, Winter & Estes 1992, Steinberg et al. 1995, Targett & Arnold 1998), although not all, herbivores. Phlorotannin concentrations in populations of *Fucus vesiculosus* from 3 sites from the northern Baltic Sea were negatively correlated with the nitrogen concentration of algal thalli (Ilvessalo & Tuomi 1989). Similar patterns were found in populations of *F. vesiculosus* in estuarine sites in Massachusetts, USA (Yates & Peckol 1993). Algae at a low-nitrogen site generally had higher phlorotannin concentrations than algae at a high-nitrogen site, although some seasonal variation in phlorotannin concentrations did occur. Yates & Peckol conducted experimental enrichments with an ammonium nitrate and phosphate fertilizer at both sites. Enrichment resulted in an increase in tissue nitrogen concentrations in algae from both sites but a decrease in phlorotannin concentrations only in algae from the low-nitrogen site. At both sites, growth rates were higher in fertilized algae, but the response was more pronounced in the low-nitrogen site. Pavia et al. (1999) found growth rates and phlorotannin concentrations to be negatively correlated in Northern Atlantic *Ascophyllum nodosum*, but both growth and phlorotannin concentrations were generally unrelated to tissue nitrogen concentrations or highly variable.

In laboratory-culture experiments, the tropical brown alga *Lobophora variegata* fertilized with different concentrations of nitrogen showed patterns that were consistent with the CNBH. Algae grown in high-nitrogen media had higher nitrogen concentrations and lower phlorotannin concentrations than algae grown in low-nitrogen media (Arnold et al. 1995). However, results

of similar experiments that measured terpene production in tropical algae under different nutrient regimes have produced contrasting results. Puglisi & Paul (1997) conducted field experiments in which they added nitrogen, phosphorus, and a combined nitrogen/phosphorus treatment to sites containing the tropical red alga *Portieria hornemannii*. They found no differences in concentrations of the terpene octadene or in triglyceride concentrations in any of their experimental treatments. Similarly, terpene concentrations in the brown alga *Dictyota ciliolata* did not change when the algae were fertilized with ammonium chloride and with plant food containing nitrogen, phosphorus and potassium (Cronin & Hay 1996a).

Previous tests of the CNBH with seaweeds have primarily been conducted on adults. However, grazing pressures on many seaweeds are thought to be most intense during juvenile stages (see reviews by Hawkins & Hartnoll 1983, Chapman 1986, Santelices 1990, and Vadas et al. 1992). In intertidal algae, high rates of mortality occur in early post-settlement periods and herbivory can be the major factor limiting survival through juvenile stages (e.g., Hawkins 1981, 1983, Lubchenco 1983, Harris et al. 1984, Keser & Larson 1984, Gunnill 1986, Dean et al. 1988, Chapman 1990).

The impact of herbivores changes as algae pass through different life history stages. For example, field experiments showed that the major cause of mortality in *Lessionia nigrescans* gametophytes <15 µm long was herbivory, but in larger sporophytes the primary source of mortality was wave action (Martinez & Santelices 1998). The palatability of algae also changes as the plants get larger. In laboratory feeding experiments, littorinid snails preferred 1 to 3 cm juvenile *Fucus serratus* to individuals that were 5 to 7 cm long (Lubchenco 1983). Juvenile *Ulva lactuca* were preferred to adult *U. lactuca* by *Littorina littorea*, but adult *Fucus serratus* were preferred to juveniles (Watson & Norton 1987). Van Alstyne et al. (1999a) found that intertidal urchins, snails, and isopods often had distinct preferences when offered tissues from juvenile and adult kelps and rockweeds, but the preferences were not predictable; herbivores could prefer adult tissues, juvenile tissues, or exhibit no preference, depending on the algal species and herbivore species being examined.

The high mortality rates of juveniles and the shifts in herbivore preferences among life history stages suggest 2 mutually exclusive resource-allocation strategies that juveniles may have evolved for surviving herbivory: (1) juveniles may allocate more resources for defense at a cost of lower growth rates, or (2) they may allocate fewer resources to defense and grow rapidly in order to 'escape in size'. Both strategies assume that defense production incurs metabolic costs, an assump-

tion that has not been rigorously tested in most systems (Angrawal & Karban 1999).

Concentrations of defensive compounds in juveniles and adults provide evidence that both strategies may be used. Small juveniles of the brown algae *Alaria marginata*, *Egregia menziesii*, *Fucus gardneri*, *Hedophyllum sessile*, and *Lessoniopsis littoralis* produced higher concentrations of phlorotannins than older or adult stages (Van Alstyne et al. 2001b). Juveniles of the siphonous green algae *Halimeda maculosa* were significantly less calcified and contained higher concentrations of the diterpenoid feeding-deterrent halimadial than adult tissues (Paul & Van Alstyne 1988). Lipid-soluble extracts from juveniles were more deterrent towards herbivorous fishes than were extracts from adults (Paul & Van Alstyne 1988). However, in other species, defense levels may be lower in juveniles than adults. Juveniles of the rockweeds *F. vesiculosus* and *F. evenescens* (Denton et al. 1990) and the kelp *Nereocystis luetkeana* (Van Alstyne et al. 2001b) had lower concentrations of phlorotannins than adults.

The CNBH suggests that resource allocation towards chemical defenses is dynamic and shifts with changes in concentrations of limiting nutrients (Bryant et al. 1983). Juvenile marine macroalgae are a useful system to test the predictions of the CNBH because juveniles have conflicting pressures to grow rapidly to escape in size from herbivores and to produce chemical defenses that deter herbivores. The purpose of this study was to determine experimentally how nutrient enrichment affected growth rates and phlorotannin concentrations in early post-settlement stages of a common intertidal macroalga, *Fucus gardneri*. Furoid brown algae or rockweeds have a haplobiontic life history (Bold & Wynne 1985). *F. gardneri* is dioecious and releases eggs and motile sperm into seawater where fertilization occurs. In some *Fucus* species, gamete release is triggered by a combination of dissolved inorganic carbon depletion during periods of low water movement and daylight (Pearson et al. 1998). Fertilization occurs within a few hours (Pearson et al. 1998) and propagules settle within a short distance of adults (Johnson & Brawley 1998). Zygotes develop directly into diploid adults.

The CNBH predicts that enrichment with nitrogen, which can be the limiting nutrient to some coastal marine macroalgae (Fujita et al. 1989, Wheeler & Björnsäter 1992, Pedersen & Borum 1996, 1997), will cause increased growth rates and decreased phlorotannin concentrations of *Fucus gardneri* embryos. Non-limiting nutrients should not affect growth or phlorotannin concentrations. To test these hypotheses with *F. gardneri* embryos, we grew embryos in media with ambient and enhanced concentrations of nitrogen, phosphorus, and iron and measured the effects of

nutrient enrichment on embryo growth rates and phlorotannin concentrations.

MATERIALS AND METHODS

Fertile receptacles from at least 25 *Fucus gardneri* were collected from the beach at the Shannon Point Marine Center, Anacortes, Washington, USA. The receptacles were cleaned of visible epiphytes and placed overnight in 5 μm -filtered seawater at 8°C. The next morning, the receptacles were removed and the eggs were collected on a 40 μm filter, rinsed 3 times with filtered seawater, then added to 4 l of sterile 5 μm -filtered seawater. The suspension was shaken and 50 ml was distributed into each of 72 100 ml glass finger-bowls. Fertilized eggs were allowed to settle and attach for 6 d before nutrient-enriched media was added to the cultures. The bowls were randomly arranged on 2 shelves of an incubator (12°C, 43 $\mu\text{E m}^{-2} \text{s}^{-1}$, 16:8 h L:D) and the media was changed weekly and the dishes rotated.

A total of 8 different types of media were distributed among the 72 bowls ($n = 9$ of for each treatment): (1) a base medium, that consisted of sterile Guillard's f/2 enriched seawater medium (McLachlan 1973) except that it lacked NaNO_3 , $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; (2) an enriched nitrogen medium, that consisted of the base medium plus 75.0 $\text{mg l}^{-1} \text{NaNO}_3$; (3) an enriched phosphorus medium that consisted of the base medium plus 5.0 $\text{mg l}^{-1} \text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; (4) an enriched iron medium, that consisted of the base medium plus 3.15 $\text{mg l}^{-1} \text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; (5) an enriched nitrogen and phosphorus medium that consisted of the base medium plus 75.0 $\text{mg l}^{-1} \text{NaNO}_3$ and 5.0 $\text{mg l}^{-1} \text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; (6) an enriched nitrogen and iron medium that consisted of the base medium plus 75.0 $\text{mg l}^{-1} \text{NaNO}_3$ and 3.15 $\text{mg l}^{-1} \text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; (7) an enriched phosphorus and iron medium that consisted of the base medium plus 5.0 $\text{mg l}^{-1} \text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 3.15 $\text{mg l}^{-1} \text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; (8) an enriched nitrogen, phosphorus and iron medium that consisted of the base medium plus 75.0 $\text{mg l}^{-1} \text{NaNO}_3$, 5.0 $\text{mg l}^{-1} \text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, and 3.15 $\text{mg l}^{-1} \text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. All media were made with seawater collected 50 m off the beach at the Shannon Point Marine Center at a depth of 5 m.

Embryos in 3 bowls from each of the 8 treatments were harvested 27 and 48 d after the cultures were started; 2 collections of embryos from each bowl were made. The first consisted of a small number of embryos that were carefully scraped off the bottoms of the bowls and fixed in 10% glutaraldehyde for later morphometric measurements; the second consisted of the remaining embryos, which were scraped from the bowl and filtered onto 13 mm-diameter glass-fiber filters

that had been lyophilized, weighed, and stored in a desiccator to prevent hydration. The filters with the embryos were frozen to -50°C , lyophilized, and weighed to the nearest 0.001 mg. They were ground in a glass tissue-homogenizer in 0.5 ml 80% MeOH, then centrifuged at 14 000 rpm. The supernatant was used to determine phlorotannin concentrations as described in Van Alstyne (1995). Phloroglucinol dihydrate was used as a standard. The data were transformed with an angular transformation and a 4-way analysis of variance was used to examine the effects of the fixed factors (1) nitrogen addition, (2) phosphorus addition, (3) iron addition, and (4) sampling time on phlorotannin concentrations.

Samples fixed in 10% glutaraldehyde were used for morphometric analyses. From each sample, measurements were conducted on approximately 25 embryos that were haphazardly selected. Only embryos with intact rhizoids were used for the analyses. Measurements were made at 40 \times using an image-analysis system with Optimas 6.2 software. They included the length of the embryo from the base of the rhizoid to the distal end, the maximum width of the pigmented portion of the embryo, the length of the rhizoid from its base to the distal end of the rhizoid, and the surface area of the pigmented portion of the embryo.

Principal-components analyses indicated that embryo length, rhizoid length, and photosynthetic surface area cumulatively explained 96.1% of the variance in the morphometric measurements. Therefore, only these 3 measurements were used in the factorial MANOVA (multivariate analyses of variance) to examine the effects of nutrient enrichment. Approximately 60% of the variance was explained by amount of photosynthetic surface area, 21% was explained by em-

bryo length, 15% was explained by rhizoid length, and only 4% was explained by the width of the embryos. MANOVA was performed on the data to examine the effects of enrichment with nitrogen, phosphorus, and iron, and the effects of the time at which measurements were made.

RESULTS

The addition of nitrogen and iron to culture media significantly affected the growth of *Fucus gardneri* embryos (nitrogen: $p = 0.0357$, iron: $p = 0.0168$, Table 1). After 3 wk in culture, embryos growing in nitrogen-enhanced media were longer, wider, and had a greater surface area (Fig. 1). This trend continued in the embryos at the 6 wk measurements (Fig. 1, Table 2). The addition of iron to the media tended to affect the shape of the embryos rather than resulting in overall increases in size (Fig. 1, Table 2). After 6 wk, embryos grown in media enriched with iron alone were, on average, shorter and wider than embryos grown in media without iron (Table 2). They also had a slightly larger photosynthetic surface area but shorter rhizoids.

Although the addition of phosphorus alone had no significant effects on embryo size ($p = 0.5641$: Table 1), there was a significant phosphorus \times iron interaction effect ($p = 0.0307$: Table 1). In the absence of phosphorus enrichment, the addition of iron resulted in embryos that were, on average, 17.0% longer and that had 22.5% longer rhizoids after 3 wk; after 6 wk, iron enrichment resulted in embryos that were 7.8% longer, 14.4% wider, with 23.2% more surface area and rhizoids that were 36.7% shorter than embryos in

Table 1. *Fucus gardneri*. Results of multivariate analysis of variance of embryo lengths, rhizoid lengths, and photosynthetic surface area of embryos grown in 8 different media enriched with nitrogen, phosphorus, and iron. Measurements were made 27 and 48 d after cultures were started. **Bold** indicates treatments that had significant effects on growth

Source	Wilks' lambda	F	Numerator df	Denominator df	p
N	0.73219256	3.2918	3	27	0.0357
P	0.92846073	0.6935	3	27	0.5641
Fe	0.68938189	4.0552	3	27	0.0168
Time	0.57681987	6.6028	3	37	0.0017
N \times P	0.94654272	0.5083	3	27	0.6799
N \times Fe	0.84495861	1.6514	3	27	0.2009
N \times Time	0.92209413	0.7604	3	27	0.5261
P \times Fe	0.72333817	3.4423	3	27	0.0307
P \times Time	0.92758395	0.7026	3	27	0.5587
Fe \times Time	0.89360211	1.0716	3	27	0.3776
N \times P \times Fe	0.92253290	0.7557	3	27	0.5287
N \times P \times Time	0.97306952	0.2491	3	27	0.8613
N \times Fe \times Time	0.98586913	0.1290	3	27	0.9420
P \times Fe \times Time	0.94839441	0.4897	3	27	0.6923
N \times P \times Fe \times Time	0.83664772	1.7572	3	27	0.1719

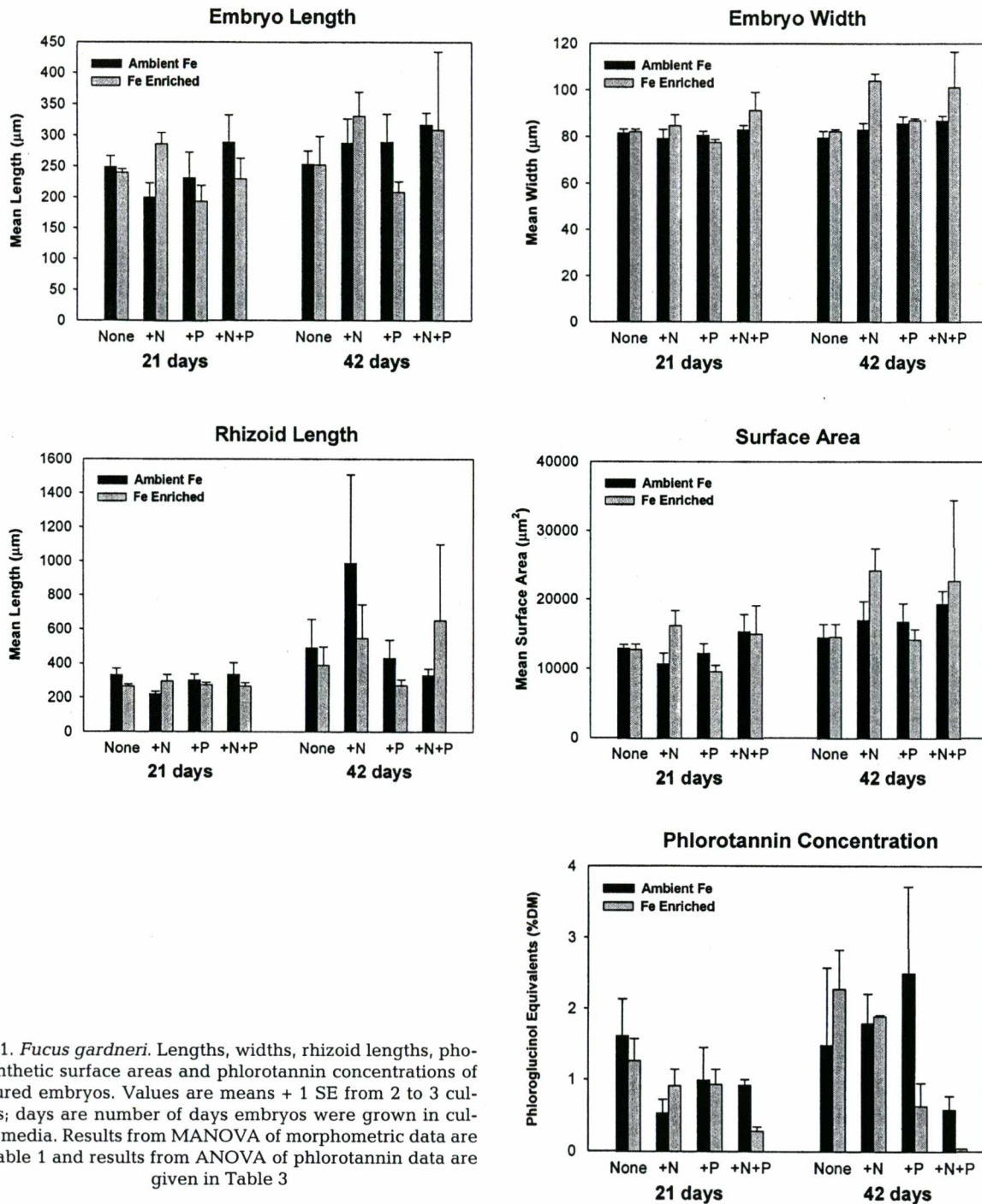


Fig. 1. *Fucus gardneri*. Lengths, widths, rhizoid lengths, photosynthetic surface areas and phlorotannin concentrations of cultured embryos. Values are means + 1 SE from 2 to 3 cultures; days are number of days embryos were grown in culture media. Results from MANOVA of morphometric data are in Table 1 and results from ANOVA of phlorotannin data are given in Table 3

unenriched media (Fig. 1). However, when the media were enriched with both phosphorus and iron, 3 wk-old embryos were 20.0% shorter, had 14.6% less surface area, and 14.6% shorter rhizoids (Fig. 1) than embryos in media without iron enrichment. Six wk-old embryos in the iron and phosphorus enriched media were 18.6% shorter, 7.2% wider, had rhizoids that

were 14.5% longer, and had 4% less surface area (Fig. 1).

Phlorotannin concentrations of embryos were significantly lower when embryos were grown in media enriched with nitrogen ($p = 0.013$) or phosphorus ($p = 0.004$, Fig. 1, Tables 2 & 3). Enrichment with iron alone had no significant effect on phlorotannin concentra-

tions (Table 3: $p = 0.189$); however, phosphorus \times iron had a significant synergistic effect on phlorotannin concentrations ($p = 0.009$). In the absence of phosphorus enrichment, iron enrichment resulted in 2.0 and 27.2% increases in phlorotannin concentrations over 3 and 6 wk, respectively (Fig. 1). However, when media was enriched with phosphorus, the addition of iron resulted in 36.6 and 64.3% decreases in mean phlorotannin concentrations over 3 and 6 wk, respectively (Fig. 1).

Phlorotannin concentrations of embryos across all experiments were not significantly correlated with changes in embryo length, width, or surface area (Fig. 2, linear regressions: $p > 0.05$); however, there was a positive correlation between phlorotannin concentration and the increase in rhizoid length (Fig. 2: linear regression: $r^2 = 15.1\%$, $p = 0.005$). Phlorotannin concentrations were significantly and positively corre-

lated with length (linear regression: $r^2 = 20.4\%$, $p = 0.001$), rhizoid length (linear regression: $r^2 = 24.9\%$, $p < 0.001$), and surface area (linear regression: $r^2 = 11.3\%$, $p = 0.015$) of the embryos (Fig. 3), suggesting that size was a more important determinant of phlorotannin levels than growth rates.

DISCUSSION

Phlorotannin concentrations in the *Fucus gardneri* embryos in our experiments tended to be positively correlated with measurements of embryo size (Fig. 2) rather than growth rates (Fig. 3). This suggests that in *F. gardneri* embryos, ontogenetic changes are more important determinants of phlorotannin levels than are trade-offs in the allocation of resources to growth and secondary metabolite production.

Table 2. *Fucus gardneri*. Changes in size and phlorotannin concentrations in embryos due to effects of nitrogen, phosphorus and iron enrichment. Values are only given for treatments that had significant effect on embryo growth or phlorotannin concentrations (Tables 1 & 3). Directions of arrows indicate whether nutrient additions had positive (\uparrow) or negative (\downarrow) effect on growth of phlorotannin concentrations

Enrichment	Weeks	Length	Width	Rhizoid length	Surface area	Phlorotannin concentration
N	3	9.6% \uparrow	3.6% \uparrow	6.5% \downarrow	18.4% \uparrow	45.6% \downarrow
	6	27.2% \uparrow	9.9% \uparrow	43.3% \uparrow	36.4% \uparrow	18.4% \downarrow
P	3	–	–	–	–	25.3% \downarrow
	6	–	–	–	–	47.9% \downarrow
Fe	3	1.0% \uparrow	1.2% \uparrow	5.9% \downarrow	4.9% \uparrow	–
	6	7.7% \downarrow	9.0% \uparrow	10.1% \downarrow	4.3% \uparrow	–

Table 3. *Fucus gardneri*. Analysis of variance of angularly transformed phlorotannin concentrations of embryos grown in 8 different media enriched with nitrogen, phosphorus, and iron. Measurements of phlorotannin concentrations were made 27 and 48 d after cultures were started. **Bold** indicates treatments that had a significant effect on phlorotannin concentrations

Source	df	SS	MS	F	p
N	1	0.008210	0.008210	7.06	0.013
P	1	0.011459	0.011459	9.86	0.004
Fe	1	0.002101	0.002101	1.81	0.189
Time	1	0.001955	0.001955	1.68	0.205
N \times P	1	0.002255	0.002255	1.94	0.174
N \times Fe	1	0.000090	0.000090	0.08	0.783
N \times Time	1	0.000007	0.000007	0.01	0.937
P \times Fe	1	0.009012	0.009012	7.75	0.009
P \times Time	1	0.003149	0.003149	2.71	0.111
Fe \times Time	1	0.000478	0.000478	0.41	0.526
N \times P \times Fe	1	0.000057	0.000057	0.05	0.826
N \times P \times Time	1	0.005259	0.005259	4.53	0.042
N \times Fe \times Time	1	0.000002	0.000002	0.00	0.970
P \times Fe \times Time	1	0.002887	0.002887	2.48	0.126
N \times P \times Fe \times Time	1	0.003546	0.003546	3.05	0.091
Error	29	0.033705	0.001162		
Total	44	0.084170			

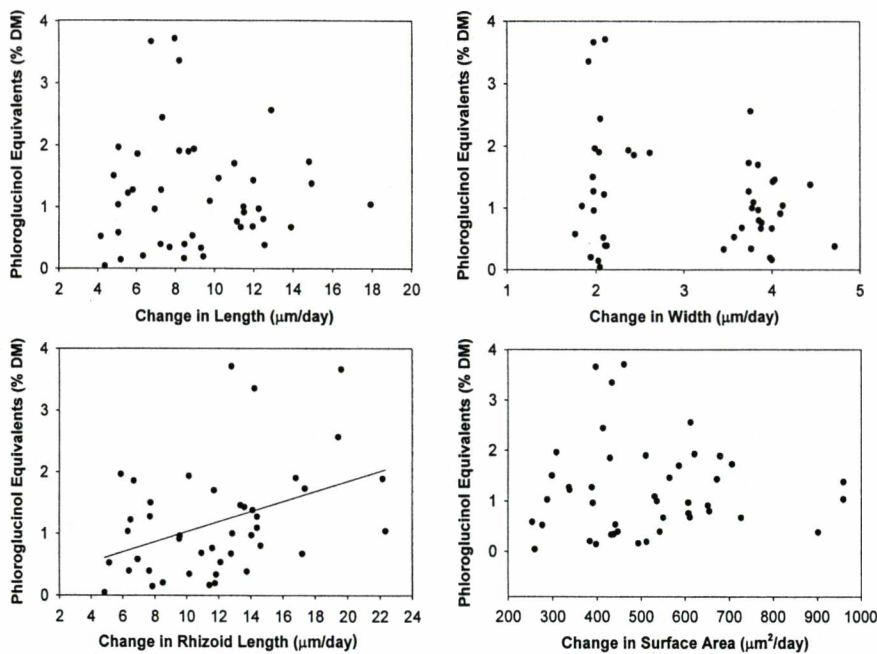


Fig. 2. *Fucus gardneri*. Phlorotannin size as a function of average daily changes in embryo length (linear regression: $p > 0.05$), width (linear regression: $p > 0.05$), rhizoid length (linear regression: $r^2 = 15.1\%$, $p = 0.005$), and surface area (linear regression: $p > 0.05$) over 3 wk period. Each data point represents results from a single culture dish measured at either 3 or 6 wk from beginning of experiment. Lines are best-fit from simple linear regression. DM = dry mass

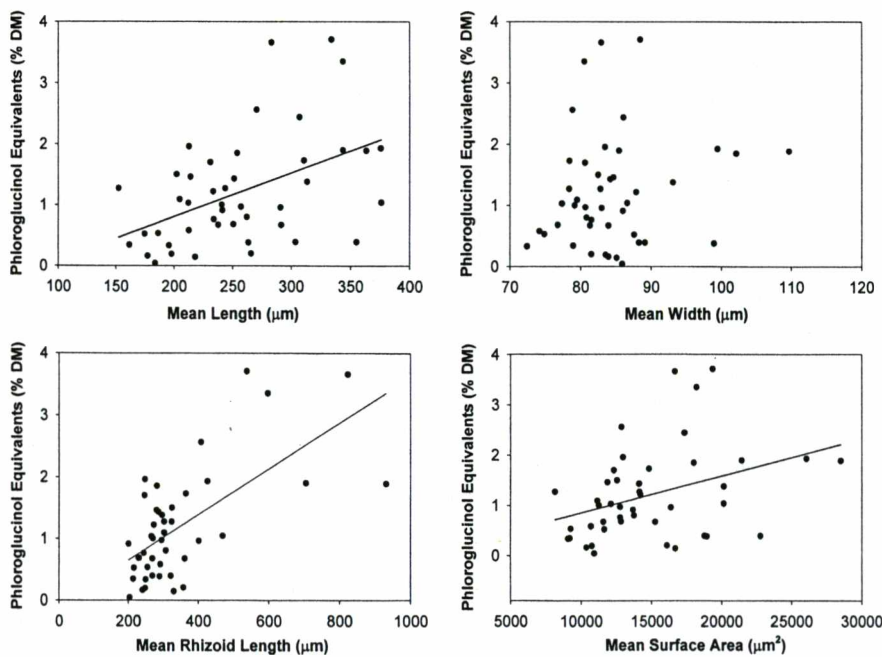


Fig. 3. *Fucus gardneri*. Phlorotannin size as a function of embryo length (linear regression: $r^2 = 20.4$, $p = 0.001$), width (linear regression: $p > 0.05$), rhizoid length (linear regression: $r^2 = 24.9$, $p < 0.001$), and surface area (linear regression: $r^2 = 11.3$, $p = 0.015$). Each data point represents results from a single culture dish measured at either 3 or 6 wk from beginning of experiment. Lines are best-fit from simple linear regression. DM = dry mass

The phlorotannin concentrations of the *Fucus gardneri* embryos in this study were low relative to concentrations in adult plants of the same species, which typically range from ~5 to 10% dry mass (DM) (Steinberg 1985, Van Alstyne 1988, 1989, Van Alstyne & Paul 1990, Targett et al. 1992, Van Alstyne et al. 1999b,c). However, the concentrations in the cultures containing some of the larger individuals were as high as 3 to 4% DM, within the range of values seen in larger individuals from the field. Van Alstyne et al. (2001b) found that concentrations of phlorotannins in 1 to 2 cm-long juvenile *F. gardneri* were significantly higher than concentrations in adults, and concentrations in 1 to 2 cm-long juveniles decreased as the plants elongated.

The lower concentrations of phlorotannins in laboratory-cultured embryos suggests 2 possibilities. The first is that embryos allocate fewer resources to phlorotannin production than larger juveniles because survival at this stage is enhanced if these resources are used for growth. If phlorotannins function as an anti-herbivore defense, they are likely to work by being distasteful during an initial attack by a grazer, and reducing subsequent attacks. Most temperate intertidal herbivores are not visual feeders, and therefore have to consume a portion of an alga to learn if it is distasteful. For example, littorinid snails will climb on damaged and undamaged *Fucus gardneri* at the same rate, but will leave the damaged *F. gardneri* that has higher phlorotannin concentrations faster than they will leave undamaged algae (Van Alstyne 1988). Embryos are unlikely to survive an initial attack by a grazer, whereas a larger juvenile might survive an initial attack although it might lose a significant proportion of its surface area. Consequently, the production of phlorotannins as chemical defenses by embryos would be of little or no benefit since they are unlikely

to survive the first attack. Larger juveniles might benefit by having high concentrations of phlorotannins if they are effective at preventing subsequent tissue loss. The value of these defenses may decrease as plants get larger and the proportion of tissue lost during a single attack decreases.

An alternative explanation for the low phlorotannin concentrations of *Fucus gardneri* embryos in this study may be that phlorotannin production can be induced by environmental factors that are not present in laboratory-culture conditions. Phlorotannin concentrations are known to increase in response to physical damage (Van Alstyne 1988, 1989, Peckol et al. 1996, Hammerstrom et al. 1998) and increased UV-light levels (Pavia et al. 1997). Without these inducers present, phlorotannin concentrations may remain at lower concentrations.

Nutrient enrichment affected both embryo growth rates and phlorotannin concentrations. The results of the nitrogen-enrichment experiments supported some predictions of the carbon/nutrient balance hypothesis (CNBH). The model predicted that an increase in the availability of a limiting nutrient should result in increases in growth and decreases in the production of carbon-based defensive compounds (Bryant et al. 1983). Of the 3 nutrients we supplemented in our growth experiments, only nitrogen produced overall increases in the size of *Fucus gardneri* embryos (Tables 1 & 2). Iron enrichment affected embryo morphology, but not size, and phosphorus enrichment had no effects on embryo growth. Previous enrichment experiments involving *Fucus* spp. embryos have found similar responses. The addition of iron alone had no effect on *F. edentatus* embryos, but did increase growth in combination with nitrogen and phosphorus (McLachlan 1977); the highest growth rates of *F. edentatus* occurred when nitrogen, phosphorus, and iron were supplemented simultaneously. Culture experiments with adult *Fucus* spp. have also shown that growth is enhanced by nitrogen addition (Rosenberg et al. 1984, Creed et al. 1997).

Nitrogen enrichment resulted in decreases in embryo phlorotannin concentrations at both the 3 and 6 wk intervals. Our results are similar to the results Arnold et al. (1995) obtained with cultures of *Lobophora variegata*, despite large differences in the nitrogen concentrations of the baseline and enriched media. Our unenriched media had nitrogen concentrations of about 7 mg l⁻¹, whereas Arnold et al.'s were 0.2 mg l⁻¹. Concentrations in our enriched media were about 20 mg l⁻¹, whereas the highest concentrations used by Arnold et al. were 5.2 mg l⁻¹. Arnold et al. (1995) found direct positive correlations between nitrogen concentrations in the media and nitrogen concentrations in the algae and a negative correlation with phlorotannin concentrations.

Decreases in phlorotannin concentrations by combinations of nutrients that had no or negative effects on growth suggest that physiological stresses may be affecting phlorotannin concentrations. Phosphorus enrichment resulted in a large, 25 to 50%, decrease in phlorotannin concentrations (Table 2), yet had no effect on growth (Table 1). Enrichment simultaneously with phosphorus and iron also resulted in sharp decreases in phlorotannin concentrations (Table 3, Fig. 1), as well as having negative effects on growth (Table 1). Van Alstyne et al. (2001a) suggest that changes in environmental factors such as nutrients can alter chemical defense concentrations in 3 ways: (1) they can provide cues for plants to alter concentrations of secondary metabolites to concentrations more appropriate for the environment at that time and place, such as the production of inducible defenses (Karban & Baldwin 1997, Tollrian & Harvell 1999), (2) they can alter the amounts of materials and energy available for defense production and how those resources are allocated to growth, reproduction and defense (Bryant et al. 1983, Coley et al. 1985, Herms & Mattson 1992, Tuomi 1992), and (3) they can stress plants, resulting in an overall lowered physiological condition and lowered chemical defense concentrations (Renaud et al. 1990, Cronin & Hay 1999a,b).

The addition of iron and phosphorus may be physiologically stressing the plants, resulting in lowered growth rates and phlorotannin concentrations. Other environmental factors that have detrimental effects on algal health can reduce concentrations of chemical defenses and affect the palatability of algae to herbivores. For example, prolonged periods of desiccation are stressful to marine algae (Renaud et al. 1990, Cronin & Hay 1999b). Sea urchins, *Arbacia punctulata*, significantly preferred to consume *Padina gymnospora* that had been desiccated for 30 to 90 min than undesiccated algae. When extracts of *P. gymnospora* were coated onto a preferred food, urchins preferentially consumed algae coated with extracts from desiccated algae over extracts from undesiccated algae. The results of Renaud et al.'s (1990) study indicate that stressed *P. gymnospora* undergo changes in their chemistry that affect urchin feeding; however, it was not determined whether these changes were in the chemical defenses produced by the algae. When *Dictyota ciliolata* was emersed for 1 h, photosynthetic rates decreased by 47% and respiration rates increased by 40% relative to undesiccated plants (Cronin & Hay 1996b). Desiccated plants were consumed at a faster rate by the sea urchin *A. punctulata* and the amphipod *Ampithoe longimana*. This was correlated with decreases in the secondary metabolites pachydictyol A, dictyol B acetate and dictyodial in desiccated algae. Similar tests of the effects of desiccation on herbivore resistance were conducted by Renaud et al. (1990) on the brown alga *P. gymnospora*.

Ultraviolet radiation also reduces the production of chemical defenses in some seaweeds. *Dictyota ciliolata* exposed to UV radiation had significantly lower concentrations of dictyol B acetate and dictyodial; however, concentrations of pachydictyol A were not different. The algae also grew more poorly, suffered higher mortality levels, but was not preferentially consumed by the amphipod *Ampithoe longimana* relative to algae that had been shielded from UV light (Cronin & Hay 1996b). These results contrast with the effects of UV light on *Ascophyllum nodosum* which increases phlorotannin concentrations in response to UV-light exposure (Pavia et al. 1997).

The reasons for the reduction in phlorotannin concentrations with phosphorus enrichment were not clear. Phosphorus enrichment did not appear to be stressing embryos in ways that affected growth rates (Table 1); however, it may have affected embryo health in ways not determined by embryo size measurements. Phosphorus is generally not considered to be a limiting nutrient to coastal seaweeds and the mechanism by which increases in phosphorus would decrease phlorotannin production are not known. The decreases in growth and phlorotannin concentrations resulting from the combined effects of iron and phosphorus are also difficult to explain. Phosphorus addition is known to have deleterious effects on adult *Fucus virsoides* in the presence of other metals such as manganese, cobalt, and zinc (Munda & Veber 1996). Furthermore, phlorotannins have been shown to chelate metals, including iron (Ragan & Glombitza 1986, Targett & Arnold 1998).

The results of these experiments show that phlorotannin concentrations in *Fucus gardneri* embryos are determined by a complex interaction of several different factors. These include the ontogenetic stage of the algae and resource-allocation shifts and physiological stresses that result from the addition of different nutrients. Because these factors can act synergistically, this study illustrates the importance of manipulating multiple factors simultaneously when looking at the role of environmental changes on plant secondary-metabolite production.

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