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NOTE

EFFECTS OF UV RADIATION ON GROWTH AND PHLOROTANNINS IN *FUCUS GARDNERI* (PHAEOPHYCEAE) JUVENILES AND EMBRYOS[1]

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Diminishing levels of atmospheric ozone are increasing UV stress on intertidal algae. Early developmental stages tend to be more susceptible to environmental stresses; however, little research has examined how these stages are protected from UV radiation (UVR). Many brown algae contain high levels of phlorotannins, which are thought to function in screening UVR. In this study, we tested the effects of ambient levels of UV-B and UV-A on growth and phlorotannin production in 1- to 2-cm juvenile and microscopic postsettlement embryos of the intertidal alga *Fucus gardneri* Silva. Algae were grown in four light treatments: 1) ambient light; 2) under cellulose acetate, which lowered light quantity but did not affect light quality; 3) under Mylar™, which filtered UV-B; and 4) under Plexiglas™, which blocked UV-A and UV-B. Over a 3-week period, UV-B inhibited and UV-A enhanced the growth of *F. gardneri* embryos, whereas the growth of juveniles was not affected. Phlorotannin concentrations of both embryos and juveniles did not differ in any of the light treatments. Our results suggest that embryos of *F. gardneri* are susceptible to UV light but develop a tolerance to it as they mature. This tolerance may result from increases in phlorotannin concentrations that occur during maturation; however, phlorotannin production in embryonic or juvenile stages is either not induced by UV light or takes more than 3 weeks to occur.

Key index words: **embryos; *Fucus*; growth; juveniles; phlorotannins; UV radiation**

Abbreviations: **MeOH, methanol; SPMC, Shannon Point Marine Center; UVR, UV radiation**

Intertidal macroalgae encounter a variety of environmental stresses, such as high light levels, variable temperatures and salinities, desiccation, and nutrient limitation (Davison and Pearson 1996). In recent years, diminishing ozone levels are allowing more UV-B radiation (280–315 nm) to reach the earth, potentially increasing UV light stresses (Stolarski et al. 1992, Kerr and McElroy 1993, El-Sayed et al. 1996, Häder 2001). Exposure to UV radiation (UVR) can have diverse effects on algae, including damaging DNA by creating cyclobutyl thymine dimers and pyrimidine adducts (Mitchell and Karentz 1993, Franklin and Forster 1997, Pakker et al. 2000b, Russell 2002), destroying chl *a* and carotenoids (Dring et al. 1996, Franklin and Forster 1997, Mattoo et al. 1999), and causing photoinhibition by disrupting the electrochemical gradient in thylakoid membranes and degrading proteins in PSII (Anderson et al. 1997, Franklin and Forster 1997, Häder and Figueroa 1997, Aguilera et al. 1999, Mattoo et al. 1999, Häder 2001, Vass et al. 2002).

Macroalgae are protected from UV damage by avoidance, repair, and screening mechanisms (Karentz 1994, El-Sayed et al. 1996, Franklin and Forster 1997, Karentz 2001). Intertidal macroalgae generally cannot avoid UVR completely because they are exposed to full sunlight upon emersion, but they can decrease their exposure by growing in turfs, under canopies, in deeper waters, or when UV levels are low (Ang 1991, Brawley and Johnson 1991, El-Sayed et al. 1996, Johnson and Brawley 1998). UV-induced DNA damage can be repaired through photoreactivation or excision repair (Karentz 1994, Karentz et al. 1994, Pakker et al. 2000a,b, Russell 2002). However, damage rates can exceed repair rates (Hoffman et al. 2003), so many macroalgae produce UV screening compounds, such as mycosporine-like amino acids (Karentz et al. 1991, Karentz 1994, 2001, Sinha et al. 1998), scytonemin (Sinha et al. 1998, Franklin et al. 1999, Karsten et al. 1999, Häder 2001, Karentz 2001, Dillon et al. 2002,) and possibly phlorotannins (Pavia et al. 1997, Pavia and Brock 2000), that decrease UV damage while allowing the alga to use PAR (400–700 nm). Early developmental stages of intertidal algae can be particularly vulnerable to UV stress (Major and Davison 1998, Wiencke et al. 2000, Coelho et al. 2001, Hoffman et al. 2003). Thallus thickness and light sensitivity are often correlated, making juveniles more susceptible to UV stress than adults (Dring et al. 1996, Franklin and Forster 1997, Hanelt et al. 1997, Aguilera et al. 1999).

The mechanisms used by juveniles and adults to cope with UVR may be similar. In brown algae, embryos and juveniles may use phlorotannins as UV screens. Phlorotannins, polyphenols that are produced by brown algae (Ragan and Glombitza 1986), can have multiple functions, the most common being serving as

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cell wall components (Schoenwaelder 2002), deterring herbivores (Targett and Arnold 1998), and screening UVR (Pavia et al. 1997). *Ascophyllum nodosum* (L) Le Jolis phlorotannins absorb UV-B light (Pavia et al. 1997) and increase in response to UVR (Pavia et al. 1997, Pavia and Brock 2000), suggesting they function as UV-induced screening compounds. Phlorotannin concentrations of *Fucus gardneri* Silva embryos grown in the laboratory in the absence of UV light are lower (< 1% by dry mass) (Van Alstyne and Pelletreau 2000) than in larger juveniles from the field (approximately 4%–5% dry mass) (Van Alstyne et al. 2001). This suggests that phlorotannin concentrations in early developmental stages of *F. gardneri* are either stage specific or induced by UV light. In *A. nodosum,* phlorotannin concentrations and growth are negatively correlated (Pavia et al. 1999), possibly because producing phlorotannins incurs metabolic costs. Thus, it may be beneficial to limit phlorotannin synthesis in the absence of high UVR.

The purpose of this study was to determine whether UV-induced phlorotannin production occurs in early developmental stages of *F. gardneri* and to measure the effects of UVR on growth. *Fucus gardneri* grows in the high to low intertidal zones of sheltered rocky shores from Alaska to central California, USA (O'Clair and Lindstrom 2000). In this study, we grew juveniles and microscopic embryos under filters to shield them from UV-A and UV-B radiation and measured the effects on growth and phlorotannins over a 3-week period.

Mature *F. gardneri* receptacles were collected from the beach at the Shannon Point Marine Center (SPMC) in Anacortes, Washington, USA (hereafter referred to as the SPMC beach). Embryos were cultured using methods similar to Van Alstyne and Pelletreau (2000). The receptacles were cleaned of visible epiphytes, desiccated overnight, and then dipped in a 1% bleach solution, rinsed in freshwater, and placed in sterile 5 μm-filtered seawater at 8° C. After 48 h, the eggs were collected on a 40-μm filter, rinsed three times with sterile 5-μm filtered seawater, and added to 750 mL of sterile filtered seawater. The suspension was gently shaken, and 30 mL was distributed into each of 24 100-mL glass bowls. The fertilized eggs attached and grew for 10 days in an incubator (12° C, 43 μmol photons $\cdot m^{-2} \cdot s^{-1}$, 16:8-h light:dark cycle) before being randomly assigned to one of four light treatments ($n = 6$ bowls per treatment). Juvenile *F. gardneri* (1–2 cm long, 1.0–1.5 cm wide) with no visible epiphytes were collected from the mid-intertidal zone of the SPMC beach. Each individual was randomly assigned to one of 18 100-mL glass finger bowls ($n = 4$ per bowl), containing 30 mL of sterile 5-μm filtered seawater. Each bowl was then randomly assigned to one of four light treatments ($n = 4$–5 bowls per treatment).

The bowls with the embryos and juveniles were submerged approximately 2.5 cm on platforms in an outdoor flowthrough seawater table (Bingham and Reitzel 2000). Filters were placed over the tank to create the four light treatments that included two controls. The first control treatment was unfiltered and exposed to full sunlight. The second control was a cellulose acetate (McMaster Carr, Los Angeles, CA, USA) filter, which reduced irradiance but did not alter light quality. A Mylar™ (McMaster Carr) filter screened UV-B, and Plexiglas™ (Ace Hardware, Anacortes, WA, USA) screened both UV-B and UV-A. Irradiance from 300 to 700 nm was measured under each filter with a Li-Cor 1800 spectroradiometer (LiCor, Lincoln, NE, USA). The relative irradiance of each spectral component (UV-B, 300–315 nm; UV-A, 316–399 nm; PAR, 400–700 nm) was determined by integrating the curves obtained for each filter and comparing them with unfiltered light. All experiments were conducted in June and July 2002 when the weather was generally clear with occasional morning fog and the seawater tables received about 8 h of direct sunlight daily. Average weighted daily UV at the National Ultraviolet Monitoring station in Port Angeles, WA (located approximately 60 km from our study site) measured from 330–363 nm with an MKIV Brewer Spectrophotometer (Kipp and Zonen, Saskatoon, SK, Canada) was $3762 \pm 928\ J \cdot m^{-2}$ (http://oz.physast.uga.edu).

Embryos and juveniles remained in the sea table for 21 days. The filtered seawater in the bowls was changed every 4 days. Embryos were collected from each bowl on days 0, 14, and 21 by gently scraping them with a pipette tip. They were then fixed in 500 μL 10% aqueous glutaraldehyde for later measurements of length and surface area. Using Optimas 6.2 image analysis software (Optimas Corporation, Bothell, WA), the length from the base of the rhizoid to the distal end of the embryo and the surface area of the pigmented portion was measured on each of 25 haphazardly selected individuals from each bowl. A comparison of embryo lengths before and after fixing in glutaraldehyde showed no significant differences (mean length ± SD before fixing: 240 ± 69.5 μm; mean length of embryos fixed for 4 days in glutaraldehyde: 233 ± 78.3 μm; *t*-test: $t = 0.839$, $df = 64$, $P = 0.404$).

After 21 days, the remaining embryos in the bowls (approximately 10 mg) were removed and collected on 13-mm preweighed lyophilized glass-fiber filters. The filters were frozen to −50° C, lyophilized, and reweighed to obtain an embryo dry mass. They were then ground in a tissue homogenizer in 1 mL 80% methanol (MeOH). Phlorotannins were measured with a Folin-Ciocalteu analysis using phloroglucinol-dihydrate as a standard (Van Alstyne 1995).

At the beginning of the experiment and after 7, 14, and 21 days, each juvenile was weighed and its length, from the distal to proximal end, and width across the widest portion of the blade were measured. After 21 days, the juveniles were collected, frozen to −50° C, lyophilized, and ground to a powder in a SPEX mixer/mill (SPEX, Metuchen, NJ, USA). The resulting powder was weighed, and approximately 15 mg of tissue was homogenized with 1 mL of 80% MeOH and approximately 0.05 g of autoclaved sand that had passed through a 0.25-mm mesh. Phlorotannin con-

centrations were measured as above. Folin-Ciocalteu reactive substances were not detected from sand ground alone in MeOH.

A multivariate analyses of variance (MANOVA) was conducted separately for embryos and juveniles with individuals nested within bowls and bowls nested within treatments (SPSS version 10.0, SPSS, Chicago, IL). Because the covariances were significant among groups (Box's test, $P < 0.05$), a Pillai-Bartlett V statistic, which is robust to moderate heteroscedasticity, was used (Johnson and Field 1993). A Student-Newman-Keuls (SNK) post hoc test was used to test for differences in embryo and juvenile growth among treatments. Phlorotannin data were analyzed with two-way analyses of variance (ANOVA) with stage and light treatment as fixed factors, after verifying that variances were homogeneous. Pearson's correlation coefficients were calculated to look for correlations between phlorotannin concentrations and growth measurements.

To isolate phlorotannins for spectral analysis, approximately 400 g of lyophilized *F. gardneri* from SPMC beach were extracted twice in MeOH under N_2 overnight in darkness at -2° C. The extracts were dried on a rotary evaporator, dissolved in water, and then partitioned three times with dichloromethane. The dichloromethane fraction was discarded, and the aqueous fraction was lyophilized, redissolved in water, filtered, and then partitioned with ethyl acetate until the ethyl acetate fractions were clear. The ethyl acetate fractions were combined, dried on a rotary evaporator, dissolved in water, and lyophilized again. The phlorotannin concentration in the solids was approximately 72%. To further purify the phlorotannins, approximately 0.1 g of the solids were dissolved in 10 mL of acidified MeOH and mixed with 1 g of polyvinylpolypyrrolidone. The slurry was centrifuged at 4500 rpm for 5 min, and the supernatant was discarded. The solids were then mixed with 10 mL of water and centrifuged at 4500 rpm for 5 min. A small portion of the supernatant was diluted in water, and the absorbance was determined from 190 to 400 nm on an Agilent 8453 diode array spectrophotometer (Agilent, Waldbronn, Germany).

TABLE 1. Relative penetration of light through filters.

	UV-B (%)	UV-A (%)	PAR (%)
No filter	100	100	100
Cellulose acetate	58.7	56.7	52.4
Mylar™	3.6	56.9	59.2
Plexiglas™	0.2	1	72.5

UV-B, 300–315 nm; UV-A, 316–399 nm; PAR, 400–700 nm.

The screens created distinctly different light environments (Table 1). Cellulose acetate did not selectively filter any wavelengths of light but decreased irradiance to a level slightly below the other filters. Mylar™ blocked most of the UV-B light, and Plexiglas™ blocked both UV-A and UV-B wavelengths. Isolated *F. gardneri* phlorotannins absorbed strongly in the UV-C region of the spectrum with peaks at 195 and 265 nm (Fig. 1). Absorption occurred to a lesser extent in the UV-B region and but not in the UV-A region.

Exposure to UVR over 21 days had a significant effect on the growth of *F. gardneri* embryos (Fig. 2; MANOVA: Pillai's V = 0.088, F = 24.966, df = 6, error df = 3260, $P < 0.001$). Embryos grown under Mylar™ screens in the absence of UV-B were 33.2% longer and had a 31.7% greater surface area than embryos grown in the presence of both UV-B and UV-A. When both UV-A and UV-B were filtered by Plexiglas™, embryos grew to be 13.5% longer and have a 6.1% greater surface area than in control treatments. Embryo growth in the two control treatments were not significantly different from each other but were lower (SNK, $P < 0.05$) than in the other two treatments. Growth rates of embryos also differed significantly over time (Fig. 2; MANOVA: Pillai's V = 0.740, F = 478.643, df = 4, error df = 3260, $P < 0.001$). Growth rates of *F. gardneri* juveniles did not differ significantly among light treatments (Fig. 3; MANOVA: Pillai's V = 0.037, F = 0.727, df = 9, error df = 525, $P = 0.685$) but did differ over time (MANOVA: Pillai's V = 0.091, F = 2.759, df = 6, error df = 348, $P = 0.12$).

Phlorotannin concentrations were higher in juveniles than embryos (Table 2; two-way ANOVA:

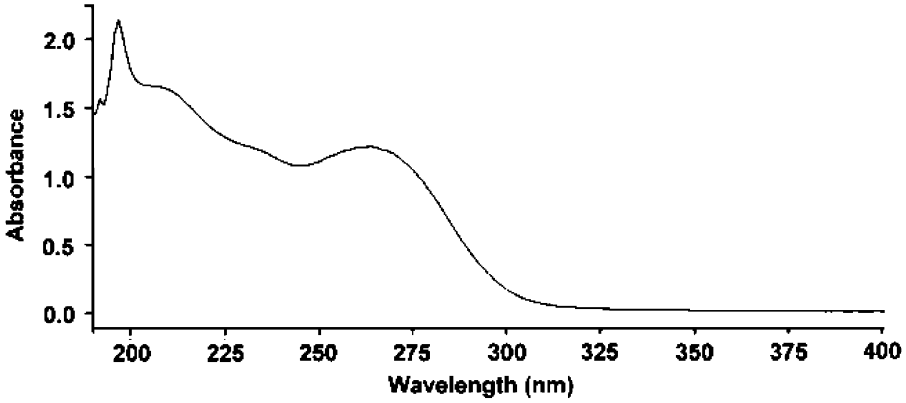

FIG. 1. Absorbance spectrum of phlorotannins isolated from *Fucus gardneri* after desorption from polyvinylpolypyrrolidone.

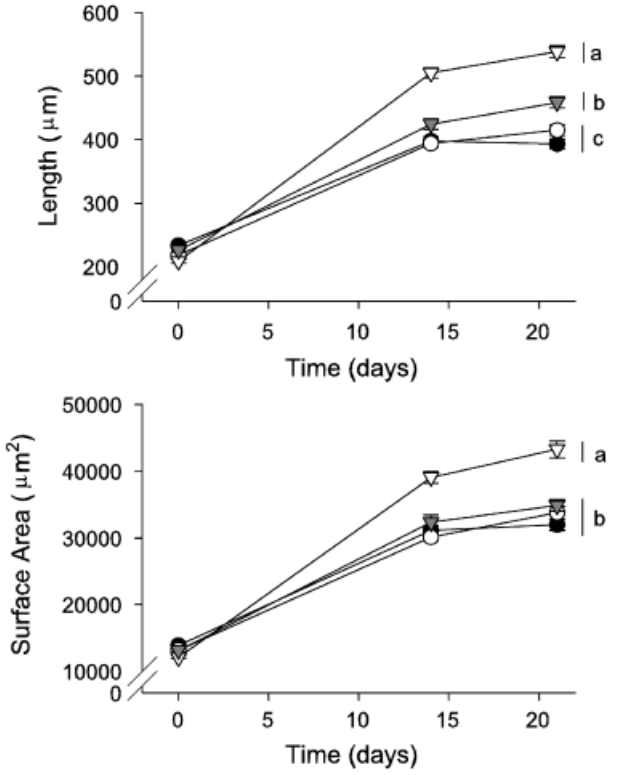

FIG. 2. *Fucus gardneri* embryo lengths and surface areas under different light treatments. Data are means ± SE ($n = 150$). Treatments had a significant effect on embryo growth (MANOVA: Pillai's Trace V = 0.088, F = 24.966, df = 6, error df = 3260, $P < 0.001$). Groups designated by different letters were significantly different in size (SNK post hoc test, $P < 0.05$). Open triangle, Mylar™ (UV-B filtered); gray triangle, Plexiglas™ (UV-A and UV-B filtered); closed circle, unfiltered control; open circle, cellulose acetate (filtered control).

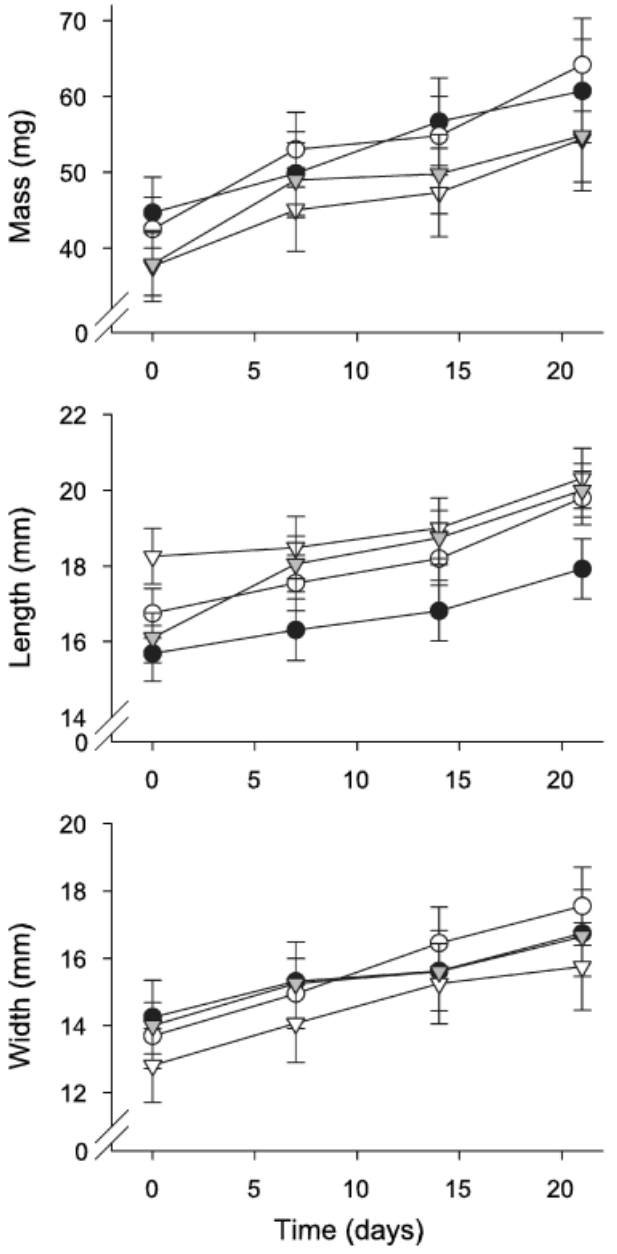

FIG. 3. *Fucus gardneri* masses, lengths, and widths over 21 days. Data are means ± SE ($n = 16$–20). Light treatments had no significant effect on juvenile sizes (MANOVA: Pillai's Trace V = 0.037, F = 0.727, df = 9, error df = 525, $P = 0.685$). Open triangle, Mylar™ (UV-B filtered); gray triangle, Plexiglas™ (UV-A and UV-B filtered); closed circle, unfiltered control; open circle, cellulose acetate (filtered control).

F = 4.211, df = 3, $P = 0.043$); however, they did not differ among light treatments (two-way ANOVA: F = 0.937, df = 3; $P = 0.427$; age × light treatment interaction: F = 0.994, df = 3, $P = 0.400$). There were no correlations between embryo surface area or length and phlorotannin concentrations (surface area: Pearson correlation coefficient = 0.223, $P = 0.295$; length: Pearson correlation coefficient = 0.200, $P = 0.349$) or between juvenile mass, length, or width and phlorotannin concentration (mass: Pearson correlation coefficient = 0.122, $P = 0.323$; length: Pearson correlation coefficient = – 0.052, $P = 0.674$; width: Pearson correlation coefficient = – 0.123, $P = 0.316$).

UVR inhibited the growth of *F. gardneri* embryos, but not juveniles, and had no effect on phlorotannin concentrations in either embryos or juveniles within 3 weeks. Embryos grew larger in the absence of UV-B than in controls, indicating that UV-B inhibited growth. Intermediate growth rates occurred when both UV-B and UV-A were filtered, suggesting that UV-A had a slight positive effect on growth. Both UV-A and UV-B can inhibit macroalgal growth (Franklin and Forster 1997, Häder and Figueroa 1997, Aguilera et al. 1999, Häder 2001, Hoffman et al. 2003); however, in some species UV-A aids in DNA repair and increases growth (Karentz 1994, El-Sayed et al. 1996, Pakker et al. 2001a,b). In prokaryotes, green microalgae, marine diatoms (Karentz et al. 1994), and rhodophytes (Pakker et al. 2000a,b), the photolyase enzyme, which mediates the reversal of cyclobutyl thymine dimers and pyrimidine adducts (Karentz

TABLE 2. *Fucus gardneri* phlorotannin concentrations (mean % of dry mass ± SD) after 21 days of exposure to light treatments.

	Unfiltered control	Cellulose acetate filter	Mylar filter	Plexiglas filter
Embryos	2.68% ± 0.96%	3.60% ± 1.28%	4.06% ± 0.78%	3.58% ± 0.83%
Juveniles	4.22% ± 1.39%	3.76% ± 1.10%	4.26% ± 1.54%	4.29% ± 1.57%

There were no significant differences in phlorotannin concentrations among light treatments (two-way ANOVA: $F = 0.937$, $df = 3$, $P = 0.427$; stage × light treatment interaction: $F = 0.994$, $df = 3$, $P = 0.400$. Embryos: $n = 6$, Juveniles: $n = 16$–20.

et al. 1994, Pakker et al. 2000b), can be activated by UV-A and blue light during photoreactivation. However, we found no evidence in the literature that photoreactivation occurs in brown algae. Thus, the cause of the positive effects of UV-A on embryo growth are not clear.

Our results suggest that juvenile *F. gardneri* have a greater tolerance to UVR than embryos because screening UVR did not affect their growth. Tolerances to other environmental stresses can increase with age as individuals become more differentiated and develop improved repair and defense mechanisms (Davison and Pearson 1996, Dring et al. 1996, Franklin and Forster 1997, Hanelt et al. 1997). The recruitment patterns in *Fucus distichus* (= *F. gardneri*) provide further evidence for the greater sensitivity of embryos to UVR. Germination and early cell division rates in *F. gardneri* are negatively correlated with UVR exposure, particularly at lower temperatures (Hoffman et al. 2003). Microscopic recruits of *F. gardneri* are found primarily during the winter, and densities of larger macroscopic juveniles tend to peak in the summer, even though reproduction occurs year round (Ang 1991). This suggests that that embryo survival is low during the summer months when several stresses, including UV levels, desiccation, and temperatures, are highest. Individuals that recruit between fall and spring will be larger and more resistant to these stresses by the time they increase in the summer. The potential for UV-B damage at early stages may also lead to preferential settlement or lower mortality rates in microhabitats that are protected from direct UV exposure. For example, survival of *Pelvetia fastigiata* J. Ag. embryos is higher under adult canopies and in red algal turfs than in exposed areas (Brawley and Johnson 1991). Although the interiors of turfs and canopies tend to be damper and have less variable temperatures than exposed surfaces, they are also protected from direct UV light. Thus, early developmental stages may benefit from reduced UV light levels in these microhabitats.

Purified *F. gardneri* phlorotannins absorbed in the UV-B range and were similar to absorbances for *A. nodosum* phlorotannins (Pavia et al. 1997). If phlorotannin concentrations were induced by UVR, there should have been higher concentrations in embryos in the two control treatments. If they relaxed in the absence of UVR, phlorotannin levels should have been lower in the juveniles grown under Mylar™ and Plexiglas™. However, our screening treatments had no effect on phlorotannin concentrations in *F. gardneri* embryos and juveniles over 3 weeks.

The effects of UV on *F. gardneri* show some similarities to those in *Ascophyllum nodosum*. Adult *A. nodosum* produce higher phlorotannin concentrations in response to artificial UV-B treatments after 2 weeks (Pavia et al. 1997) and to ambient UV-B treatments after 7 weeks but not after 2 or 4 weeks (Pavia and Brock 2000). If the response time to ambient light is the same in *F. gardneri* and *A. nodosum*, 3 weeks may have been too short to detect phlorotannin increases in *F. gardneri*. However, it is questionable whether responses taking longer than 3 weeks would provide protection rapidly enough to enhance the growth or survival of early stages.

The longer 7-week induction period in *A. nodosum* in response to UV light (Pavia and Brock 2000) may occur because UV-B is a minor but chronic stress with cumulative effects. More rapid responses may require stronger stimuli, such as those provided by physical damage. Phlorotannin induction in *F. gardneri* can occur in less than 2 weeks in response to mechanical damage (Van Alstyne 1988). In kelps, phlorotannin induction from grazing and mechanical damage is detectable within 1 to 3 days after the stimulus occurs (Hammerstrom et al. 1998). Concentrations return to prestimulus levels within 5 to 7 days after the stimulus is terminated. Alternatively, UV light may be stimulating phlorotannin production, but the phlorotannins may be transported and bound to cell walls, making them difficult to extract. In the embryos of some brown algal species, phlorotannins are deposited in newly forming cell walls (Schoenwaelder and Clayton 1998) and have been suggested to be important cell wall constituents (Schoenwaelder 2002).

UV-induced phlorotannin production has not been studied in many taxa. Different brown algal species may react differently to UV exposure, and *F. gardneri* may not alter its phlorotannin concentrations in response to changes in UV light. *Fucus gardneri* embryos are often found under canopies of adults and may not need protection from phlorotannins until they are larger. The elevated phlorotannin concentrations in older *F. gardneri* juveniles and adults (Van Alstyne et al. 2001) could be sufficient to protect the algae from UVR. In brown algae, phlorotannins are thought to have multiple functions, including cell wall formation, adhesion to surfaces, herbivore deterrence, wound

healing, antifouling, and UV absorption (Targett and Arnold 1998, Schoenwaelder 2002). Thus, altering phlorotannin concentrations in response to one stress may have ecological consequences for other functions (Berenbaum and Zangerl 1999). Given the number of functions that phlorotannins have and the variety of environments they are found in, it would not be surprising if the cues for induction or relaxation differ among species, life-history stages, or habitats.

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