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Geographic variation in polyphenolic levels of Northeastern Pacific kelps and rockweeds

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Abstract Brown algal polyphenolic compounds are secondary metabolites whose functions may include protecting plants from pathogens or damage by UV radiation, and deterring feeding by herbivores. We present here the first analysis of spatial variation (at scales from tens of meters to hundreds of kilometers) in concentration of these compounds in two orders of brown algae from the northeastern Pacific Ocean. In kelps (order Laminariales), variation among sites was significant in only 25% of species examined and was consistent within families (high in the Alariaceae and low in the Laminariaceae and Lessoniaceae). In rockweeds (order Fucales, family Fucaceae), site variation was high in three of four species examined. Both the proportion of high polyphenolic kelp species and the magnitude of spatial variation within species from both kelps and rockweeds were much higher than would have been predicted from previous studies in other regions. In one kelp (*Laminaria groenlandica*), significant differences between sites occurred at scales of only tens of meters. No latitudinal clines were observed. Differences in phenolic concentrations of kelps spanned nearly an order of magnitude in one species, *Hedophyllum sessile*. Phenolic levels were significantly higher in members of the Fucales than the Laminariales, but showed no significant differences between intertidal and subtidal species.

Introduction

Allelochemicals are natural products produced by one species that elicit physiological or behavioral responses in other species (Dicke and Sabelis 1988). Their importance in mediating ecological interactions among marine organisms has been the focus of much recent research (Hay and Fenical 1988; Van Alstyne and Paul 1989; Hay and Steinberg 1992; Paul 1992; Targett and Arnold 1998). Many studies have found that the biological activity of allelochemical natural products can be concentration-dependent (e.g. Hay et al. 1987, 1988; Harvell et al. 1988; Paul and Van Alstyne 1988; Steinberg 1988; Paul and Pennings 1991; Meyer and Paul 1992; Steinberg and van Altena 1992; Winter and Estes 1992; Van Alstyne et al. 1994; Steinberg et al. 1995). Thus, variation in the levels of natural products may significantly affect the outcomes of the ecological interactions they mediate.

Polyphenolic compounds are marine natural products whose antifeeding activity towards many, though not all (Steinberg and van Altena 1992; Boettcher and Targett 1993), herbivores is concentration-dependent (Steinberg 1988; Steinberg and van Altena 1992; Winter and Estes 1992; Steinberg et al. 1995; Targett and Arnold 1998). They are also referred to as phlorotannins because they bind proteins and are ultimately derived from phloroglucinol (Ragan and Glombitza 1986; Steinberg 1992). Phenolic compounds have been hypothesized as serving a number of functions, including grazer deterrence (Ragan and Glombitza 1986; Steinberg 1992) and protection from pathogens and UV damage (Targett and Arnold 1998). Concentrations of polyphenolic compounds in marine brown algae range from undetectable to nearly 20% of an alga's dry mass (Ragan and Glombitza 1986).

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Spatial variation in levels of brown algal polyphenolic compounds has been examined across very large spatial scales, such as between hemispheres (Steinberg 1989, 1992; Steinberg et al. 1995) and between temperate and tropical habitats (Steinberg 1986; Van Alstyne and Paul 1990; Targett et al. 1992). Variation in phenolic levels has also been examined across regional scales (tens to hundreds of km) for several species of Australasian brown algae (Steinberg 1989), *Lobophora variegata* in Caribbean and Atlantic waters (Targett et al. 1995), *Fucus vesiculosus* in Atlantic waters (Targett et al. 1992), and *Ascophyllum nodosum* in Europe (Pavia and Aberg 1996). Steinberg (1989) reported that phenolic levels of Australasian kelps and rockweeds often differed significantly among sites, but within a species the highest phenolic concentration was generally less than twice the lowest concentration. In *L. variegata*, phenolic levels differed six-fold between North Carolina, USA, and Aklins Island, Bahamas (Targett et al. 1995). Phenolic concentrations in *F. vesiculosus* differed by an order of magnitude between Delaware, USA, and Nova Scotia, Canada (Targett et al. 1992). In contrast, Pavia and Aberg (1996) found little variation in phenolic levels of *A. nodosum* at sites located 1000 km apart, but significant variation at scales of meters up to 1 km.

Because extensive studies of spatial variation in phenolic levels at similar scales have not been conducted in northern Pacific temperate habitats, we conducted a survey of phenolic levels in brown algae from the west coast of the USA. In this study, we posed the following questions: (1) Within species, do differences in phenolic levels occur among sites and, if so, does this variation form a latitudinal cline? and (2) Do phenolic levels in vegetative tissues among diverse species vary in a site-specific manner, suggesting localized environmental control of levels of these compounds? To address these questions we measured phenolic concentrations in kelps and rockweeds collected over a 2 wk period from nine sites ranging from southern California to northern Washington, USA (Fig. 1).

Materials and methods

Collection sites

Kelps and rockweeds were collected during low tides or by SCUBA at nine sites: five in California, one in Oregon, and three in Washington (Fig. 1). The Catalina Island (33°23′N; 118°21′W) collections were from the intertidal zone at the northern edge of Avalon, California, and by SCUBA in a shallow *Macrocystis pyrifera* bed at depths from 2 to 10 m (Fig. 1). The San Simeon (35°39′N; 121°13′W) site was an intertidal cobble beach. Algae were collected at 3 to 5 m depth off Lover's Point (36°38′N; 121°56′W) at the Pacific Grove site. The Pigeon Point (37°12′N; 122°24′W) site was a wave-exposed intertidal promontory ~10 km north of Davenport, California. Collections were made at low tide at Mouda Point (41°08′N; 124°10′W), an exposed rocky shore ~25 km north of Eureka, California, and at Boiler Bay (44°50′N; 124°04′W), ~5 km north of Depoe Bay, Oregon. The three collections made from the sites in Washington include: (1) an intertidal collection at Tatoosh Island (48°23′N; 124°44′W), (2) a collection by SCUBA at 5 to 8 m depth near the site of the former cantilever pier (hereafter referred to as the "cantilever pier site") and off the floating dock of the Friday Harbor Laboratories (48°32′N; 123°02′W) on San Juan Island, and (3) a collection from the intertidal zone at Cattle Point (48°27′N; 122°58′W), a moderately wave-exposed site at the southern tip of San Juan Island.

To examine smaller-scale spatial variation in phenolic levels, we sampled populations of subtidal kelps that were within ~50 m of one another at the Friday Harbor Laboratories: a population of *Laminaria groenlandica* from the floating dock and a population of *L. groenlandica* at 5 to 8 m depth at the "cantilever pier site". *L. complanata* was also collected from the second site.

The algae were immediately wrapped in damp paper towels and placed on ice for overnight mailing to Ohio, where they were stored at 6 °C for no more than one day before they were extracted. We found in controlled experiments at the Friday Harbor Laboratories that phenolic concentrations did not decrease in the algae for 3 to 4 d if the algae were kept cool and damp.

Phenolic analyses

We sampled ~1 g of tissue from each of several areas of kelps: meristems, vegetative tissues one-third up the blade, two-thirds up the blade, and at the distal ends of the blade (Fig. 2). In rockweeds, a single sample was taken from a non-meristematic vegetative portion of the blade. If a gram of tissue was not available we used as much tissue as possible.

Individual samples were divided into halves. One piece was weighed, dried in a 60 °C oven for 48 h, and then reweighed to

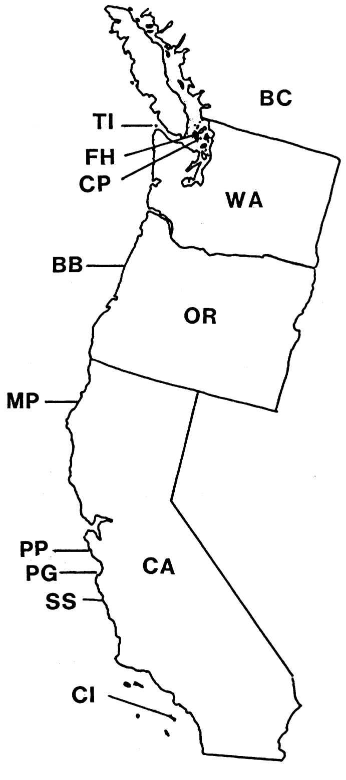

Fig. 1 Sites along west coast of North America (*BC* British Columbia, Canada; *CA* California, USA, *OR* Oregon, USA) where brown algae were collected to examine variation in phenolic concentrations over large spatial scales (*BB* Boiler Bay, Oregon; *CI* Catalina Island, California; *CP* Cattle Point, San Juan Island, Washington; *FH* Friday Harbor, San Juan Island, Washington; *MP* Mouda Point, California; *PG* Pacific Grove, California; *PP* Pigeon Point, California; *SS* San Simeon, California; *TI* Tatoosh Island, Washington; *WA* Washington, USA)

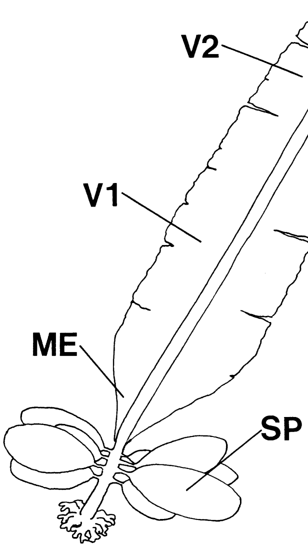

Fig. 2 *Alaria marginata.* (*DI* distal end of blade; *ME* vegetative meristematic tissue; *SP* sporophyll; *V1* vegetative tissue one-third of way up the blade; *V2* vegetative tissue two-thirds of way up the blade)

obtain a dry:wet mass ratio. The second piece was weighed, placed in 15 ml of 80% methanol, and kept as long as 10 mo at −4 °C in darkness before being homogenized. To determine if levels of phenolic compounds would decrease over time at −4 °C in aqueous methanol, we conducted Folin–Ciocalteu assays on 25 extracts immediately after collection and again 10 mo later. There was no evidence of a decrease in the concentration of Folin–Ciocalteu-reactive compounds.

We measured phenolic levels with a modified (Van Alstyne 1995) Folin–Ciocalteu method (Folin and Ciocalteu 1927). This assay is nearly identical to the Folin–Denis assay which is routinely used to quantify levels of phenolic compounds in brown algae (Ragan and Glombitza 1986; Van Alstyne 1995; Targett and Arnold 1998). The Folin–Ciocalteu assay also quantifies non-phenolic hydroxylated aromatic compounds; however, non-phenolic compounds are thought to make up < 5% of the total Folin–Ciocalteu-reactive compounds in marine brown algae (Van Alstyne 1995). Tissue samples that had been stored in methanol were ground with a Virtis "23" homogenizer and filtered through a Whatman GF/A glass-fiber filter. The extract (0.05 ml) was diluted 1:20 with distilled water, reacted with 1.00 ml of 40% Folin–Ciocalteu reagent for 5 min, then made alkaline with the addition of 1.00 ml of 2*N* Na_2CO_3. Absorbance was read at 765 nm after the solution had developed for 30 min at 50 °C. Phloroglucinol dihydrate was used as a standard.

Statistical analyses

We created a blade-composite average for each kelp sample; the average was calculated as a simple mean of the phenolic concentrations of the four areas of the tissue sampled. To determine if there was variation among sites in vegetative phenolic levels, the blade-composite averages (kelps) or blade phenolic concentrations (rockweeds) were transformed with an angular transformation so that the data would meet assumptions of normality and homogeneity of variances necessary for analyses of variance (Sokal and Rohlf 1995). One-way analyses of variance were conducted for each species that was collected at more than one site. If there were significant differences among sites ($P < 0.05$), a Tukey's test was used to determine which sites had levels significantly different from the others. A simple linear regression was used to examine the relationship between latitude and the angularly transformed average phenolic levels at a site.

To determine if phenolic levels varied in similar ways among sites, we conducted a two-way analysis of variance on the blade-composite averages of *Alaria marginata*, *Egregia menziesii*, and *Hedophyllum sessile* from Boiler Bay, Tatoosh Island, and Cattle Point. These species and sites were chosen because all three species were collected from all three sites and they all exhibited among-site differences in vegetative phenolic levels. The factors in the analysis of variance were species and sites. Blade-composite averages were transformed with an angular transformation before analysis.

Results

Vegetative polyphenolic levels (Tables 1 and 2) differed significantly among sites in 4 of the 12 kelp species and 3 of the 4 rockweed species that were collected at more that one site (Table 3). In kelps, the tendency for phenolic levels to differ among sites followed taxonomic relationships at the family level. Site-to-site differences in vegetative phenolic levels were found among all three members of the Alariaceae that were examined (Table 3). Only 1 of the 7 members of the Laminariaceae, *Hedophyllum sessile*, and none of the Lessoniaceae exhibited between-site differences (Table 3). The tendency not to exhibit geographic variation in phenolic levels was most common in low phenolic kelps. It should be noted, however, that in most of these species the phenolic levels were so low as to be indistinguishable from the background "noise" caused by non-phenolic Folin–Ciocalteu-reactive compounds. Seven of the eight kelps with no site-to-site differences in vegetative phenolic levels had low vegetative phenolic levels (Table 1); *Laminaria farlowii* had consistently high vegetative phenolic levels, but was sampled only at two sites, Pacific Grove and Pigeon Point (Table 1).

Phenolic levels differed among sites in the rockweeds *Fucus gardneri*, *F. spiralis*, and *Pelvetiopsis limitata*, but not in *Pelvetia fastigiata* (Table 3). The largest among-site difference in the Fucales was a nearly threefold difference in phenolic levels of *F. spiralis* from Boiler Bay and Cattle Point (Table 2).

Differences in phenolic levels among sites were not a function of latitude. Latitude and average phenolic concentrations were not correlated (linear regression: $P > 0.46$) in species collected at ≥3 sites.

Phenolic concentrations of *Alaria marginata*, *Egregia menziesii*, and *Hedophyllum sessile* at Boiler Bay, Tatoosh Island, and Cattle Point differed significantly among species ($P = 0.001$) and among sites ($P < 0.001$). There was a significant species x site interaction effect ($P < 0.001$, Table 4). Phenolic levels were generally lowest at Tatoosh Island and higher at Boiler Bay and Cattle Point (Fig. 3). *A. marginata* and *E. menziesii* had higher phenolic levels at Boiler Bay than Cattle Point. *H. sessile* at Boiler Bay had lower levels than at Cattle Point and an unusually high degree of variation. Phenolic levels were also higher in rockweeds

Table 1 Concentrations (means ± 1 SD) of phenolic compounds (% dry mass) in vegetative tissues of north-eastern Pacific kelps. (Sample sizes in parentheses)

Taxon/location	Habitat	Polyphenols
Family Alariaceae		
Alaria marginata		
Mouda Point, California	low intertidal	0.93 ± 0.57 (5)
Boiler Bay, Oregon	low intertidal	2.88 ± 0.67 (5)
Tatoosh Island, Washington	low intertidal	0.55 ± 0.25 (5)
Cattle Point, Washington	low intertidal	1.59 ± 0.67 (5)
Friday Harbor, Washington	subtidal, 0–0.5 m	2.96 ± 0.50 (5)
Alaria nana		
Pigeon Point, California	mid intertidal	3.43 ± 1.55 (5)
Tatoosh Island, Washington	mid intertidal	0.92 ± 0.15 (9)
Egregia menziesii		
Catalina Island, California	subtidal, 2–5 m	2.16 ± 0.60 (4)
San Simeon, California	low intertidal	0.40 ± 0.12 (6)
Pacific Grove, California	subtidal, 3–5 m	0.58 ± 0.23 (2)
Pigeon Point, California	low intertidal	0.68 ± 0.11 (4)
Mouda Point, California	low intertidal	0.48 ± 0.26 (6)
Boiler Bay, Oregon	low intertidal	1.62 ± 1.16 (8)
Tatoosh Island, Washington	low intertidal	0.33 ± 0.11 (8)
Cattle Point, Washington	low intertidal	0.36 ± 0.07 (7)
Eisenia arborea		
Catalina Island, California	subtidal, 2–5 m	5.02 ± 0.73 (6)
Family Laminariaceae		
Agarum fimbriatum		
Friday Harbor, Washington	subtidal, 0–0.5 m	3.24 ± 1.14 (5)
Costaria costata		
Boiler Bay, Oregon	low intertidal	0.34 ± 0.04 (5)
Cattle Point, Washington	low intertidal	0.35 ± 0.05 (5)
Cymanthere triplicata		
Tatoosh Island, Washington	low intertidal	0.35 ± 0.02 (5)
Cattle Point, Washington	low intertidal	0.38 ± 0.03 (5)
Hedophyllum sessile		
Boiler Bay, Oregon	mid intertidal	1.44 ± 2.23 (4)
Tatoosh Island, Washington	mid intertidal	0.31 ± 0.10 (5)
Cattle Point, Washington	mid intertidal	2.76 ± 0.23 (5)
Laminaria complanata		
Friday Harbor, Washington	subtidal, 5–8 m	2.26 ± 1.00 (5)
Laminaria dentigera		
San Simeon, California	low intertidal	0.37 ± 0.08 (6)
Pigeon Point, California	low intertidal	0.30 ± 0.05 (5)
Mouda Point, California	low intertidal	0.42 ± 0.09 (5)
Boiler Bay, Oregon	low intertidal	0.32 ± 0.19 (5)
Laminaria farlowii		
Pacific Grove, California	subtidal, 3–5 m	4.60 ± 0.46 (6)
Pigeon Point, California	low intertidal	4.78 ± 0.24 (5)
Laminaria groenlandica		
Tatoosh Island, Washington	low intertidal	1.08 ± 0.86 (5)
Friday Harbor, Washington	subtidal, 0–0.5 m	0.85 ± 0.04 (5)
Laminaria setchelli		
Tatoosh Island, Washington	low intertidal	0.44 ± 0.49 (5)
Laminaria sinclairii		
San Simeon, California	low intertidal	0.37 ± 0.05 (6)
Pigeon Point, California	low intertidal	0.36 ± 0.09 (5)
Mouda Point, California	low intertidal	0.50 ± 0.20 (5)
Family Lessoniaceae		
Lessoniopsis littoralis		
Mouda Point, California	low intertidal	0.37 ± 0.12 (5)
Boiler Bay, Oregon	low intertidal	0.28 ± 0.06 (5)
Macrocystis pyrifera		
Catalina Island, California	subtidal, 5–10 m	1.04 ± 0.37 (4)
Pacific Grove, California	subtidal, 3–5 m	1.02 ± 0.26 (6)
Postelsia palmaeformis		
Pigeon Point, California	mid intertidal	1.31 ± 0.22 (5)

Table 2 Concentrations (means ± 1 SD) of phenolic compounds (% dry mass) in vegetative tissues of northeastern Pacific rockweeds. (Sample sizes in parentheses)

Taxon/location	Depth	Polyphenols
Family Cystoseiraceae		
Cystoseira neglecta		
Catalina Island, California	subtidal, 5–10 m	1.37 ± 0.78 (9)
Cystoseira osmundacea		
Pacific Grove, California	subtidal, 3–5 m	1.60 ± 0.63 (6)
Halidrys dioica		
Catalina Island, California	subtidal, 5–8 m	4.14 ± 3.20 (6)
Family Fucaceae		
Fucus gardneri		
Pigeon Point, California	mid intertidal	2.87 ± 1.35 (4)
Boiler Bay, Oregon	mid intertidal	5.21 ± 0.77 (5)
Tatoosh Island, Washington	mid intertidal	4.59 ± 0.71 (5)
Cattle Point, Washington	mid intertidal	3.55 ± 0.41 (5)
Fucus spiralis		
Boiler Bay, Oregon	mid intertidal	4.30 ± 0.57 (5)
Cattle Point, Washington	mid intertidal	1.55 ± 0.26 (5)
Hesperophycus harveyanus		
Catalina Island, California	mid intertidal	1.09 ± 0.29 (3)
Pelvetia fastigiata		
San Simeon, California	mid intertidal	5.98 ± 0.99 (9)
Pigeon Point, California	mid intertidal	5.24 ± 0.76 (5)
Pelvetiopsis limitata		
Pigeon Point, California	mid intertidal	2.69 ± 0.77 (5)
Mouda Point, California	mid intertidal	4.50 ± 0.54 (5)
Boiler Bay, Oregon	mid intertidal	4.41 ± 0.54 (5)
Tatoosh Island, Washington	mid intertidal	2.75 ± 0.90 (5)
Family Sargassaceae		
Sargassum palmeri		
Catalina Island, California	subtidal, 2–5 m	1.31 ± 0.44 (6)

Table 3 Results of one-way analyses of variance to determine whether there were significant differences in phenolic levels among sites for species collected at more than one site. For each species, sites are given from lowest to highest mean phenolic level [*Sites with same superscripts* had mean phenolic levels that were not significantly different (based on Tukey's test that was conducted if there was a significant site effect from the ANOVA for that species, Minitab, Release 10, $P < 0.05$)] All phenolic concentrations were transformed with angular transformation prior to analysis so that data would meet assumptions of normality and homogeneity of variances necessary for conducting analyses of variance (Sokal and Rohlf 1995) (*Site codes* as in Fig. 1)

Species	F	P	Sites
Order Laminariales			
Family Alariaceae			
Alaria marginata	18.02	<0.001	TI^{a} MP^{a,b} CP^{b} BB^{c} FH^{c}
Alaria nana	35.01	<0.001	TI^{a} PP^{b}
Egregia menziesii	10.26	<0.001	TI^{a} CP^{a} SS^{a} MP^{a} PG^{a,b} PP^{a,b} BB^{b,c} CI^{b,c}
Family Laminariaceae			
Costaria costata	0.02	0.981	CP BB TI
Cymanthere triplicata	0.73	0.417	TI CP
Hedophyllum sessile	7.80	0.008	TI^{a} BB^{a,b} CP^{b}
Laminaria dentigera	1.19	0.344	PP BB SS MP
Laminaria farlowii	0.72	0.418	PG PP
Laminaria groenlandica	0.02	0.901	TI FH
Laminaria sinclairii	1.47	0.266	MP SS PP
Family Lessoniaceae			
Lessoniopsis littoralis	2.49	0.153	BB MP
Macrocystis pyrifera	0.00	0.974	PG CI
Order Fucales			
Family Fucaceae			
Fucus gardneri	6.82	0.004	PP^{a} CP^{a,b} TI^{a,b} BB^{b}
Fucus spiralis	97.01	<0.001	CP^{a} BB^{b}
Pelvetia fastigiata	2.20	0.185	PP SS
Pelvetiopsis limitata	8.33	0.001	PP^{a} TI^{a} MP^{b} BB^{b}

Table 4 *Hedophyllum sessile*, *Alaria marginata* and *Egregia menuiesii* Two-way analysis of variance of phenolic levels in kelp at Boiler Bay, Oregon, Cattle Point, Washington, and Tatoosh Island, Washington. All phenolic concentrations were transformed with angular transformation prior to analysis (*SS* sum of squares)

Source	(*df*)	SS	*F*	*P*
Species	(2)	0.023693	9.08	0.001
Site	(2)	0.055300	21.02	< 0.001
Species × site	(4)	0.039920	7.65	< 0.001
Error	(43)	0.056082		
Total	(51)	0.175543		

(*Fucus gardneri*, *F. spiralis*, and *Pelvetiopsis limitata*) from Boiler Bay than from other sites (Table 2).

Phenolic concentrations differed across locations that were separated by < 100 m. Meristematic phenolic levels in subtidal *Laminaria groenlandica* from the floating dock at the Friday Harbor Laboratories were almost twice as high as those from conspecifics at "cantilever pier" (Fig. 4; one way ANOVA: $P < 0.001$; Bonferroni test: $P < 0.05$); however, subtidal *L. complanata* from the "cantilever pier site" had significantly higher phenolic concentrations than either the subtidal or the dock populations of *L. groenlandica* (Fig. 4; one way ANOVA: $P < 0.001$; Bonferroni test: $P < 0.05$).

Mean phenolic levels (calculated with across-site averages for each species) were significantly higher in members of the Fucales than in members of the Laminariales (two-way ANOVA on angularly transformed data, order effect: $P = 0.035$, Table 5). Phenolic levels were not significantly different in intertidal versus subtidal species (two-way ANOVA on angularly-transformed data, location effect: $P = 0.723$, Table 5). There was a non-significant (two-way ANOVA on angularly transformed data, interaction effect between order and location: $P = 0.180$; Table 5) trend for phenolic levels to be higher in intertidal rockweeds than subtidal rockweeds, and higher in subtidal than intertidal kelps.

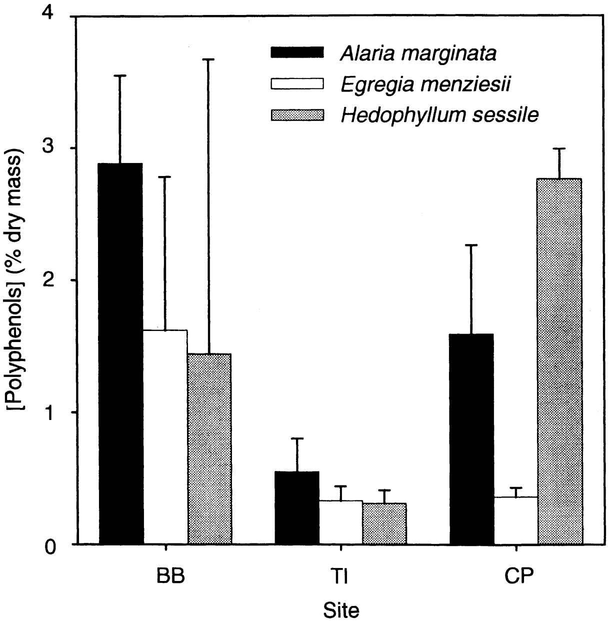

Fig. 3 *Alaria marginata*, *Egregia menziesii*, and *Hedophyllum sessile*. Concentrations (means ± 1 SD, $N = 4$ to 8) of phenolic compounds (% of dry mass) in specimens from Boiler Bay, Oregon (*BB*), Tatoosh Island, Washington (*TI*), and Cattle Point, Washington (*CP*). Results of statistical analyses are presented in Table 4

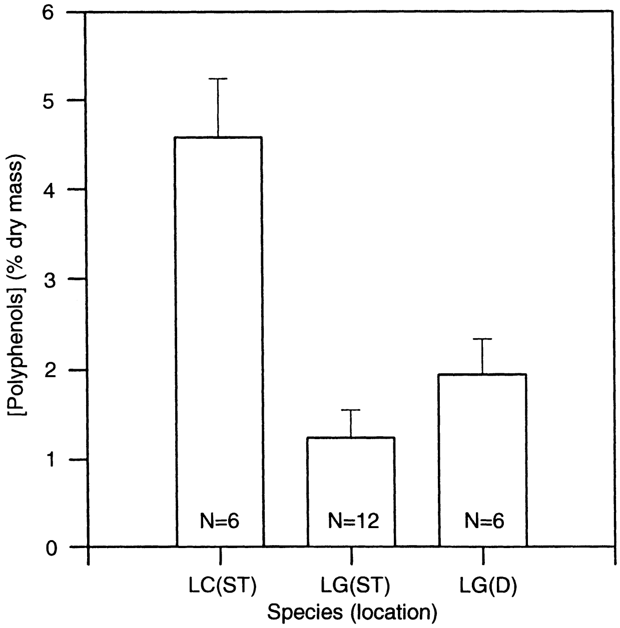

Fig. 4 *Laminaria complanata* (*LC*) and *L. groenlandica* (*LG*). Phenolic concentrations (mean ± 1 SD as % dry mass) of meristematic tissues from specimens from floating dock (*D*) at Friday Harbor Laboratories, Friday Harbor, Washington, and from subtidal (*ST*) site (5 to 8 m depth off site of former "cantilever pier") ≃50 m away from D. Sample size is given at base of each bar

Table 5 Laminariales and Fucales. Concentrations (means ± 1 SD) of phenolic compounds (% dry mass) in intertidal and subtidal species. Values are averages of means for each species across all sites where they were collected (two-way analysis of variance on angularly transformed data; order effect: $P = 0.035$, location effect: $P = 0.723$, order × location effect: $P = 0.180$)

	Laminariales	Fucales
Intertidal species	1.00 ± 0.53 ($N = 4$)	3.45 ± 1.65 ($N = 5$)
Subtidal species	1.77 ± 1.65 ($N = 13$)	2.11 ± 1.36 ($N = 4$)

Discussion

Studies striving to understand ecological processes frequently begin with an examination of spatial or temporal patterns in the distribution of key ecological parameters. The ecological role and selective consequences of polyphenolic compounds in marine algae are probably multifaceted and extremely important, yet we know very little about the patterns of distribution of these compounds among taxa of algae from the north Pacific, and even less about spatial and temporal variation of polyphenolic compounds within species. In this study, we have documented that kelps from the northeast Pacific contain these compounds more frequently and at higher concentrations than would have been expected from patterns described in previous publications. A review of brown-algae phlorotannins (Ragan and Glombitza 1986) lists only 4 of 19 (21%) species on the west coast of North America as having vegetative phenolic levels >2% of the alga's dry mass. Three of four species (75%) at the Friday Harbor site had phenolic levels >2% by dry mass (Table 3). Eight of the 17 kelp species (47%) we examined had vegetative tissues with levels >2% by dry mass in at least one location (Table 3). Most of the measurements described in Ragan and Glombitza's (1986) review paper were conducted with a Folin–Denis analysis with phloroglucinol used as a standard, a method very similar to that used for measuring phenolic levels in this study. Therefore, it is unlikely that methodological differences could account for the differences in phenolic levels reported from this study and others. The differences are more probably due to the larger numbers of species and locations sampled in this study compared to past studies. Concentrations of phenolic compounds from the Fucales were similar to values previously reported from west coast rockweeds (Steinberg 1985; Ragan and Glombitza 1986; Van Alstyne 1988, 1989; Van Alstyne and Paul 1990; Irelan and Horn 1991; Targett et al. 1992; Winter and Estes 1992).

The magnitude of spatial variation seen in many species in this study was also high compared to values reported by Steinberg (1989) and Pavia and Aberg (1996). For example, in *Hedophyllum sessile* (Table 1), there was nearly an order of magnitude difference in phenolic levels between Tatoosh Island and Cattle Point, sites separated by only ~130 km. Concentrations of phenolic levels in *Egregia menziesii* were over six times higher at Catalina Island than at Tatoosh Island (Table 1). These values are comparable to the high degrees of spatial variation reported for the tropical alga *Lobophora variegata* (Targett et al. 1995) and the Atlantic species *Fucus vesiculosus* (Targett et al. 1992).

Some data suggest that phenolic levels vary in similar ways across sites (Table 4), but further work is needed to unequivocally determine this. Phenolic levels in *Alaria marginata*, *Egregia menziesii*, and *Hedophyllum sessile* were lowest at Tatoosh Island, and except for *H. sessile*, were highest at Boiler Bay (Fig. 3). Other species were not collected at all three sites but showed similar patterns. Phenolic levels of *Fucus gardneri* and *Pelvetiopsis limitata* were higher at Boiler Bay than Tatoosh Island (Table 2). Phenolic levels of *A. nana* were lower at Tatoosh Island (Table 1), and phenolic levels of *F. spiralis* were higher at Boiler Bay (Table 2) than at other locations.

For the brown algal kelps and rockweeds examined in this study, phenolic levels tended to differ significantly among sites in species within the order Fucales and within the family Alariaceae of the order Laminariales (Table 3), but not in the families Laminariaceae and Lessoniaceae. This suggests that the response of phenolic levels to environmental conditions, either through localized selection or phenotypic plasticity, may be phylogenetically constrained.

Phenotypic plasticity in phenolic concentrations has been previously described in the fucoid brown algae *Fucus gardneri* (Van Alstyne 1988), *F. vesiculosus* (Yates and Peckol 1993; Peckol et al. 1996), and *Ascophyllum nodosum* (Pavia et al. 1997) and in the kelps *Agarum fimbriatum*, *Pleurophycus gardneri*, *Laminaria complanata*, and *L. groenlandica* (Hammerstrom et al. 1998). However, changes in phenolic levels in response to changes in environmental conditions do not occur in all brown algae. Steinberg (1994) found no evidence of increased phenolic levels in response to simulated herbivory in the kelp *Ecklonia radiata* and the fucoid brown alga *Sargassum vestitum*, suggesting that the ability of brown algal phenolic levels to vary in response to changing environmental conditions is species-specific.

Although this study was designed only to document patterns of spatial variation in phenolic levels rather than to discern causes of these patterns, the patterns we saw allow some causes to be eliminated. Because concentrations did not follow a latitudinal cline, length of photoperiod is unlikely to be a causative agent, although phenolic levels have been shown to be influenced by light intensity (Cronin and Hay 1996), particularly in the UV range (Pavia et al. 1997). There was no tendency for species at more wave-exposed sites to have higher or lower phenolic levels than species at less wave-exposed sites. This is in agreement with previous measurements of phenolic levels from *Fucus gardneri* from wave-exposed and wave-protected areas within a single site (Van Alstyne 1995). Phenolic levels have been shown to be influenced by or correlated with grazing pressure (Van Alstyne 1988; Peckol et al. 1996; Hammerstrom et al. 1998), nitrogen levels (Ilvessalo and Tuomi 1989; Yates and Peckol 1993; Arnold et al. 1995; Peckol et al. 1996) and salinity (Pedersen 1984). The design of our study did not allow us to assess these factors.

In summary, phenolic concentrations of kelps and rockweeds from the west coast of North America varied greatly over a wide range of spatial scales. Significant variation in phenolic concentrations has been reported from virtually every possible spatial scale: among tissue types in an individual (Geiselman 1980; Steinberg 1984, 1985, 1989; Johnson and Mann 1986; Tugwell and Branch 1989; Van Alstyne 1989; Pfister 1992), among sites at scales ranging from tens of meters to hundreds of kilometers (Table 3; and Steinberg 1989), and between global regions (Steinberg 1986, 1992; Van Alstyne and Paul 1990; Steinberg and van Altena 1992; Targett et al. 1992; Steinberg et al. 1995). Temporal variation in concentrations of phenolic levels has also been documented on scales ranging from weeks (Van Alstyne 1988) to months (Ragan and Glombitza 1986 and references cited therein; Steinberg and van Altena 1992).

The causes and consequences of this high degree of variability are undoubtedly complex, but they need to be considered when making generalizations about the ecological and evolutionary significance of phenolic metabolites.

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