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Seasonal variation in kelp phlorotannins in relation to grazer abundance and environmental variables in the Alaskan sublittoral zone

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   Phlorotannins are common metabolites produced in kelps that can have deterrent functions against grazers. The factors dictating seasonal patterns of phlorotannin content in northeastern Pacific kelps are not well understood. This study assessed density and grazing of the gastropod *Lacuna vincta* on the annual canopy-forming kelp *Nereocystis luetkeana* and the perennial understory species *Agarum clathratum*, *Saccharina latissima* and *S. groenlandica* in Kachemak Bay, Alaska. In addition, we assessed seasonal patterns of environmental variables as possible drivers of phlorotannin concentrations. Phlorotannins occurred in all species, with overall lowest levels in *N. luetkeana*, and with different seasonal patterns among the four species. *Lacuna vincta* was most dense on *N. luetkeana* thalli in the summer and had highest grazing rates on this low-phlorotannin species. However, correlations between *L. vincta* density and phlorotannin content of each kelp species were not significant. Except for *N. luetkeana*, there were no correlations between phlorotannin levels and environmental variables. We suggest that kelp life history traits may be more important for phlorotannin patterns in these kelp species than grazers or environmental drivers.

Key Words: environmental variables; grazers; kelps; life history; phlorotannins

INTRODUCTION

   Kelps (Order Laminariales) form the basis of essential habitats in temperate coastal ecosystems because of their high productivity and function as spawning and nursery grounds for many invertebrate and fish species (Steneck et al. 2002). However, intense grazing can severely compromise kelp abundance and function (Scheibling et al. 1999). Mesograzers of the nearshore marine environment such as gastropods (Iken 1999, Granado and Caballero 2001) are capable of affecting the growth and reproductive abilities of individual macroalgal species (Dean et al. 1984, Dethier et al. 2005) and even the composition and density of macroalgal communities (Carney et al. 2005). Mesograzer habitat and food choice is often attributed to algal morphological characteristics like shape, size and

tissue toughness (Littler and Littler 1980, Steneck and Watling 1982, Hay et al. 1994), and to chemical properties related to nutritive value (Cruz-Rivera and Hay 2003) and anti-herbivore defense (Paul et al. 2001, Amsler 2008).

   Kelps usually contain phlorotannins, phloroglucinol-based polymers present in most brown algae (Ragan and Glombitza 1986, Amsler and Fairhead 2006), which have primary metabolic roles in wound healing (Lüder and Clayton 2004) and cell wall construction (Schoenwaelder and Clayton 1999). Phlorotannins also have been assigned secondary functions such as protection against UV radiation (Pavia et al. 1997), bacterial and fungal growth and fouling (Ragan and Glombitza 1986, Wikström and Pavia 2004, Iken et al. 2009), and grazing damage (for reviews

see Targett and Arnold 1998, Amsler and Fairhead 2006). The function and effectiveness of phlorotannins as feeding deterrents may differ by algal and herbivore species, with some studies demonstrating grazing reduction or inhibition (Geiselman and McConnell 1981, Pavia and Toth 2000*a*) and others showing negligible responses or even increased herbivory (Steinberg and van Altena 1992, Deal et al. 2003, Kubanek et al. 2004).

Phlorotannin content in brown algae can be regulated by a variety of abiotic and biotic variables, with large species-specific variability in responses to these factors (reviewed by Amsler and Fairhead 2006), which may act synergistically to affect distribution of phlorotannins within species and within thalli (e.g., Hay 1996, Targett and Arnold 1998). Phlorotannin content may be influenced by temporal variability in physical factors such as irradiance (Pavia and Toth 2000*b*, Cruces et al. 2012), salinity (reviewed by Ragan and Glombitza 1986), nutrients (Yates and Peckol 1993, Cronin and Hay 1996, Svensson et al. 2007), and water movement (Dayton 1985). Additionally, the risk of attack by grazers may affect patterns of phlorotannin content (Rhoades 1979, Amsler 2001). For example, the presence of grazers or mechanical wounding may affect patterns of phlorotannin production if the macroalgal species in question demonstrates inducible defenses (Hammerstrom et al. 1998, Pavia and Toth 2000*a*, Jormalainen et al. 2003, Hemmi et al. 2004).

In the present study, we investigated seasonal phlorotannin content of the annual, canopy-forming kelp species *Nereocystis luetkeana* (Mertens) Postels et Ruprecht, and the perennial, understory species *Agarum clathratum* Dumortier, *Saccharina latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl & G. W. Saunders and *S. groenlandica* (Rosenvinge) C. E. Lane, C. Mayes, Druehl & G. W. Saunders in the Northeast Pacific (Alaska). We analyzed phlorotannin content in relation to seasonal environmental variables (light and nutrients). We further determined seasonal field densities and laboratory grazing by the gastropod *Lacuna vincta* on these kelp species. These kelp and grazer species were chosen due to their ubiquity within the study area (Dubois 2006, Chenelot and Konar 2007, Konar et al. 2009). The grazer is known to be able to decimate algal biomass and to be a structuring force for macroalgal communities (Fralick et al. 1974, Johnson and Mann 1986, Chenelot and Konar 2007). Our objectives were to assess relationships between phlorotannin levels, environmental conditions and grazing rates, and to determine if these relationships differed between seasons in species with different life history strategies.

MATERIALS AND METHODS

Study species and sites

Field studies occurred off the western end of Hesketh Island (59°30.3′ N, 151°31.8′ W) within Kachemak Bay, south-central Alaska. The study site was located on exposed shores approximately 5 m below mean lower low water. Common kelp species in the study area include the canopy-forming annual kelp *Nereocystis luetkeana* and the perennial understory kelps *Agarum clathratum*, *Saccharina latissima* and *S. groenlandica*. The perennial kelp species may persist through the winter as complete individuals or as stipes (O'Clair and Lindstrom 2000, Chenelot 2003). The gastropod grazer *L. vincta* is seasonally abundant and has been reported to have significant grazing impact on canopy (Duggins et al. 2001, Chenelot 2003, Carney et al. 2005) and understory kelp thalli (Fralick et al. 1974, Johnson and Mann 1986, Krumhansl and Scheibling 2011).

Grazer and kelp surveys

Collection of kelp thalli occurred at Hesketh Island every two weeks in summer (June to September of 2004 and 2005) and monthly in winter (December 2004 to March 2005). *L. vincta* densities were measured on the same sampling dates during 2005. Gastropod density was determined within three 100 cm² quadrats per blade (one blade haphazardly chosen for *N. luetkeana*) of three randomly selected individuals per kelp species.

The same kelp individuals observed for gastropod densities were then removed from the substrate below the holdfast and kept in cold seawater in the dark for transport (n = 3 thalli per species). Thallus portions (<1.5 g each) were sampled for phlorotannin analysis from the stipe and blades. Excised tissue was weighed and kept frozen at -20°C until phlorotannin analysis. In addition, larger amounts of blade material (~500 g) were collected for each species and kept frozen for phlorotannin standard preparation.

Phlorotannin analysis

Preparation of phlorotannin standards and analysis of kelp tissue phlorotannin content followed the 2, 4-dimethoxybenzaldehyde (DMBA) method described by Stern et al. (1996). Briefly, phlorotannin standards were made by grinding (Power Gen 500 homogenizer; Fisher Scientific, Hampton, NH, USA) and extracting frozen kelp tis-

sue three times in 80% methanol for 12 h. The combined extracts were filtered (8 µm cellulose filters, Whatman; Fisher Scientific, Hampton, NH, USA), evaporated under reduced pressure at 35°C and further purified using subsequent elutions with toluene and acetone (Stern et al. 1996). Each standard was freeze dried for 48 h and then ground to a fine powder. Two phlorotannin standards were made for each species. Replicate standard curves for both standards for each species were determined using a 0-500 µg µL^{-1} range of phlorotannin mass to methanol volume. The use of a mean slope from multiple replicates accounted for some procedural variability in standard curves, though differences among replicates were very low.

For phlorotannin determination in kelps, thallus samples (stipe and blade for seasonal collections, only blade for feeding assay collections – see below) were homogenized separately and 80% methanol-extracted overnight. Phlorotannin measurements were conducted on a solution of the DMBA assay working reagent (Stern et al. 1996), 200-400 µL of extract (depending upon kelp species) and *N, N*-dimethylformamide. Solutions were incubated and absorbance determined spectrophotometrically at 510 nm (Thermospectronic Genesys 5; Cole-Parmer, Vernon Hills, IL, USA) against blanks of the same volume in all assays (Stern et al. 1996). Calculation of phlorotannin content as percent dry mass (% DM) was based on the ratio of wet : dry mass for stipes and blades of each kelp species (n = 6) after drying at 60°C for 48 h.

Feeding assays

Laboratory feeding assays with *L. vincta* were conducted during the summer (August) of 2004 and 2005 to test for the palatability of kelp blades of the four species in non-choice experiments. Grazers were collected from areas adjacent to the study site and allowed to clear guts for 24 h in running seawater tanks. Prior to the start of each experiment, ten individuals per kelp species were collected and three 1.5 ± 0.1 g blade pieces excised adjacent to each other from the meristematic region of each kelp individual. One of these pieces was used in the feeding treatment, the second in the control treatment and the third was frozen for corresponding phlorotannin analysis (for methods see above). Fifty *L. vincta* of average size (~6 mm) were placed in a treatment container (n = 10) with a piece of kelp blade. The quantity of *L. vincta* was based on the maximum density observed in the field on a blade area similar to that used in feeding assays. A paired container per kelp species and replicate held only a match-

ing blade segment (no grazers) as a control to account for autogenic weight changes due to growth or decay (Peterson and Renaud 1989). Containers were floated in flow-through seawater tanks to maintain ambient temperature regimes, and water within containers was replaced every 24 h. Relative mass change after 72 h was determined by the difference in wet mass exposed to grazers minus the mass change of ungrazed control blade segments.

Environmental variables

Nutrient concentrations and light attenuation were measured at each field sampling. Water samples obtained ~1 m above the substrate were used for analysis of nitrate, ammonium, phosphate and silicate concentrations using an Alpkem model RFA-300 continuous nutrient analyzer (Alpkem Co., Clackamas, OR, USA). Light profiles were taken with a LiCor 193SA spherical sensor (Li-Cor, Lincoln, NE, USA) to determine irradiance (µmol m^{-2} s^{-1}) just above and below the water surface and at 5 m depth. Since *N. luetkeana* blades are close to or at the water surface for a large proportion of their life cycle, percent light attenuation at 1 m depth was used for this species, while the 5 m depth measurements were taken to approximate attenuation within the understory kelp canopy. Temperatures from a HOBO data logger (Onset Computers) at the study site were used to establish "summer" (10.3 ± 0.10°C, June-September) and "winter" (4.88 ± 0.07°C, December-March) seasons.

Statistical analyses

Prior to statistical assessments, parametric assumptions for analyses of variance (ANOVA) were evaluated using residual plots and Shapiro-Wilk tests (Zar 1999). *L. vincta* grazer density data were left untransformed and phlorotannin content data were arcsine-square root transformed to improve error variance. Grazer density on blades of each of the four kelp species was analyzed for between-season differences using single-factor ANOVA (season as the orthogonal factor). Single-factor ANOVA were also used to separately assess between-season differences in phlorotannin content in stipe and blades of each of the four kelp species. Untransformed tissue mass and phlorotannin data from palatability assays were analyzed by single-factor ANOVAs with kelp species as the fixed factor. All post-hoc assessments of significant effects and interactions were made using the Tukey-Kramer method. Between-season comparisons of environmental data were conducted using single-factor ANOVA (season
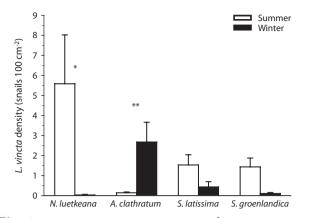
Fig. 1. *Lacuna vincta* density (snails 100 cm^{-2}; mean ± 1 SE) on blade tissue of *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *Saccharina groenlandica* during the summer and winter at Hesketh Island. Significant differences in density between seasons are denoted by *0.01 < p < 0.05 and **p ≤ 0.01.

as the orthogonal factor) on untransformed irradiance and log-transformed nitrate, ammonium, phosphate and silicate. Pearson's product-moment correlations were used to separately determine the relationship between blade phlorotannin content with grazer density and environmental variables (light and nutrients) for each kelp species. Phosphate and silicate were excluded from this analysis due to high correlation (p < 0.01) with nitrate. All statistical tests were conducted using SAS, v9.1 (SAS Institute Inc., Cary, NC, USA) at α = 0.05.

RESULTS

Seasonal grazer density

Seasonal patterns of *L. vincta* were not consistent among kelp species. During summer, snail density was significantly higher than during winter on *N. luetkeana* (p = 0.03), and higher but not significantly on *S. latissima* and *S. groenlandica*. Conversely, *L. vincta* density was significantly higher during winter than summer on *A. clathratum* blades (p < 0.01, Fig. 1).

Phlorotannin content

Phlorotannin content in stipes and blades of the four kelp species was not consistently higher in either summer or winter (Fig. 2). *N. luetkeana* was the only kelp species that demonstrated a significant difference in phlorotannin content between seasons in both thallus parts, with higher content in summer in stipe and blade (Table
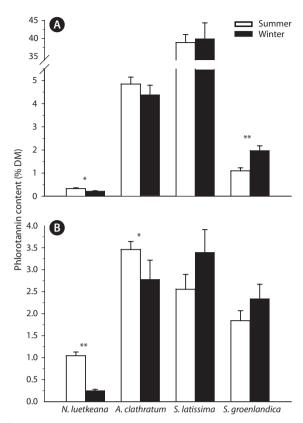

Fig. 2. Phlorotannin content (% dry mass [DM]; mean ± 1 SE) of *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *Saccharina groenlandica* stipe (A) and blade tissue (B) (n = 5-36 per thallus part) during the summer and winter at Hesketh Island. Significant differences in phlorotannin content between seasons are denoted by *0.01 < p < 0.05 and **p ≤ 0.01. Note difference in y-axis scales between thallus parts.

1, Fig. 2). *A. clathratum* had similar phlorotannin concentrations between seasons in stipes and significantly higher concentrations in summer in blades. Conversely, *S. latissima* and *S. groenlandica* phlorotannin content was typically higher during winter in both thallus parts, but with the only significant difference in *S. groenlandica* stipes (Table 1, Fig. 2).

Feeding assays

As much as 30% of the total wet mass of kelp tissue was consumed by *L. vincta* within 72 h (Fig. 3A). *Lacuna vincta* consumed significantly more blade tissue of *N. luetkeana* than of any understory species (p < 0.05). Grazing was lowest on the two *Saccharina* species. Phlorotannin content of tissues used in feeding experiments also varied significantly between kelp species (p < 0.01). Blade phlorotannins were generally low (<2.0% DM) in all kelp species, with concentrations in *N. luetkeana* and *S. latis-*
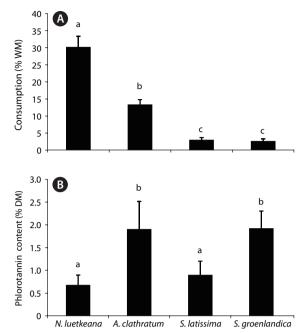
Fig. 3. *Lacuna vincta* grazing (% WM ± 1 SE) (A) and phlorotannin content (% dry mass [DM]; mean ± 1 SE) (B) of *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *Saccharina groenlandica* blade segments (n = 10 per species). Letters above bars indicate significant differences among species (p < 0.05).

sima less than 1% DM (Fig. 3B). The greatest consumption occurred in the species containing the lowest phlorotannin content (*N. luetkeana*), but no overall relationship between snail grazing and tissue phlorotannin content existed (p > 0.05).

Environmental variables

Irradiance was higher during summer and decreased in winter by ~80-90% at 1 and 5 m depths (Table 2). With the exception of ammonium, all other nutrient concentrations were significantly different between seasons (Table 2), with higher concentrations in winter than summer (p < 0.01).

Phlorotannin relationships with grazer density and environmental variables

Pearson's product-moment correlations between blade phlorotannin content and *L. vincta* density were not significant for any kelp species (Table 3). Correlation analyses of blade phlorotannin content of the four kelp species with irradiance, nitrate, and ammonium dem-

Table 1. Results of single-factor ANOVA of phlorotannin content (% dry mass [DM]) in *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *Saccharina groenlandica* stipe and blade during summer (June-September) and winter (December-March)

Source of variation	Stipe			Blade		
	df	F	p	df	F	p
N. luetkeana	1	6.23	**0.02**	1	57.06	<0.01
Error	50			49		
A. clathratum	1	0.32	0.58	1	4.42	0.04
Error	50			52		
S. latissima	1	0.02	0.90	1	2.60	0.11
Error	51			47		
S. groenlandica	1	12.1	**<0.01**	1	1.32	0.26
Error	48			49		

Significant values at p < 0.05 are shown in bold.

Table 2. Subtidal irradiance, nitrate, ammonium, phosphate, and silicate concentrations (mean ± 1 SE) measured during summer (June-September) and winter (December-March)

Season	Irradiance (μmol m⁻² s⁻¹)*	Nitrate (μM)**	Ammonium (μM)	Phosphate (μM)**	Silicate (μM)**	n
Summer	300.69 ± 71.81 (1 m) 85.45 ± 16.19 (5 m)	1.45 ± 0.31	0.45 ± 0.07	0.60 ± 0.04	5.28 ± 0.54	9 (irradiance) 13 (each nut.)
Winter	27.40 ± 9.25 (1 m) 15.52 ± 4.46 (5 m)	11.89 ± 0.63	0.22 ± 0.07	1.36 ± 0.05	22.56 ± 1.37	4 (irradiance) 5 (each nut.)

Sample sizes are shown for each season and variable. Significant differences in a variable between seasons are denoted by *0.01 < p < 0.05 and **p ≤ 0.01.

onstrated significant relationships only for *N. luetkeana* (Table 3). *N. luetkeana* phlorotannins were positively correlated with irradiance at 1 m (r = 0.653, p = 0.02) and negatively correlated with nitrate at 5 m (r = -0.699, p < 0.01). No significant relationship existed between *N. luetkeana* phlorotannins and ammonium. None of the correlations between *A. clathratum*, *S. latissima* and *S. groenlandica* blade phlorotannins and environmental variables at 5 m were significant and varied in terms of the direction of the relationship between factors (Table 3).

DISCUSSION

Northeastern Pacific kelps from this study exhibited detectable phlorotannin concentrations in stipe and blade tissues throughout the duration of field surveys. Mean annual phlorotannin values of all kelp species approximate those measured previously in northern Pacific or Atlantic brown algae (Ragan and Glombitza 1986, Targett et al. 1992, Van Alstyne et al. 1999), though the remarkably high content in *S. latissima* stipes is unprecedented. The ecological and physiological significance of these high phlorotannin values is as of yet unexplained. However, it is unlikely that methodological problems with stipe phlorotannin assays caused inflated values since the same laboratory procedures were used to produce phlorotannin values for all kelp species and thallus parts, all of which are within range of those reported in the scientific literature. We also repeated these measures several times with identical results. Higher phlorotannin concentrations in stipes, which is the perennial thallus part in *S. latissima* (O'Claire and Lindstrom 2000), or holdfasts than in blades has been observed before in several macroalgal species (e.g., Ragan and Glombitza 1986, Tugwell and Branch 1989, Iken et al. 2007). The large variability in phlorotannin content in the studied kelps is also similar to other observations of substantial differences within (e.g., Pavia et al. 2003, Fairhead et al. 2005, Iken et al. 2007) and between (e.g., Ragan and Glombitza 1986, Van Alstyne et al. 1999) species.

L. vincta was overall more abundant in the summer than winter, indicating seasonal rather than continuous recruitment in the study area (also see Maney and Ebersole 1990, Martel and Chia 1991). Gastropods during summer were on average more abundant on the low-phlorotannin containing thalli of *N. luetkeana* and less frequent on the thalli of those species containing higher phlorotannins (especially *A. clathratum*), despite *N. luetkeana* being, on average, the least abundant of the four kelp species within the study area (Dubois 2006). In winter, snail densities were highest on *A. clathratum*, possibly because this perennial species maintains much of its blade structure during the winter. In contrast, most of the annual *N. luetkeana* thalli disappear in winter and the two *Saccharina* species mostly overwinter with reduced blade portions. Hence, seasonal kelp abundance might influence *L. vincta*'s preference for *A. clathratum* in the winter. In the northern Gulf of St. Lawrence, Canada, however, *L. vincta* was more abundant on *A. cribrosum* than several other macroalgae also in the summer (Bégin et al. 2004).

In our palatability assays, *L. vincta* grazed significantly more on *N. luetkeana* blades with the lowest phlorotannin levels of the studied kelp species, similar to the high grazing rates on juvenile *N. luetkeana* previously reported for the study area (Chenelot and Konar 2007). While these relationships may indicate grazer avoidance of phlorotannin-rich kelps, consumption by *L. vincta* in feeding assays was not consistently proportional to phlorotannin content of each kelp species (Table 3). Therefore, other or additional factors, such as tissue toughness or nutritive value, may have affected kelp palatability (Chavanich

Table 3. Results of Pearson's product-moment correlations of blade phlorotannin content (% dry mass [DM]) in *Nereocystis luetkeana*, *Agarum clathratum*, *Saccharina latissima* and *Saccharina groenlandica*, with grazer density, irradiance (n = 13 per species), nitrate, and ammonium (at 5 m; each n = 17 per species)

Kelp species	Grazers (snails 100 cm^{-2})		Irradiance (μmol m^{-2} s^{-1}) (1 or 5 m)		Nitrate (μM)		Ammonium (μM)	
	r	p	r	p	r	p	r	p
N. luetkeana	0.316	0.16	0.653	**0.02**	-0.699	**<0.01**	0.247	0.34
A. clathratum	-0.001	0.50	0.467	0.11	-0.432	0.08	0.237	0.36
S. latissima	0.141	0.33	-0.269	0.37	0.120	0.65	-0.287	0.26
S. groenlandica	-0.069	0.42	-0.286	0.34	-0.200	0.44	-0.414	0.10

Analyses include data from summer and winter of 2004 and 2005. Phosphate and silicate are not included due to high autocorrelation (p ≤ 0.01) with nitrate. Significant values at p < 0.05 are shown in bold.

and Harris 2002). Based on the lack of a consistent relationship between *L. vincta* and phlorotannin content in the four kelp species in both field and laboratory observations, it is plausible that this grazer is not significantly deterred by just the presence of phlorotannins in kelps. Phlorotannins can have concentration-dependent effects on gastropods (Johnson and Mann 1986, Pavia and Toth 2000*a*), and mean phenolic levels of 2% DM have previously been suggested as the lower concentration limit that deters grazing (Hay et al. 1994). When offered phenolic-rich and -poor algal species, the gastropod *Tegula funebralis* preferentially grazed on individuals with phlorotannin content less than 1.65% DM (Steinberg 1985). *L. vincta* avoided *Saccharina longicruris* meristematic blade tissue (approximately 5.5% DM phlorotannins) but grazed on all other tissue types with phlorotannin levels less than 1% DM (Johnson and Mann 1986). Most kelp species tested in the present study had blade phlorotannin concentrations of 1-3% DM, which may have been too low to elicit a feeding deterrent effect. Unfortunately, experiments using various concentrations of isolated phlorotannins could not be performed in the present study because *L. vincta* did not respond to artificial food pellets. Therefore, a causal relationship between phlorotannins as quantitative chemical deterrents and *L. vincta* grazing could not be established.

Some macroalgal species are able to induce chemical defense levels such as phlorotannins in the presence of grazers, particularly small mesograzers (Amsler 2001, Toth and Pavia 2007). Hence, alternative to the explanation that *L. vincta* densities were not strongly driven by the presence and concentration of phlorotannins in the studied kelps, it also is possible that kelps did not respond appreciably to the presence of *L. vincta* by increasing their blade phlorotannin content. This explanation could indicate that the studied kelp species may not have the ability to induce phlorotannin production in response to grazers, or just not in blade tissue (Taylor et al. 2002). *L. vincta* also could not be an appropriate grazer to trigger induction in these kelp species as induction can be grazer-specific (Amsler 2001). Inducible phlorotannin production has been identified for some brown algae (Van Alstyne 1988, Toth et al. 2005, Toth 2007, see Toth and Pavia 2007 for overview), including several kelp species (Hammerstrom et al. 1998, Molis et al. 2006, 2008), but not in other kelp species (Steinberg 1994, Toth and Pavia 2002, Macaya and Thiel 2008). Varying macroalgal responses of phlorotannin induction may be due to differences in the mechanism of wounding by particular grazers (reviewed by Amsler 2001). Alternatively, variation in

ambient environmental variables such as irradiance and nitrogen levels may be important in triggering phlorotannin production (Pavia and Toth 2000*b*, 2008, Cronin 2001, Jormalainen et al. 2003).

The analysis of environmental factors demonstrated that *N. luetkeana* phlorotannins were significantly negatively correlated with nitrate and positively with light attenuation. In contrast, none of the phlorotannin concentrations in the perennial understory species were significantly related to any of the irradiance or nutrient measurements. Hence, if environmental variables affected phlorotannin production in our study species, then this was not based on general but on species-specific patterns. As an annual species, *N. luetkeana* has limited time to attain its mature thallus length and complete its life cycle, and the necessary fast growth rates are strongly dependent on the availability of sufficient light and nutrients. Possibly, overall low phlorotannin content in this species is due to an r-selected life history where energy is mostly allocated towards growth and reproduction instead of defenses. However, excess photosynthesis (high light conditions) but slowed growth (nitrogen limitation) in the summer may result in the production of carbon-based compounds such as phlorotannins in an annual kelp such as *N. luetkeana*. This is in agreement with some plant resource allocation theories, such as the carbon-nutrient balance hypothesis, which explains phenotypic expression of defenses being driven by resource availability (Bryant et al. 1983, Tuomi et al. 1991, Stamp 2003). However, the predictive and explanatory values of this particular hypothesis are debated (e.g., Hamilton et al. 2001, Koricheva 2002), and given the low overall phlorotannin concentrations in *N. luetkeana* and the obvious lack of grazer deterrence, we conclude that phlorotannin patterns in this species are not likely to be driven by evolutionary mechanisms of defense allocation as predicted by the carbon-nutrient balance hypothesis. Likewise, the ecological meaning of seasonal variation of the overall low phlorotannin content (<1% DM) as grazer defense is probably low, although we cannot discount that phlorotannins play other roles in assisting with *N. luetkeana* fitness.

Conversely, none of the seasonal phlorotannin patterns in the three perennial species were related to environmental variables, and overall phlorotannin concentrations were higher than in the annual *N. luetkeana*. These perennial species may exhibit more of a K-selected life strategy, where competitive and other biological pressures can drive the production of mostly constitutive (consistently expressed) defenses, especially in the pe-

rennial thallus parts (Herms and Mattson 1992, Åström and Lundberg 1994, Pavia et al. 2002, Toth et al. 2005); such constitutive defenses are less variable in response to grazer abundance and environmental conditions than induced defenses. In support of this, the low feeding rates of *L. vincta* on the understory species seems to indicate that phlorotannins in these species indeed may have some grazer deterrent function.

The results from the current study do not indicate a strong correlation between kelp phlorotannin content and *L. vincta* density and grazing on the four studied kelp species within Kachemak Bay. Likewise, seasonal environmental variables seem to play a minor, if any, role in kelp phlorotannin concentrations. Since the perennial understory species had much higher concentrations and in some cases inverse seasonal pattern of phlorotannin content as compared to the annual *N. luetkeana*, it seems that life history strategy may be a stronger determinant of phlorotannin concentrations in these North Pacific kelps than grazers or environmental conditions.

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