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Phlorotannins versus other factors affecting epiphyte abundance on the kelp *Ecklonia radiata*

Abstract We examined factors affecting the abundance and distribution of epiphytes (fouling) on the sublittoral kelp *Ecklonia radiata*. We first assessed the importance of phlorotannins as chemical defences against epiphytes by (a) correlating epiphyte loads on different parts of the thallus with the phlorotannin content of those tissues, and (b) experimentally testing the effects of variation in phlorotannin concentration against the settlement and growth of gametes of *Ulva lactuca*, a common epiphyte in the system. Tissue phlorotannin content was, at best, only weakly related to epiphyte loads, with r^2 values typically <0.10. Inhibition of *Ulva* gametes only occurred at concentrations >10 mg l^{-1}, which is 5 orders of magnitude greater than levels of phlorotannins in the water column around beds of *E. radiata*, and 1–3 orders of magnitude greater than estimated levels in the boundary layer at the surface of the plant. We concluded that phlorotannins have a negligible impact on patterns of epiphytism on *E. radiata*, and proceeded to investigate other factors influencing the distribution and abundance of epiphytes. In our samples the relative age of different parts of the thallus was strongly correlated with epiphyte abundance, with epiphyte densities greatest on the oldest tissue and least on the youngest. Distal parts of the thalli also had greater epiphyte loads than basal parts. Field experiments in which kelp tissue was suspended at two heights in an *E. radiata* forest for varying lengths of time confirmed the importance of the length of time that the tissue was in the water, and its height in the water column, to the development of an epiphyte community. Comparison of epiphyte loads on tissue from primary (smooth) and secondary (rough) laminae in these experiments indicated that surface rugosity also affected fouling. Macroherbivores were rare on *E. radiata*, and abundances of mesofauna and epiphytes were positively related, suggesting that grazers were not important determinants of patterns of epiphyte abundance. Although phlorotannins have been previously suggested to play an important role as defences against epiphytes, we suggest that water-soluble compounds such as phlorotannins are less likely to be effective defences against epiphytes than non-polar metabolites, which can adhere to the surface of the producing organism.

Key words Fouling · epiphytes · phlorotannins · chemical defences · *Ecklonia radiata*

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Introduction

A wide diversity of secondary metabolites are produced by benthic marine macroalgae (Hay and Fenical 1988). As in terrestrial systems (Rosenthal and Berenbaum 1992), these compounds are thought to function primarily as defences against natural enemies of the algae, including herbivores, fouling organisms (epiphytes), competitors (allelopathy), and pathogens (reviews in Davis et al. 1989; Hay and Steinberg 1992; Paul 1992). However, the majority of ecologically relevant studies on algal chemical defences have focused on their interaction with herbivores (Hay and Fenical 1988; Hay and Steinberg 1992), rather than on their effects against other kinds of natural enemies. This bias is due in part to differences in the methodological ease of different studies; it is generally much easier to measure the concentration of compounds contained within the thallus of an alga, and their effect against herbivores, than it is to measure (for example) the levels of compounds on algal surfaces and their effects against epiphytes.

However, a broader view of the effects of putative algal chemical defences is clearly desirable (Van Alstyne and Paul 1988; Schmitt et al. 1995). Natural enemies of algae other than herbivores can have a significant impact on the plants, and thus mechanisms by which algae deter these other organisms are likely to be ecologically im-

portant. For example, epiphytes (fouling) on marine plants restrict the light available to the host, thereby decreasing photosynthesis (Sand-Jensen 1977), increase the drag on the host and its susceptibility to breakage (Dixon et al. 1981) or being torn off the substrata (D'Antonio 1985; Dayton 1985), and may also decrease the reproductive output of algae (D'Antonio 1985). From an evolutionary perspective, if chemical defences in algae function primarily against one or a few herbivores, then the possibility exists for reciprocal evolutionary responses by the algae and their herbivores (coevolution). However, if the compounds have multiple functions – e.g. against epiphytes and herbivores – then the likelihood of particular compounds co-evolving specifically in response to particular herbivores is reduced (Schmitt et al. 1995). Finally, because of the current interest in the development of commercial antifouling compounds from natural sources such as seaweeds (Clare 1995), an understanding of how these compounds function in nature is also useful in an applied context.

Brown algae (Division Phaeophyta) are the most abundant benthic algae in most temperate algal communities, and the most common secondary metabolites in these plants are phlorotannins, which are polymers of the simple phenolic phloroglucinol (1,3,5-trihydroxybenzene). These compounds can occur in concentrations as high as 20% (dry mass) in some brown algae (Ragan and Glombitza 1986; Steinberg 1989). Although, as for most algal secondary metabolites, the emphasis in phlorotannin research has been on their effects on herbivores, their effects on epiphytes have been studied to some extent. For example, Conover and Sieburth (1966) showed that phlorotannins in tide pools could reach concentrations high enough to kill settling propagules. Langlois (1975) found that exudates containing phlorotannins from both *Ascophylum nodosum* and *Fucus spiralis* decreased the survival of voticellids (ciliates). Fletcher (1975) reported strong allelopathic effects of phlorotannins from the crustose brown alga *Ralfsia spongiocarpa* against potential fouling algae. Sieburth and Conover (1965) showed that polyphenolic extracts from *Sargassum natans* disabled the active tentacles of two hydroid species and also affected most of the other fouling organisms tested, including bacteria, nematodes, and copepods.

There are some difficulties, however, with previous studies of the effects of phlorotannins on epiphytes. First, measurements of phlorotannins based on whole extracts of plant tissue (Sieburth and Conover 1965) are unlikely to accurately estimate the concentration of compounds that epiphytes actually encounter at or near the surface of the thallus. Second, attempts at more realistic measurements of the effective concentrations of these compounds, typically done by measuring exudation rates of phlorotannins (e.g. Sieburth and Conover 1965, Langlois 1975), in the laboratory, may have often significantly overestimated release rates of phlorotannins. Exudation rates increase as a result of harvesting, handling and changing pressure or temperature conditions, all of which are likely to occur in the collection and use of plants for laboratory studies (Moebus and Johnson 1974; Jennings and Steinberg 1994). Finally, studies based on correlations between variation in the concentration of phlorotannins along a thallus (Sieburth and Conover 1965) with variation in fouling have not always taken into account variation in other factors – e.g. the age of different parts of the thallus, position in the water column – which can also affect the settlement, growth and survival of the epibiota (Davis et al. 1989).

In this study we examine factors affecting the distribution and abundance of epiphytes on the sublittoral kelp *Ecklonia radiata*. Because of previous research on the effects of phlorotannins on brown algal epiphytes, we initially ask what role phlorotannins play as epiphyte deterrents. *E. radiata* is a particularly appropriate species with which to address this question because (a) phlorotannin levels are high [up to 15% dry weight in specific tissues (Steinberg 1989)] and (b) we have previously (Jennings and Steinberg 1994) measured *in situ* exudation of phlorotannins from *Ecklonia radiata.* Because of their highly polar nature, phlorotannins released by the alga should quickly diffuse into the water column (Ragan and Glombitza 1986), and thus these exudation rates allow us to make realistic estimates of the concentration of phlorotannins that epiphytes are likely to encounter in the surrounding water column generally or in the boundary layer near the surface of the kelps. Neither correlative nor experimental data indicated an important role for phlorotannins as defences against epiphytes on *E. radiata*, and we proceeded to examine other factors which could affect the distribution and abundance of epiphytes, including; the height of the tissue in the water column, its age, its surface roughness, and the abundance of grazers.

Materials and methods

Study sites

Field sampling of epiphyte loads and phlorotannin levels of *E. radiata* was carried out at two locations near Sydney, New South Wales, Australia (34° 0′S, 151° 11′E) during 1992–1994; Cape Banks, a moderately exposed coastal site; and Nielsen Park, Vaucluse, a more protected site within Sydney Harbour. *E. radiata* forms well developed forests at both sites. Manipulative field experiments were done at Cape Banks.

Measurement of epiphyte loads and phlorotannin content

The relative abundance of different species of epiphytes on *E. radiata* and thallus phlorotannin content were measured at Cape Banks and Nielsen Park for five seasons during 1992–1993. At each location for each season, 20 mature (i.e. with well developed primary and secondary laminae as per Kirkman 1984) *E. radiata* plants were randomly selected, harvested and brought back to the University of New South Wales for analysis. Tissue samples approximately 3 cm^2 in area were cut from the plant using a razor

blade. Samples were taken at five positions along the thallus: stipe, meristem (at the base of the primary laminae), basal secondary laminae, older primary laminae at the top of the plant and distal secondary laminae (Fig. 1). These positions were chosen because they vary in phlorotannin content (Steinberg 1989; Runcie 1991), represent a range of different ages of plant tissue, and because initial visual inspections indicated variation in epiphyte loads among these tissues.

The percent cover and species (or higher taxonomic groupings in some instances) composition of macroepiphytes on each tissue sample (both sides) was measured by comparing the samples to percent coverage diagrams (ranging from 0 to 100%, with intervals of 5%) under a dissecting microscope, and assigning each sample of kelp thallus to an appropriate percent cover category. This "comparative" technique was validated in two ways. First, for one sampling date for $n = 100$ samples we compared this comparative percent cover method with a quantitative measurement (e.g. Dethier et al. 1993) of percent cover using an acetate grid and the point intersect technique. Secondly, using this comparative method we compared estimates of percent cover for $n = 40$ samples between two independent observers.

Once the epiphyte measurements were complete (within two hours) the samples were carefully scraped clean of all epiphytes using a scalpel, divided into two pieces, and both pieces weighed. Care was taken to avoid removing kelp tissue during this procedure. One piece was dried at 50°C for 24 h for eventual dry weight determination, and the duplicate piece was placed in a flask containing 50 ml of methanol (70%). The samples were homogenised and extracted in the dark for 24 h, and the levels of phlorotannins in each sample then measured by the Folin-Denis technique (Steinberg 1985, 1989).

Because phlorotannins in species of *Ecklonia* are concentrated in the surface (meristodermal) cell layers of the plants (Tugwell and Branch 1989; P.D. Steinberg, unpublished work) and because levels of phlorotannins per unit surface area of plant tissue may be a more relevant measure for epiphyte settlement than phlorotannins measured as % dry weight, we also recorded the surface area of the samples in spring 1992 and summer 1993. This allowed us to measure phlorotannins as milligrams per square centimeter.

Sampling design and statistical analysis

Phlorotannin levels or the abundance of epiphytes taken from different positions on the same plant may not be independent of one another. Thus, for each of the 20 plants harvested at each season at each location, only one sample was taken from one position for each plant, e.g. a sample from the stipe only from $n = 4$ plants, or a sample from the meristem only from four plants. These samples were then analysed for variation in the percent cover of epiphytes and phlorotannin levels by three-factor (season × location × position) analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Paired *t*-tests were used to analyse data on assessing the validity of the epiphyte analysis technique. The relationship between phlorotannin levels and epiphyte loads was analysed *via* regression. All percentage data were transformed by arcsin $\sqrt{p}$ prior to analysis. Analyses were done using the statistical packages StatView II and DataDesk for Macintosh computers.

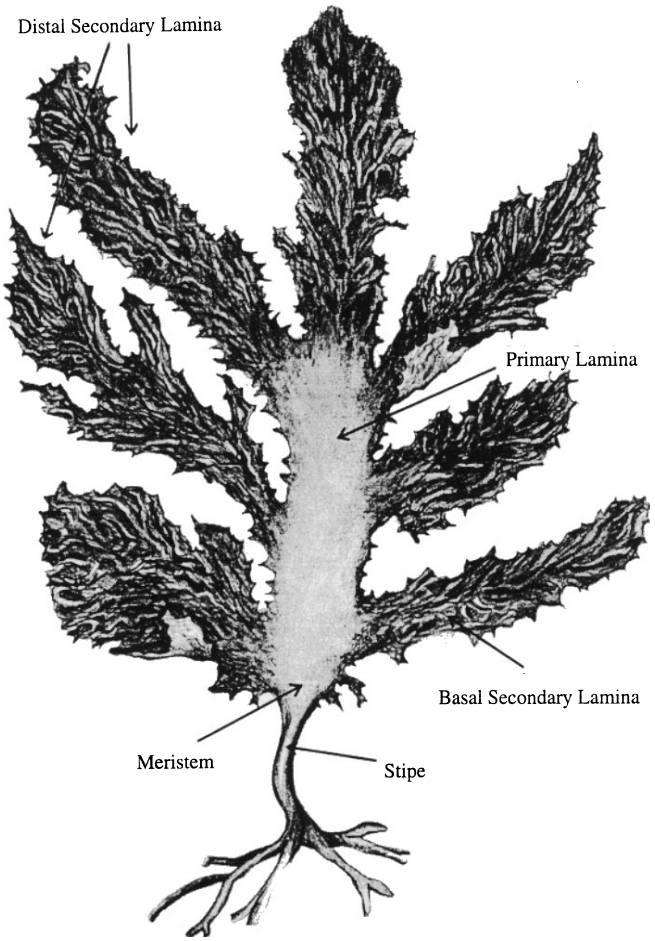

Fig. 1 *Ecklonia radiata*. A mature plant showing the five positions of the thallus sampled during this study

Inhibition of germination and growth of algal gametes by phlorotannins

The effects of phlorotannins on the germination and growth of gametes of the green alga *Ulva lactuca* were tested using a modification of the bioassay in de Nys et al. (1995). *U. lactuca* was chosen as the test organism because it is a common epiphyte in the system, occurring on both *Ecklonia radiata* and other macroalgae (R. de Nys, unpublished work), and because its life history (it is fertile on a fortnightly cycle) and ease of culturing makes it ideal for repeated assays.

Phlorotannin fractions were extracted following Ragan and Glombitza (1986) as modified by Steinberg and Van Altena (1992). These fractions are estimated to be 95–99% pure phlorotannins (based on ^{13}C nuclear magnetic resonance spectroscopy; see Van Altena and Steinberg 1992 for published spectra), but contain a mixture of polymer types and sizes. Phlorotannins extracted from the local fucoid *Sargassum vestitum* were used in addition to those from *E. radiata* in order to compare the effects of phlorotannins from different algae. Phlorotannins from both species were dissolved in ethanol and sterilised nutrient enriched sea water (1 ppm ethanol in seawater) for use in the assays. Dilutions were made up at eight concentrations between 1000 mg l^{-1} and 100 ng l^{-1}. These concentrations span the levels of phlorotannins in the water surrounding *E. radiata* kelp beds at Cape Banks and Nielsen Park (Jennings and Steinberg 1994), estimated levels in the boundary layer around the plants (see Discussion), as well as most previous estimates of exudation in brown algae (Ragan and Glombitza 1986).

To conduct the assay, fertile thalli of *U. lactuca* (identified by discolouration around the tip of the frond) were collected in the field, brought back to the laboratory, washed three times in sterile seawater and left to dry for 2 h. Swarmer release was induced by placing the fronds in beakers of sterilised seawater. Positively phototactic swarmers were concentrated and identified as either zoospores or gametes based on their number of flagella (Fletcher 1989; de Nys et al. 1995). In this assay gametes rather than zoospores were used, as gametophytes were much more common than sporophytes in the field. Gametes readily grow into macroscopic (haploid) thalli in culture (Fletcher 1989). In order to remove

contaminants, gametes were twice "raced" towards a light source across a watchglass containing sterile seawater and then added to 50 ml of sterile seawater prior to use in an assay. In order to obtain a uniform suspension for addition to test wells the solution was stirred mechanically.

An aliquot of the swarmer suspension (500 µl) was added to each well and the test wells placed in the dark to allow even settlement of spores. After 1 h, 1 ml of the test solution (phlorotannin dilution), sterilised seawater + ethanol control solution, or sterilised seawater was added to each well and the plates incubated for five days at 28°C in a 15: 9 hour light-dark cycle. After 5 days germlings were counted. All test solutions and controls were replicated four times (n = 4 wells), and five fields of view (3.5 mm^2) were counted for each well using an inverted binocular microscope. The data were analysed as a three-factor nested ANOVA with factors concentration, phlorotannin type, and wells nested within (concentration × phlorotannin type).

Alternative factors affecting epiphyte distribution and abundance

Age of tissue

To determine whether the age of algal tissue was likely to have a significant effect on epiphyte loads, we compared the epiphyte loads on different positions of the kelp (Fig. 1) to the relative ages of those positions. The main intercalary meristem of *E. radiata* is at the juncture of the stipe and primary lamina (Fig. 1) and thus the youngest parts of the thallus are the meristem and the new primary and secondary laminae. The oldest parts of the plant are the distal primary and secondary laminae and the stipe (especially the basal part). Epiphyte loads were measured at each of five positions known to be of different ages (Fig. 1) using the methods above, and correlated with the age of the tissue. In addition, to assess whether *E. radiata* differs in age between Cape Banks and Nielsen Park, we also measured the height and stipe diameters of kelp at each location.

Hydrodynamic factors

Our initial sampling indicated that epiphytes were more common on distal parts of the thalli than on basal parts, suggesting that height in the water column may be an important factor affecting the distribution of epiphytes. We also hypothesised that longevity of the tissue (= age) in the water, and its texture or rugosity, would significantly influence epiphytes. Two experiments were done to examine these factors:

In the first experiment, pieces of primary laminae (smooth tissue) and secondary laminae (rugose tissue) approximately 10 cm^2 (projected surface area) were suspended in the water column using a rope with a float attached to the top and the bottom attached to the substratum. The tissue was either suspended close to the bottom (at stipe height) or at approximately 1 m from the bottom (average height of the plants at this location). Each piece of tissue was on an individual rope (n = 6 for each tissue type at each height). The samples were collected after 3 weeks, and analysed for epiphyte composition and cover.

To examine the effects of time in the water, a similar experiment was conducted in which pieces of stipe were suspended at two heights in the water (close to the substratum and ~ 1 m above) and samples collected after 2 weeks and after 4 weeks. Stipes (n = 6 at each height for each duration) were used for this experiment because they tended to maintain their integrity better than the lamina tissue. Results from both experiments were analysed via two-factor ANOVA.

The effect of herbivores

Herbivores may also influence the distribution of epiphytes on *E. radiata* if they occur or feed disproportionately on different parts of the kelp. Accordingly, surveys of both macro (>2 cm) and mesoherbivores (<2 cm, >1 mm) on *E. radiata* were done at both Cape Banks and Nielsen Park.

During summer 1994 at each location macroinvertebrate grazers (e.g. sea urchins, gastropods) were surveyed along four 10-m transects. For each plant falling on the transect any macroinvertebrates and their position on the plant was recorded.

Mesoinvertebrates (typically small crustacea and gastropods; Brawley 1992) were sampled as follows (Duffy 1990; Duffy and Hay 1994): Sections of individual *E. radiata* (n = 8) were enclosed in plastic bags and sealed tightly with a cable tie. These sections were cut underwater and were of three types: the stipe; the lower portion of the plant encompassing the newer primary and secondary laminae; and the upper portion of the plant with the older more epiphytised laminae. The water from the collecting bags was poured through a 100-µm sieve and the fauna retained. The kelp in each bag was weighed and soaked in fresh water for 1 h, then agitated for 1 min. The water from the first wash was poured through the sieve and the agitation process repeated. All invertebrates were then preserved in 5% formalin (Duffy and Hay 1994).

Organisms were classified under a dissecting microscope at 12–40 × magnification into the following categories; gammarid amphipods, caprellid amphipods, copepods, ostracods, gastropods, and "other". Abundance of organisms is presented as number/100 g algae. Results were analysed by two-factor ANOVA followed by Tukey's test.

Results

Validation of epiphyte measurement technique

There was no significant difference (paired t-test, df = 99, P = 0.421) between subjective estimates of the percentage cover of epiphytes using percent cover diagrams as a guide and measurements of the same samples using the point intersect method, and we concluded that the subjective technique provided an adequate estimate of epiphyte abundance while also allowing us to process samples much more efficiently than the point intersect method. There was also no significant difference between estimates of percent cover between observers for the same samples (paired t-test, df = 39, P = 0.326).

Distribution and abundance of epiphytes on *E. radiata*

The distribution and abundance of epiphytes on *E. radiata* showed a clear pattern (Fig. 2, Table 1), with position on the plant having the strongest effect on epiphyte loads. The distal secondary laminae consistently had the greatest cover of epiphytes (~35% in some seasons), followed by the older primary laminae. No epiphytes were found on the basal secondary laminae, and meristems were also rarely epiphytised. There was no overall difference in epiphyte loads between Cape Banks and Nielsen Park; however, epiphytes were much more common on stipes at Nielsen Park than at Cape Banks during most seasons (Fig. 2, Table 1; Tukey's tests). Abundance of epiphytes also varied seasonally (Table 1) and the significant three-way interaction indicated that there were a variety of more complex differences in the abundance of epiphytes on the kelp.

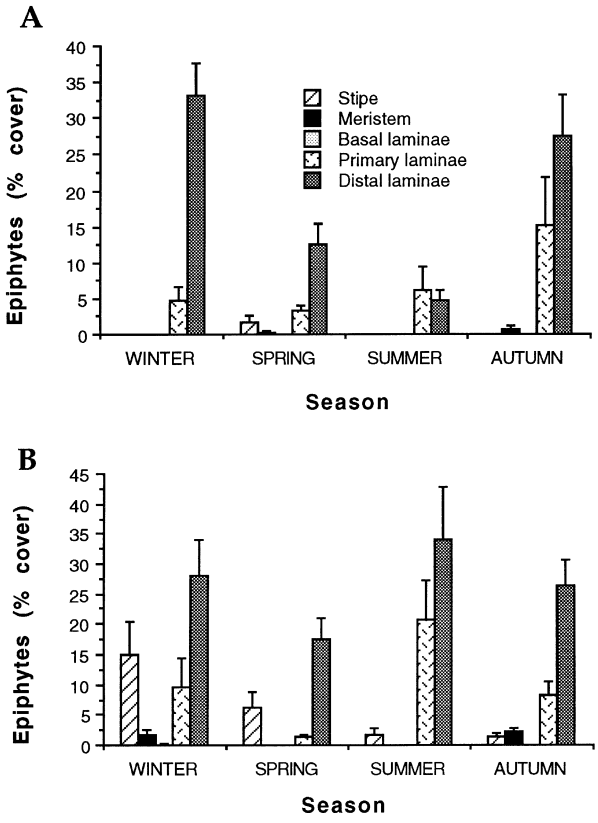

Fig. 2 Total percent cover of epiphytes at five positions on the thallus of *E. radiata* for four seasons during 1992–1993 at **A** Cape Banks and **B** Nielsen park. Data are mean + SE with $n = 4$ at each position for each location

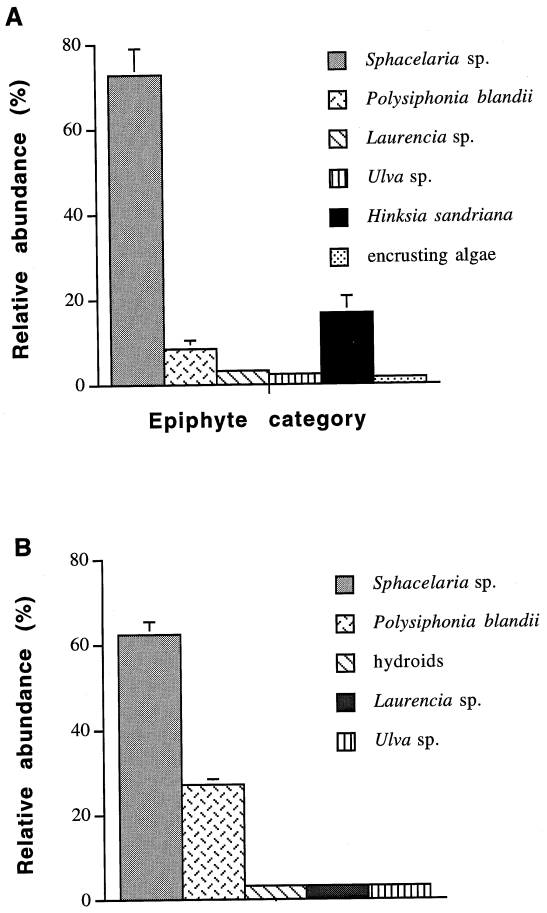

Fig. 3 Relative abundance of major categories of epiphytes on *E. radiata* plants from **A** Cape Banks and **B** Nielsen Park. Data are mean + SE (for clarity of presentation, SE not shown for categories where SE < 0.2%) with $n = 80$ for each category

The focus of this research was on the overall abundance of epiphytes on *E. radiata*, and variation in the species composition of epiphytes on different parts of the plants, or in different seasons, etc. was not analysed in detail. Species composition of epiphyte communities were reasonably similar at Cape Banks and Nielsen Park, with the algae *Sphacelaria* sp. and *Polysiphonia blandii*, together making up >75% of the total cover of epiphytes at both locations. *Hinksia sandriana* was common at Cape Banks but not at Nielsen Park. Other species found included *Ceramium* sp., *Laurencia* sp., *Ulva* sp. Sessile invertebrates were rare, and consisted primarily of hydroids (Fig. 3).

Tissue phlorotannin content (% dry weight)

The position on the thallus that samples were taken from also dominated patterns of variation in the percentage dry weight of phlorotannins in *E. radiata*

Table 1 Three factor ANOVA's for percent cover of epiphytes, phlorotannin content (% dry wt) and phlorotannin content (mg/cm^2) for *Ecklonia radiata* at Cape Banks and Nielsen Park during 1993–1994

Source	Epiphytes			Phlorotannins (% dry wt)			Phlorotannins (mg/cm^2)		
	df	*F*-test	*P* value	*df*	*F*-test	*P* value	*df*	*F*-test	*P* value
Location	1	0.547	0.4608	1	0.286	0.5939	1	31.801	0.0001
Season	3	4.947	0.0028	3	66.698	0.0001	1	24.377	0.0001
Location × Season	3	1.612	0.1903	3	10.445	0.0001	1	29.786	0.0001
Position	4	63.302	0.0001	4	123.308	0.0001	4	21.427	0.0001
Location × Position	4	6.801	0.0001	4	0.477	0.7525	4	5.549	0.0007
Season × Position	12	1.555	0.1142	12	2.151	0.0184	4	15.146	0.0001
Loc × Seas × Pos	12	2.340	0.0099	12	4.852	0.0001	4	4.682	0.0023
Error	119			119			60		

(Fig. 4, Table 1). Levels were consistently highest in the basal secondary laminae (overall mean 5.84 ± SE 0.75), with the next highest levels in the distal secondary laminae (mean 3.46 ± SE 0.39) (Fig. 4, Table 1), although these differences were not always significant for all seasons at both locations (Tukey's test). Phlorotannin levels in meristems, non-meristematic primary laminae, and stipes generally had similar concentrations (means, 1.93 ± 0.44, 1.86 ± 0.32 and 1.4 ± 0.34 respectively) all of which were typically lower than those of the secondary laminae (Fig. 4). Beyond these general patterns, there were a variety of complex, finer scale differences in the % dry weight of phlorotannins in the kelp (Table 1; note three-way interaction).

Tissue phlorotannin content (mg/cm^2)

All main factors and all interactions were significant when phlorotannins were analysed as mg phlorotannins/cm^2 (Table 1), indicating a complex pattern of variation for this parameter. However, qualitative patterns in phlorotannin content per unit area were quite different than those for percentage dry weight, with stipes and primary laminae generally having the highest concentration of phlorotannins/cm^2 (0.075 ± 0.032 and 0.073 ± 0.015 respectively) (Fig. 5). The basal secondary laminae typically had the lowest concentrations (0.026 ± 0.003) (Fig. 5, Tukey's test) although there was considerable overlap in levels among different parts of the thallus.

Relationship between epiphyte loads and tissue phlorotannin content

There was no significant relationship between the percentage dry weight phlorotannin content and percentage cover of epiphytes when all data (n = 160) from all seasons and both locations were analysed (Fig. 6A). A second analysis regressing phlorotannins as mg/cm^2 against percentage cover of epiphytes using all data (n = 80) from both locations was significant (Fig. 6B; P = 0.018). However, the regression explained less than 2% of the variance in epiphyte abundance (r^2 = 0.014).

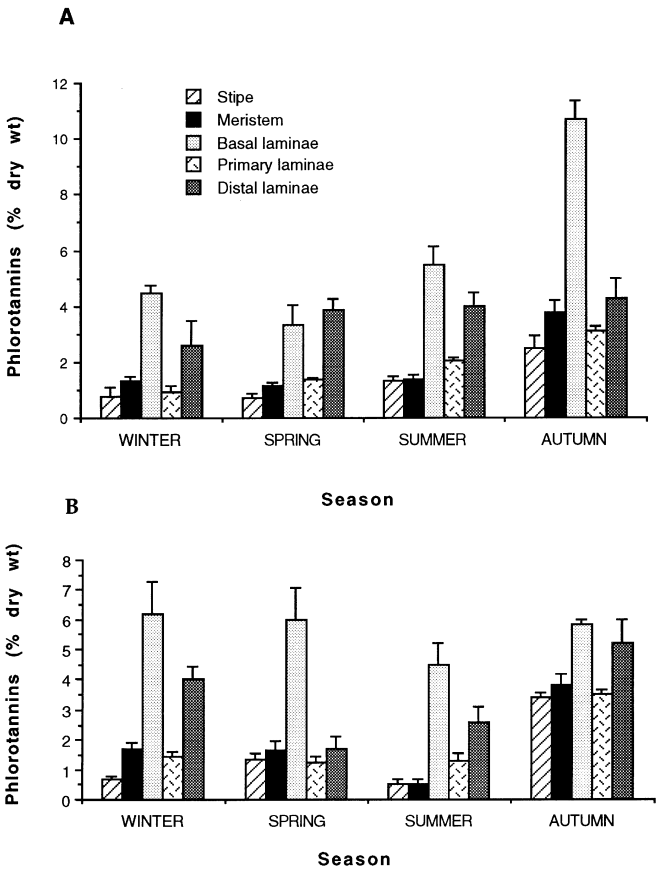

Fig. 4 Phlorotannin content of *E. radiata* tissue (as a percentage of dry tissue weight) at five positions for four seasons during 1992–1993 at **A** Cape Banks and **B** Nielsen Park. Data are means + SE with n = 4 at each position for each season at each location

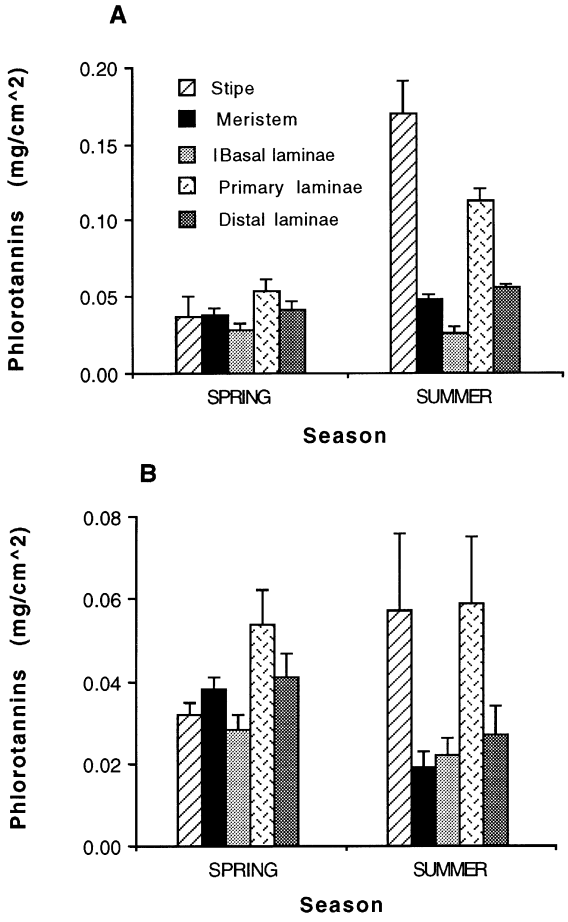

Fig. 5 Phlorotannin content of *E. radiata* tissue (expressed as mg of phlorotannins /cm^2 of plant tissue) at five positions for two seasons during 1992–1993 for plants from **A** Cape Banks and **B** Nielsen Park. Data are mean + SE with n = 4 at each position for each season for each location

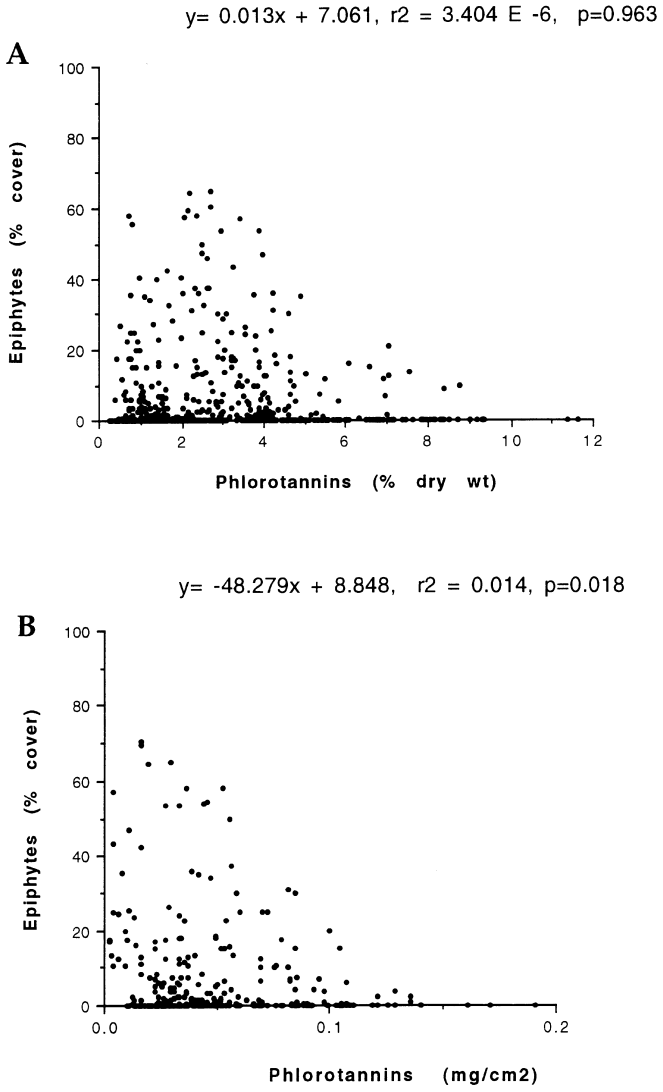

Fig. 6 Regression analyses of epiphyte loads as a function of *E. radiata* tissue phlorotannin content. **A** All data for % dry wt phlorotannins ($n = 160$). **B** All data for mg of phlorotannins/cm^2 ($n = 80$)

Finer scale regression analyses of epiphyte abundance against percentage dry wt and mg/cm^2 of phlorotannins for each location for each season were also done (Table 2). Only 2 of the 12 analyses were significant ($P < 0.05$), and one of these (Nielsen Park in autumn, Table 2A) showed a positive relationship between phlorotannin levels and epiphytes. Slopes of the regressions were just as likely to be positive as negative, and the amount of variance in epiphyte abundance explained by variation in phlorotannin levels was typically very low (r^2 between 0.002 and 0.118).

Ulva growth and germination assay

Phlorotannins from both *E. radiata* and *Sargassum vestitum* only inhibited the germination and growth of gametes of *Ulva lactuca* at concentrations above 10 mg l^{-1} (Fig. 7). Initially the data were analysed as a three-factor ANOVA, with phlorotannin type, concentration and wells nested within (phlorotannin type × concentration). However, the factor wells was not significant ($P = 0.99$) and was pooled, resulting in a two-way ANOVA. The only significant effect was Concentration ($F_{(9, 380)} = 113.2$, $P < 0.0001$ for the factor), indicating that the gametes responded similarly to phlorotannins from the two species of brown algae. Tukey's test indicated that all concentrations ≤ 10 mg l^{-1} had significantly greater germination than 100 and 1000 mg l^{-1}.

Alternative factors affecting epiphyte distribution and abundance

Age of tissue

The primary meristematic region of *E. radiata* is intercalary, so that new tissue forms in the middle region of the plant and moves distally. Thus the oldest portions of the plant are the distal parts of the primary or secondary laminae, and the stipe (particularly near the holdfast).

Table 2 **A** Regression analysis for epiphyte loads and phlorotannin content (% dry wt) of *Ecklonia radiata* tissue at Cape Banks and Nielsen Park for four seasons during 1993–1994; $n = 20$ for all analyses. **B** Regression analysis for epiphyte loads and phlorotannin content (mg/cm^2) of *Ecklonia radiata* tissue at Cape Banks and Nielsen Park for two seasons during 1993–1994; $n = 20$ for all analyses

Site	Season	Regression	P	r^2
A				
Cape Banks	Winter	$y = -0.293x + 7.835$	0.7140	0.002
Cape Banks	Spring	$y = 0.329x + 2.775$	0.4498	0.007
Cape Banks	Summer	$y = 0.551x + 0.897$	0.1749	0.023
Cape Banks	Autumn	$y = -0.943x + 12.481$	0.3103	0.013
Nielsen Park	Winter	$y = -1.150x + 15.63$	0.3113	0.017
Nielsen Park	Spring	$y = -0.499x + 5.176$	0.3864	0.011
Nielsen Park	Summer	$y = 3.842x + 16.181$	0.0624	0.063
Nielsen Park	Autumn	$y = 2.799x - 4.643$	0.0018	0.118
B				
Cape Banks	Spring	$y = -59.016x + 5.831$	0.1361	0.028
Cape Banks	Summer	$y = 20.11x + 0.926$	0.3202	0.013
Nielsen Park	Spring	$y = 15.21x + 0.899$	0.1760	0.012
Nielsen Park	Summer	$y = -144.81x + 13.98$	0.0493	0.049

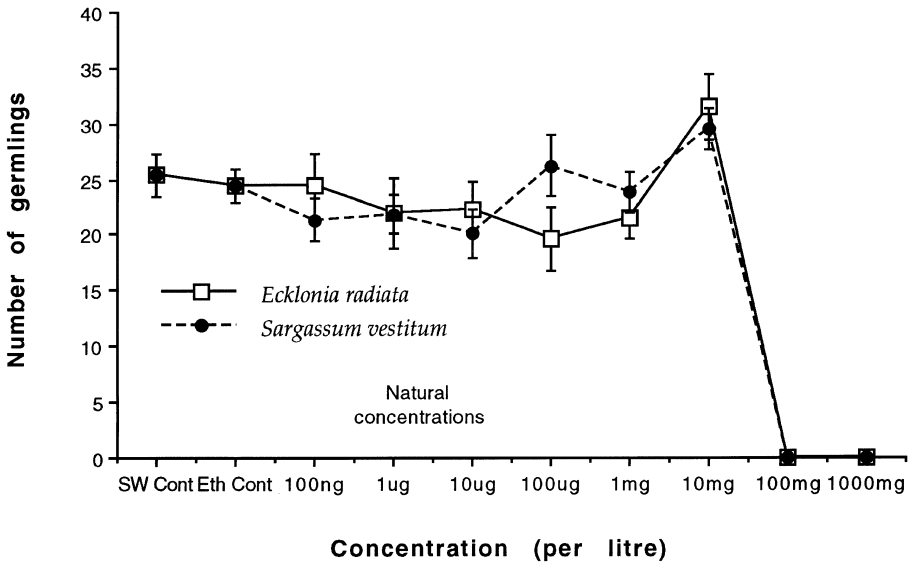

Fig. 7 Growth and germination of *Ulva lactuca* spores exposed to eight concentrations of phlorotannins from *E. radiata* and *Sargassum vestitum*, sea water, or sea water plus ethanol (controls). Data are mean ± SE with $n = 4$ wells × $n = 5$ fields of view for each concentration for each phlorotannin species. Natural concentrations of phlorotannins are estimated to be hundreds of nanograms to a few micrograms in the water column in general (Jennings and Steinberg 1994) and tens (perhaps hundreds) of micrograms in the boundary layer

These three parts of the kelp consistently had the highest abundances of epiphytes (Fig. 2), indicating a positive correlation between the age of the tissue – e.g. its length of time in the water – and epiphyte abundance.

The difference between epiphyte abundances on stipes at Cape Banks and Nielsen Park may also have been due to differences in the age of the plants. *E. radiata* at Nielsen Park were significantly larger (mean height 141.3 cm, SE = 6.3 cm) than those at Cape Banks (mean height 95.8 cm, SE = 4.7 cm; unpaired *t*-test, $t = 2.91$, $df = 99$, $P < 0.01$) and also had greater stipe diameters (Nielsen Park mean 15.5 mm, SE = 1.2 mm; Cape Banks mean 8.2 mm, SE = 0.5; unpaired *t*-test, $df = 99$, $t = 2.87$, $P < 0.01$). This is consistent with a difference in the age of plants from the two locations, although these differences may also have been due to a difference in growth rate between the two locations.

Hydrodynamic factors

Pieces of *E. radiata* tissue suspended higher in the water column consistently had much higher epiphyte abundances than pieces lower down (Fig. 8A,B; Tables 3 and 4). Tissue that was in the water for four vs. two weeks also had substantially higher epiphyte abundances (Fig 8A, Table 3). The type of tissue suspended also had an effect on epiphyte loads (Fig. 8B, Table 4), with the more rugose secondary laminae more highly epiphytized than the smoother primary laminae.

Abundance of herbivores

Two (individuals) macrograzers (1 *Turbo torquata*, 1 *Tripneustes gratilla*) were found on *Ecklonia radiata* at Cape Banks, and three at Nielsen Park (1 *T. torquata* , 2 *T. gratilla*).

The numerically dominant mesofauna on *E. radiata* at both locations were gammarid and caprellid amphi-

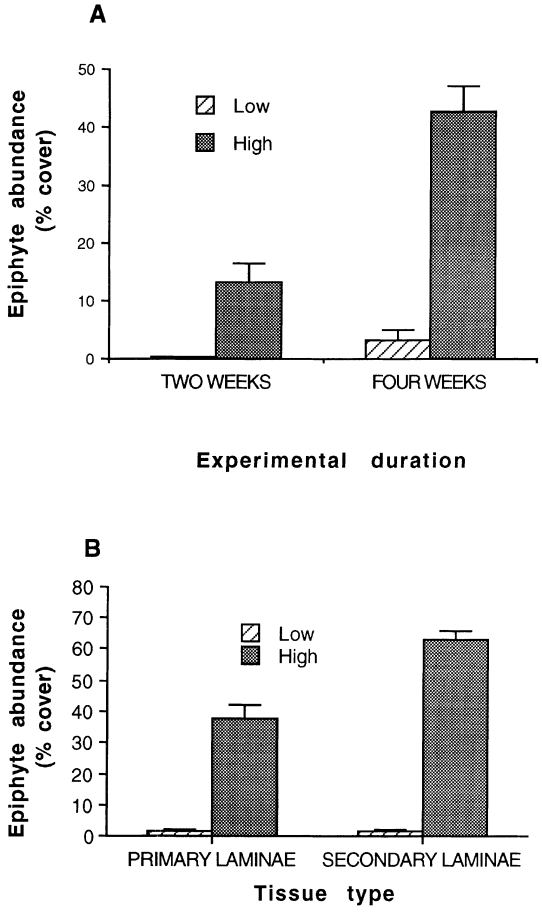

Fig. 8 **A** Percent cover of epiphytes on pieces of *E. radiata* stipe suspended at two heights in the water column for periods of 2 and 4 weeks. **B** Percent cover of epiphytes on primary and secondary lamina tissue suspended at two heights in the water column for 3 weeks. Experiments carried out at Cape Banks during spring 1993. Data are mean + SE with $n = 6$

Table 3 Two-factor ANOVA for percent cover of epiphytes on *E. radiata* at Cape Banks suspended at two heights in the water column for periods 2 and 4 weeks

Source	*df*	Mean Square	*F*-test	*P*
Time	1	0.172	23.925	0.0004
Height	1	0.654	91.139	0.0001
Time × Height	1	0.027	3.765	0.0762
Error	20	0.086		

Table 4 Two-factor ANOVA for percent cover of epiphytes on primary and secondary lamina tissue of *E. radiata* suspended at two heights in the water column at Cape Banks for 3 weeks

Source	*df*	Mean Square	*F*-test	*P*
Tissue type	1	0.052	6.900	0.0162
Height	1	2.519	332.483	0.0001
Tissue × Height	1	0.056	7.436	0.0130
Error	20	0.008		

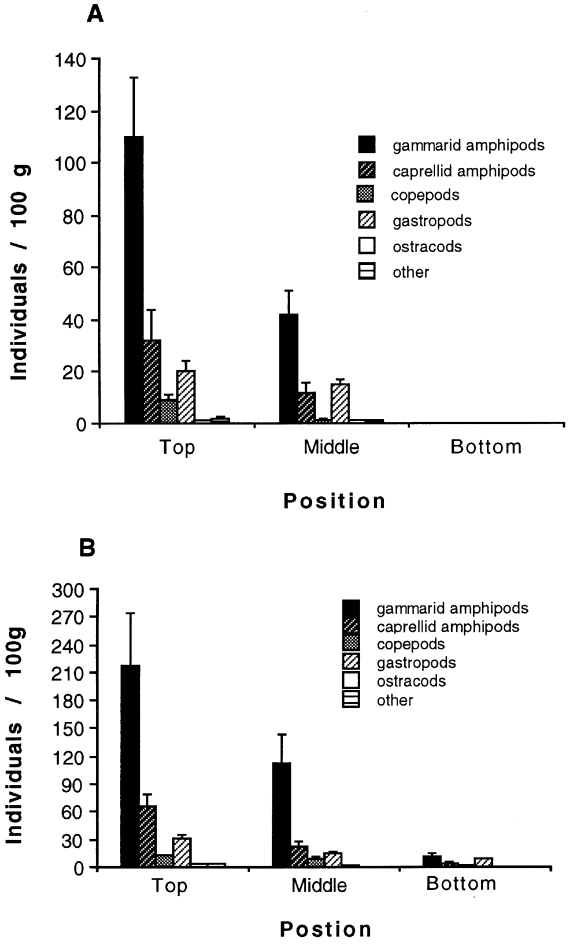

Fig. 9 Mesofauna (number of individuals/100 g wet wt plant tissue) collected from three sections of *E. radiata* plants at **A** Cape Banks and **B** Nielsen Park during summer 1994. Data are mean + SE with $n = 7$ for each plant section at each location

Table 5 Two-factor ANOVA for total numbers of mesofauna on three sections (*positions*) of *E. radiata* at two locations, Cape Banks and Nielsen Park

Source	*df*	Mean Square	*F*-test	*P*
Location	1	166194.38	11.709	0.0016
Position	2	125856.93	8.867	0.0007
Loc × Pos	2	39534.45	2.785	0.0750
Error	36	14195.36		

pods, and gastropods (Fig. 9). Harpacticoid copepods, ostracods and polychaetes also occurred in reasonable abundance. The abundance of mesofauna measured as total organisms per 100 g of algae was significantly higher on the top sections of the plants than on the middle or bottom portions, and was also higher at Nielsen Park than at Cape Banks (Table 5, two-factor ANOVA, followed by Tukey's test where appropriate). Abundance of gammarid amphipods and the gastropods, the two groups of mesofauna that were most likely to feed on the epiphytes (Brawley 1992), followed the same overall pattern as that for total mesofauna.

Discussion

Factors affecting distribution and abundance of epiphytes on *Ecklonia radiata*

There was a substantial community of epiphytes on the thalli of *E. radiata* at both Cape Banks and Nielsen Park, with the mean percent cover of epiphytes at times exceeding 30% on some tissues. Epiphytes were consistently most abundant on the older and more distal primary and secondary laminae, and absent or rare on young tissue such as the meristem or basal secondary laminae. Stipes of plants at Nielsen Park, but not Cape Banks, were moderately epiphytized. Species composition of the epiphyte community was similar between the two sites, with *Sphacelaria* sp. and *Polysiphonia blandii* making up over three-quarters of the total cover of epiphytes (across all seasons) at each site.

Phlorotannin levels in *E. radiata* also varied significantly among different tissues, locations, or sites. However, in contrast to previous studies (Sieburth and Conover 1965; Conover and Sieburth 1966; Langlois 1975) or suggestions (Foster 1992), we found little evidence that phlorotannins were affecting the distribution or abundance of epiphytes on these kelp. There were no, or only very weak, correlations between phlorotannin content (measured as percent dry weight or as mg/cm^2) of the plants and the abundance of epiphytes, and in experimental tests, extracted phlorotannins from *E. radiata* and *Sargassum vestitum* only inhibited the growth and germination of gametes of *Ulva lactuca* at concentrations >10 mg l^{-1}.

Such concentrations are unrealistically high relative to the concentrations of phlorotannins that settling

propagules will encounter. As propagules swim or settle through the water column, they will first encounter phlorotannins exuded into, and diluted by, the surrounding water column. For phlorotannins from *E. radiata*, we estimated that such levels are $<1\ \mu g l^{-1}$ (Jennings and Steinberg 1994), 5 orders of magnitude below effective concentrations of phlorotannins against *U. lactuca* in our assays.

As the propagules approach the plant, they will pass through a narrow boundary layer where water flow is reduced, and then contact the actual surface of the plant. Because phlorotannins are polar metabolites which are highly soluble in water, they are unlikely to adhere to the surface of a submerged plant, and measuring any phlorotannins that do adhere to a surface submerged in seawater is a difficult problem [using filter paper or cotton swabs (Schmitt et al. 1995) we have been unsuccessful in measuring any detectable phlorotannins on the surface of submerged, or briefly emersed, *E. radiata*]. The reapplication of phlorotannins to an experimental test surface which is then submerged is also problematic. This contrasts with non-polar algal metabolites, which adhere to surfaces and can be both measured and tested underwater (de Nys et al. 1995; Schmitt et al. 1995).

In the boundary layer concentrations of phlorotannins should be higher than in the water column in general. Although boundary layer phenomena are an active area of research in marine ecology, the focus in this regard for macroalgae has been on the effects of boundary layers in limiting nutrients or carbon coming into the plant (Wheeler 1980, Koch 1993, Gonen et al. 1995), rather than on its effects on exuded secondary metabolites going out. Crucial parameters such as diffusion coefficients are generally not known for secondary metabolites such as phlorotannins. However, if we assume (a) a boundary layer of 1 mm on a lamina of *E. radiata* (e.g. Wheeler 1980; the thickness of this layer, and the relative proportion of the laminar and turbulent components, will vary with current speed, turbulence, and laminal rugosity); (b) average exudation of phlorotannins of 5.5 $\mu g\ g^{-1}$ dry wt h^{-1} (Jennings and Steinberg 1994); and (c) a surface area of 65 $cm^2\ g^{-1}$ dry wt *E. radiata* (D. Carson, unpublished work), then by making assumptions about the rate of diffusion of phlorotannins out of the boundary layer, we can estimate boundary layer concentrations. If all the phlorotannins exuded from a kelp during one hour remain in the boundary layer for that hour, then the concentration of phlorotannnins in the boundary layer would be on the order of 1 mg l^{-1}. This residence time for phlorotannins is unlikely, given that realistic changes in current speeds – and consequent changes in carbonate fluxes in the boundary layer – can cause changes in the degree of saturation of photosynthetic rates in seconds to minutes [Koch (1993); Gerard (1987) has in fact argued that the typical flow regimes experienced by many algae would preclude the establishment of boundary layer conditions which limit diffusion enough to limit photosynthesis]. More realistic assumptions, in which phlorotannins persist in the boundary layer for periods of minutes or less, would result in boundary layer concentrations of tens of micrograms per liter or lower. Such concentrations are 3–4 orders of magnitude lower than those which were effective against *U. lactuca*.

We have studied only one species of (a dominant, phenolic-rich) kelp, and only experimentally tested phlorotannins against one (cosmopolitan) epiphyte species. However, we believe that our results are broadly relevant to previous studies of the antifouling effects of phlorotannins. In particular, our results suggest that previous tests of the effects of phlorotannins on epiphytes have often used much higher test concentrations than propagules are likely to encounter in the field. For example, Sieburth and Conover (1965) showed that polyphenolic extracts at 250 mg l^{-1} from *Sargassum natans* 'froze' the active tentacles of two hydroid species, and concentrations of 8 g l^{-1} negatively affected most other fouling organisms tested. McLachlan and Craigie (1964) found that *Fucus vesiculosus* exudates at concentrations of 150 mg l^{-1} inhibited growth of *Porphyridium* sp. and concentrations of 25 mg l^{-1} inhibited growth of *Monochrysis lutheri*. These concentrations are comparable to those which inhibited gametes of *U. lactuca* in this paper, but are orders of magnitude higher than concentrations which are likely to occur in sublittoral algal beds (Jennings and Steinberg 1994; this study). The only study of which we are aware suggesting that phlorotannins have deleterious effects against epiphytes at realistic concentrations is that of Langlois (1975), who found that tens to hundreds of micrograms per liter of crude exudates from *Ascophylum nodosum* and *Fucus spiralis* inhibited epiphytic vorticellids (ciliates). However, the experiments of Langlois (1975) were done in beakers in the laboratory, conditions which may have artificially concentrated phlorotannins in the boundary layer surrounding the algae.

Thus our data, and a consideration of previous studies, suggests that phlorotannins may not be important deterrents of epiphytes in our system or in other sublittoral algal systems. We specify sublittoral algae because the effectiveness of phlorotannins as antifoulants may be greater in intertidal brown algal communities, where (a) phlorotannins can accumulate in high concentrations in tide pools (Conover and Sieburth 1966), (b) algae can exude "spikes" of high levels of phlorotannins as they undergo cycles of immersion and emersion (Carlson and Carlson 1984), and (c) boundary layer effects on algae in non-turbulent tidepools are likely to be much greater than on sublittoral algae exposed to constant flow and turbulence (Koch 1993).

Consistent with a lack of effect of phlorotannins on epiphytes, we found that factors other than chemical deterrence adequately explained variation in the distribution and abundance of epiphytes on *E. radiata*. The pitfalls of failing to consider other factors which affect epiphytes abundance and which may covary with inhibitory chemicals has been commented on by other authors (e.g. Davis et al. 1989; Schmitt et al. 1995). In

our study, most of the variation in epiphytes on *Ecklonia radiata* was adequately explained by an increase in epiphyte loads on older tissue – presumably reflecting simple accumulation and growth of epiphytes over time – or tissue that was higher in the water column. The effect of height in the water column was probably due both to the filtering effect that kelp canopies can have on propagules of marine organisms (Gaines and Roughgarden 1987), and the heavy shading of lower portions of the thallus (the majority of epibiota on *E. radiata* were algae). A final alternative factor examined – herbivory – did not appear to have important effects on epiphytes in our system, although herbivores can influence epiphyte densities on other algae (D'Antonio 1985; Brawley and Fei 1987; Wahl 1989; Duffy 1990; Wahl and Hay 1995). However, individual species or taxa of herbivores may be influencing the abundance of epiphytes on *E. radiata* in ways that were not revealed by the taxonomic level or temporal pattern at which we sampled.

Chemical defences of algae against epiphytes

The majority of research on the ecology of secondary metabolites in macroalgae has focused on their role as defences against herbivores (reviews by Hay and Fenical 1988; Hay and Steinberg 1992; Paul 1992). As indicated in the Introduction, a broader view of the effects of these metabolites against other natural enemies of the plants – epiphytes, pathogens, competitors – is clearly desirable. However, our understanding of the overall importance of algal secondary metabolites against natural enemies other than herbivores is constrained by a number of factors.

First, comparatively (very) few studies have been done. Second, a multifactor approach has generally not been taken, and thus in some instances it is difficult to distinguish the effects of chemicals on epiphytes from the effects of other factors (Davis et al. 1989). This contrasts to a number of studies of herbivory, in which the interactive effects of secondary chemistry and other algal characteristics (nutritional value, morphology) have been studied (Steinberg 1985; Pennings and Paul 1992; Hay et al. 1994).

Third, there are a number of methodological problems in studies of algal chemical defences against epiphytes, bacteria, or competitors that have not always been addressed (this study; Davis et al. 1989, Schmitt et al. 1995). Foremost among these is that appropriate measurement and manipulation of the active metabolites for studies of epiphytism is more difficult, and requires different techniques, than do studies of chemical defences against herbivores. Herbivores consume algal tissue; thus measurement of the tissue content of secondary metabolites is an appropriate measure for herbivores. Such measurements are probably inappropriate for most studies of the antibacterial, anti-epiphyte, or allelopathic activity of such compounds (we found little or no relationship between tissue content of phlorotannins and epiphyte loads). In these interactions, for compounds to be effective they will most likely have to either be (a) absorbed onto the surface of the plant, or (b) exuded in high enough amounts for an effective concentration to be maintained in the water.

Few studies have properly addressed this issue of presentation of compounds by algae, either by measuring the compounds in the field or actually manipulating them experimentally in a realistic way. Schmitt et al. (1995) showed that when extracts from the surface of *Dictyota menstrualis* containing diterpene alcohols were applied to test surfaces they inhibited settlement of a naturally occurring epiphytic bryozoan. In this and a companion paper (Jennings and Steinberg 1994), we have achieved realistic estimates of phlorotannin levels in the field, and then measured the effects of these concentrations on an ecologically relevant epiphyte of the kelps. We (de Nys et al. 1995; Dworjanyn, S., de Nys, R., Steinberg, P. unpublished work) have also measured concentrations of individual furanones on the surface of the red alga *Delisea pulchra*, and then tested these concentrations against macro- and micro- epiphytes, demonstrating strong inhibitory effects. Several authors have also demonstrated allelopathic (de Nys et al. 1991) or antifouling (Davis 1991; Wahl et al. 1994) effects of non-polar metabolites or extracts against ecologically relevant species, without measuring concentrations of the compounds on the surface of the producing organisms.

These studies on algal antifouling, while few in number, suggest that different classes of metabolites may be differentially effective against epiphytes. Structure/function relationships are one of the most problematic aspects of marine chemical ecology, and minor structural changes to metabolites can drastically alter their effects on herbivores (Hay and Steinberg 1992, p. 382) or epiphytes (de Nys et al. 1995). However, the way in which compounds must be presented by an alga to be effective against epiphytes may give us some insight into the antifouling activities of at least different broad classes of compounds. Non-polar metabolites (e.g. terpenoids, acetogenins) exuded onto the surface of an alga are likely to persist there for much longer than water soluble metabolites such as phlorotannins, which should rapidly (if not immediately) be dissolved into the water (although the process may be slowed if compounds can be retained in mucous at the surface of the plant). Thus the amounts of water soluble metabolites that would need to be exuded to maintain an effective concentration near the surface of the plant – even given boundary layer effects – are large. This effect would be exaggerated if water soluble molecules such as phlorotannins are only active at higher concentrations against epiphytes than non-polar molecules, as appears to be the case for their effects against herbivores (Hay and Steinberg 1992). In all the studies described above where compounds or extracts were in fact effective against fouling organisms at realistic concentrations, the active compounds were non-polar metabolites.

A final difference between studies of chemical defences against epiphytes *vs.* against herbivores is that there are few studies on the impact of epiphytes on algae. The often severe consequences to algae of attack by herbivores are well documented (Lubchenco and Gaines 1981; John et al. 1992). The effects of fouling on the fitness of seaweeds, while demonstrated in a few instances (e.g. Sand-Jensen 1977; Dixon et al. 1981; D'Antonio 1985), are much less well known. Schmitt et al. (1995) have pointed out that the more diverse the functions for a given secondary metabolite, the less likely there will be coevolution between specific natural enemies (e.g. individual herbivore species) and specific chemical defences. However, we know of no instance where there is a clear link between the production of secondary metabolites and a reduction of epiphyte loads which has a positive consequence to the fitness of an alga. Thus, it is difficult at this stage to evaluate the importance of fouling as a selective agent on production of algal secondary metabolites.

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