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Experimental evidence for the effects of polyphenolic compounds from *Dictyoneurum californicum* Ruprecht (Phaeophyta : Laminariales) on feeding rate and growth in the red abalone *Haliotis rufescens* Swainson

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Abstract: The effects of polyphenolic compounds from brown algae on grazing and growth rate of the California red abalone *Haliotis rufescens* Swainson were examined. Abalone consumed three phenolic-poor algal species, *Laminaria sinclarii* (Harvey) Farlow, *Macrocystis pyrifera* Agardh, and *Nereocystis luetkeana* Postels et Ruprecht (mean phenolic content = 0.52% dry mass), at a greater rate than two phenolic-rich species, *Dictyoneurum californicum* Ruprecht and *Cystoseira osmundacea* Agardh (mean phenolic content = 4.60% dry mass). This inverse relationship between phenolic content and consumption rate also existed after the algae were macerated and the liquid portion of the blended slurry incorporated in agar discs. However, the correlation between grazing rate and phenolic content improved in this latter experiment, thus suggesting that abalone grazing was deterred significantly by the morphology of *L. sinclarii* and, to a lesser extent, of *M. pyrifera*. Polyphenolics extracted from *D. californicum* reduced abalone grazing rates by 90% when incorporated into agar discs at a concentration of 6 mg · ml⁻¹. Although abalone were unable to maintain body mass when fed ad libitum on macerated *M. pyrifera* incorporated into agar discs, polyphenolics from *D. californicum* further inhibited shell growth when added to the discs at 5 mg · ml⁻¹. The abalone ate less of the phenol-containing discs than of the discs lacking phenolics. Our results support findings of several prior studies that polyphenolic compounds from brown algae deter grazing by coastal zone herbivores in the northeast Pacific Ocean.

Key words: Deterrence; *Dictyoneurum californicum*; Herbivore; Kelp; Polyphenol; Red abalone

INTRODUCTION

Plant secondary compounds deter grazing by many terrestrial and marine herbivores (Swain, 1977; Harborne, 1978; Rosenthal & Janzen, 1979; Geiselman & McConnell, 1981; Targett & McConnell, 1982; Faulkner, 1984; Steinberg, 1985, 1988; Targett et al., 1986; Hay et al., 1987a,b; Hay & Fenical, 1988). Most of the identified compounds in marine brown algae (Division Phaeophyta) are terpenoids and polyphenolics. Terpenoids are known to deter feeding in warm temperate/tropical systems (Hay et al., 1987a,b, 1988; Hay & Fenical, 1988) whereas polyphenolics are the best-documented

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defensive compounds in cold-temperate zone/boreal algae (Steinberg, 1985, 1988). Polyphenolics, a class of compounds consisting of phloroglucinol (1,3,5-trihydroxybenzene) and its polymers, have a wide range of molecular weights and structures (Glombitza, 1977; Ragan & Glombitza, 1986). These compounds probably exist in all orders of phaeophytes and occur in concentrations as high as 15% of a plant's dry mass (Glombitza, 1977; Ragan & Craigie, 1978; Steinberg, 1989).

Two emerging patterns from these studies are that: (1) polyphenolics deter many herbivorous marine invertebrates (Geiselman & McConnell, 1981; Steinberg, 1984, 1985, 1988; Johnson & Mann, 1986; Van Alstyne, 1988); and (2) greater concentrations of polyphenolics result in greater deterrence of herbivory (Geiselman & McConnell, 1981; Steinberg, 1988). These findings are consistent with predictions of the plant apparency model (Feeny, 1976; Rhoades & Cates, 1976), i.e., phenolic compounds are broadly acting quantitative (dosage-dependent) defenses that are characteristic of apparent plants. However, the applicability of the plant apparency model has been questioned because other secondary compounds also show dosage-dependent deterrence (Hay & Fenical, 1988), some herbivores are not deterred by phenolics (Zucker, 1983; Bernays, 1981) and their role as digestibility reducers is disputed (Menhansho et al., 1983; Martin & Martin, 1984; Bernays et al., 1989).

The broad objective of this study was to measure deterrence effects of polyphenolics from the kelp *Dictyoneurum californicum* Ruprecht and several other phaeophyte species on feeding and growth of the red abalone *Haliotis rufescens* Swainson. Although brown algal polyphenolics have been shown to deter grazing in several prior studies of other herbivores and plants, we judged these particular species interesting for several reasons. We selected a kelp species because: (1) no study of deterrence by phenolics has been done using kelps and algal models incorporating phenolics extracted from kelps (such studies have been done with fucoids [Steinberg, 1985, 1988]); and (2) although kelps comprise a major component of the macro-algal biomass in temperate zone marine systems, most species are phenolic-poor and thus apparently have poor defenses against herbivores (Steinberg, 1985, 1989). *D. californicum* was selected because it is the only common phenolic-rich kelp species in central California. Red abalone was chosen because: (1) it is an abundant and economically important species with well documented feeding preferences (Leighton, 1968); (2) the effects of plant secondary metabolites on haliotids are largely unstudied (Shepard & Steinberg, unpubl. data); and (3) the haliotids are drift feeders and as such they have little or no fitness-reducing effects on their prey, thus excluding them as potential players in the co-evolution of plant defense systems.

MATERIALS AND METHODS

HERBIVORE

The California red abalone, a large herbivorous gastropod, occurs in low littoral and sublittoral habitats from Sunset Bay, Oregon, to Bahia Tortuga, Baja California. It feeds

primarily on drift algae and readily consumes phaeophytes (Leighton, 1966, 1968). The experimental animals were spawned and raised at the California Department of Fish and Game Granite Canyon Marine Laboratory. All abalone were 1–2 yr old and of similar size at the time of our experiments.

ALGAE

We used five species of phaeophytes, *Dictyoneurum californicum* Ruprecht, *Laminaria sinclarii* (Harvey) Farlow, *Macrocystis pyrifera* Agardh, and *Nereocystis luetkeana* Postels et Ruprecht (Order Laminariales), and *Cystoseira osmundacea* Agardh (Order Fucales). *Laminaria dentigera* (Harvey) Farlow was used in place of *L. sinclarii* for experiments conducted during winter when blades of *L. sinclarii* were difficult to obtain. The species' ranges overlap from San Luis Obispo, California, to Seaside, Oregon (Abbott & Hollenberg, 1976). All the algal species occur in rocky subtidal habitats and are commonly encountered by red abalone.

PHENOLIC COMPOUNDS

Brown algal phenolics are polymers of phloroglucinol joined by ether and carbon-carbon bonds (Ragan & Glombitza, 1986). The polyphenolic compounds were extracted from fresh *D. californicum* by the methods of Ragan & Glombitza (1986). We did not use a dialysis membrane to separate compounds of different molecular weights because we wished to evaluate the effects of all the plant's polymers on the herbivore. Studies of kelp and fucoid polyphenolics (Ragan & Craigie, 1976; McInnes et al., 1985, Ragan & Glombitza, 1986) indicate molecular sizes ranging from a single molecule of phloroglucinol to 10^4 phloroglucinol units. The extraction technique and probable structures for some of the compounds are given by Ragan & Craigie (1976), Glombitza et al. (1980), Ragan (1985), and Ragan & Glombitza (1986).

GRAZING EXPERIMENTS

Three grazing experiments, done at Long Marine Laboratory, University of California at Santa Cruz, California, were conducted to determine if polyphenolics from *D. californicum* deterred grazing by red abalone. The experimental units were 22-l aquaria, each of which contained five pre-weighed red abalone of comparable size and age. Two additional control aquaria, containing food (algae or agar discs) but no abalone, were used to determine changes in the food mass for reasons other than abalone grazing. The abalone were starved for 2 days prior to each feeding trial to maximize grazing rate during the experimental period. Treatments were applied at random to experimental units. Red abalone in each experiment were offered one food type in a given feeding trial (*no choice* as opposed to *multi-choice*) because we wished specifically to assess deterrent effects of phenolics rather than red abalones' preference for one food over another. The algae and discs were weighed before and after each feeding trial, corrections were made based on controls, and the mass of consumed food was determined.

Algal grazing experiment

This experiment tested the hypothesis that a negative correlation did not exist between consumption rate and phenolic content. Abalone grazed on blades of each of the five species of algae for 48 h and then we determined the grazing rates. Prior to each feeding trial the blades of a pooled collection of plants were analyzed for total phenolic content (percent dry mass) by the Folin-Denis technique (Swain & Hillis, 1959; Ragan & Jensen, 1977). Absorbances were measured at 725 nm and phloroglucinol was used as a standard.

Algal extract grazing experiment

This experiment partially tested the hypothesis that algal morphological differences did not account for different grazing rates on the algae. Abalone were allowed to graze for 48 h on agar discs that were made from a concentrated seawater extract of each alga, and grazing rates were measured. The extract was prepared by homogenizing an algal blade in filtered seawater, decanting the liquid portion, adding agar to the decanted liquid, and heating this solution to a boil. The amount of agar ranged from 2.6–4.0% w:v as recommended by Steinberg (1988). The solution was cooled to 50 °C, poured into 60 × 15-mm Petri dishes, and allowed to harden.

We determined total phenolic content of the extract by the Folin-Denis technique prior to addition of agar. The phenolic content of agar discs is reported in $\text{mg} \cdot \text{ml}^{-1}$ rather than percent dry mass because the dry mass to wet mass ratio of the agar discs is much lower than that of kelps. However, approximate averages of total phenolic content were 3.5% dry mass for the phenolic-rich and 0.3% dry mass for phenolic-poor species.

Phenolic addition experiment

In this experiment we measured consumption rates on agar discs to which phenolics extracted from *D. californicum* were added. Abalone grazed on agar discs made from *M. pyrifera* and purified phenolics from *D. californicum*. Similar methods have been used in previous studies of the deterrent effects of algal phenolics on grazing (Geiselman & McConnell, 1981; Steinberg, 1988). *M. pyrifera*, a phenolic-poor kelp, is a preferred food of red abalone (Leighton, 1966). Polyphenolics from *D. californicum* were added in concentrations of 0, 2 and 6 $\text{mg} \cdot \text{ml}^{-1}$. Total phenolic contents of the 2 and 6 $\text{mg} \cdot \text{ml}^{-1}$ discs were ≈ 9.1 and 16.7% dry mass, respectively.

GROWTH EXPERIMENT

After determining that phenolics deterred grazing by red abalone, we wished to know if these compounds reduced growth rate. Thus, we tested the hypothesis that *D. californicum* phenolics do not inhibit growth of red abalone. Eight experimental units (22-l aquaria) were used in this experiment. Each contained five tagged abalone whose shell

length and body mass were measured at the onset. The aquaria were kept dark to promote maximum growth (Ebert & Houk, 1984).

The diets used in the growth experiment were agar discs similar to those used in the phenolic addition experiment; however, particles of *M. pyrifera* were added (20% w : v) because we thought agar and a seawater extract of *M. pyrifera* might not be sufficient to sustain the animals. Discs in the growth experiment contained polyphenolics at concentrations of 0 and 5 mg · ml⁻¹ (6.7% dry mass).

Phenolic-poor and -rich treatments were applied at random and in equal numbers to the experimental units. Shell length and body mass of each animal were measured after 88 days, and growth rates derived from these data were compared between the treatments. Each aquarium received new discs every 12–14 days as pilot tests indicated that leaching of phenolics from the discs during this period was negligible. Disc mass was measured at the times of introduction to and removal from the aquaria.

STATISTICAL ANALYSES

Each trial of the algal and algal extract grazing experiments was replicated six times. Each trial of the phenolic addition grazing experiment was replicated four times. Consumption rates were transformed to arcsine square-roots and the treatment effects tested using a single-factor ANOVA. When null hypotheses were rejected, Duncan's multiple range test was used to make specific treatment comparisons (Sokal & Rohlf, 1987). Because our work was completed prior to the publication of Peterson & Renaud (1989), we did not use their recommended approach of incorporating variation among control units in the calculation of experimental error. Although this increases the probability of incorrectly rejecting the null hypotheses, the reported treatment effects in all cases are so highly significant that the revised technique probably would not have altered any of our statistical inferences.

We averaged percent change of shell length and of body mass of the five animals in each experimental unit of the growth experiment, transformed these data to arcsine square-roots, and evaluated with a *t* test the hypothesis that red abalone fed phenolic-poor and phenolic-rich discs grew at similar rates. We also measured mass of food consumed in each aquarium, transformed these data to arcsine square-roots, and compared consumption rates between phenolic-poor and phenolic-rich foods with a *t* test.

RESULTS

PHENOLIC ANALYSES

Of the five algal species used in the algal grazing experiment, *D. californicum* had the highest total phenolic content and *N. luetkeana* the lowest (Table I). Polyphenolic concentrations differed significantly among all species except *M. pyrifera* and *L. dentigera* ($P < 0.05$). However, we classified *N. luetkeana*, *M. pyrifera*, and *L. dentigera* as

phenolic-poor and *D. californicum* and *C. osmundacea* as phenolic-rich species because none of the algal species contained intermediate phenolic levels. Our measurements of phenolic concentration are similar to values reported by Steinberg (1985) for these species.

Agar discs used in the algal extract grazing experiment ranged in phenolic content from $3.42 \text{ mg} \cdot \text{ml}^{-1}$ (*D. californicum*) to $0.08 \text{ mg} \cdot \text{ml}^{-1}$ (*M. pyrifera*). As with the natural algae, variation was low in phenolic content within species and between species within the phenolic-poor and phenolic-rich groups, and high between the phenolic-poor and phenolic-rich groups (Table I). Despite the fact that discs made from different algal species required different amounts of seawater, total phenolics per dry mass of the natural algae and total phenolics per volume of disc correlated highly among species ($r = 0.99$).

TABLE I

Total phenolic content of algae (percent dry mass) and agar discs made from each alga ($\text{mg} \cdot \text{ml}^{-1}$) as determined by Folin-Denis procedure with phloroglucinol standard. Designation of high and low consumption rates are from grazing experiments with natural algae and agar discs containing macerated algae (Fig. 1). *N*, numbers of plants analysed. Data are $\bar{x} \pm 1 \text{ SE}$.

Species	N	Phenolic concentration	
		Natural algae	Agar discs
High consumption rate species			
<i>L. dentigera</i>	3	0.50 ± 0.01	0.11 ± 0.18
<i>M. pyrifera</i>	3	0.81 ± 0.10	0.03 ± 0.01
<i>N. luetkeana</i>	3	0.25 ± 0.01	0.08 ± 0.01
\bar{x}	9	0.52 ± 0.10	0.15 ± 0.03
Low consumption rate species			
<i>D. californicum</i>	3	5.49 ± 0.19	3.42 ± 0.15
<i>C. osmundacea</i>	3	3.17 ± 0.05	2.60 ± 0.07
\bar{x}	6	4.60 ± 0.43	3.01 ± 0.20

ALGAL GRAZING EXPERIMENT

Red abalone grazed phenolic-poor algae at significantly greater rates than phenolic-rich algae (Fig. 1; $F = 88.1$, $P < 0.0001$). The phenolic-poor species were consumed at an average rate of $0.51\% \text{ body mass} \cdot \text{h}^{-1}$ whereas phenolic-rich species were consumed at an average rate of $0.06\% \text{ body mass} \cdot \text{h}^{-1}$. Consumption rates differed among all species except *D. californicum* and *C. osmundacea* (Fig. 1). *N. luetkeana*, the most phenolic-poor species, was consumed at 18 times the rate of *D. californicum*, the most phenolic-rich species. This evidence, although correlative, is consistent with Steinberg's (1985) results for other northeast Pacific phaeophytes and their herbivores as well as his conclusion that algal species consumed at low rates are chemically defended by polyphenolics.

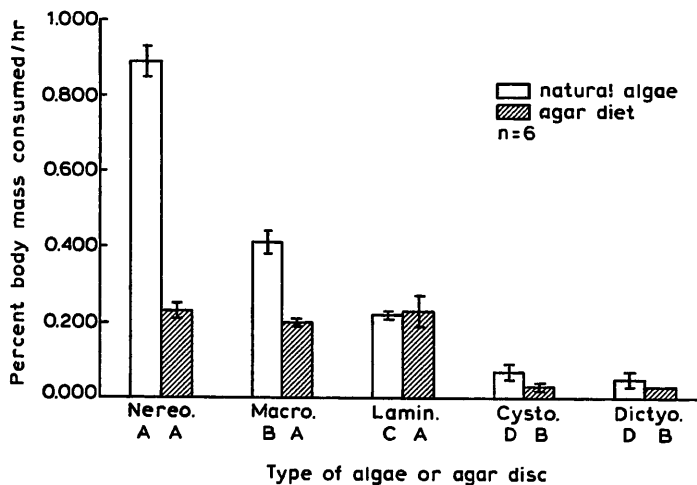


Fig. 1. Consumption rates by red abalone ($\bar{x} + SE$) of natural algae and macerated algae incorporated into agar discs. Differences among species for each treatment group (natural algae and agar extracts) were tested with a single factor ANOVA followed by Duncan's multiple range test. Treatments with same letter could not be shown to differ significantly ($P < 0.0001$).

ALGAL EXTRACT GRAZING EXPERIMENT

Algal morphology has been shown to affect herbivore grazing preferences (Steneck & Watling, 1982; Watson & Norton, 1985), and results of the algal grazing experiment do not exclude the possibility that algal structure, while possibly correlated with total phenolic levels, was the mechanism of deterrence. We addressed this possibility in the algal extract grazing experiment by solidifying the liquid portion of seawater extracts from each algal species into agar discs of similar size, toughness and shape. Thus, the discs retained water-soluble components of each species while eliminating their gross morphological differences. Red abalone grazed discs made from phenolic-poor species at greater rates than discs made from phenolic-rich species (Fig. 1: $F = 41.21$, $P < 0.0001$). The discs made from phenolic-poor species had an overall phenolic content of $0.15 \text{ mg} \cdot \text{ml}^{-1}$ and were grazed on average at a rate of 0.22% body mass $\cdot \text{h}^{-1}$; those made from the phenolic-rich species had an overall phenolic content of $3.01 \text{ mg} \cdot \text{ml}^{-1}$ and were grazed on average at a rate of 0.03% body mass $\cdot \text{h}^{-1}$.

Taken together, results from the algal grazing and the algal extract grazing experiments suggest that abalone consumption rates were influenced by differences other than phenolic content among the phenolic-poor species. This is because grazing rates on phenolic-poor species did not co-vary with phenolic content similarly between these experiments (Fig. 1). Red abalone grazed phenolic-poor algae at different rates (maximum 75% > minimum) but when these species were made morphologically similar, they were consumed at more similar rates. Alternative explanations are that other secondary compounds, or different nutritional values of the phenolic-poor algae were responsible for differential consumption rates in the algal grazing experiment.

PHENOLIC ADDITION GRAZING EXPERIMENT

Polyphenolics from *D. californicum*, when added to agar discs made from the preferred kelp *M. pyrifera*, significantly deterred red abalone grazing. Discs containing polyphenolics at 2 and 6 mg · ml⁻¹ reduced grazing by 52.7 and 90%, respectively, below grazing rates on discs without phenolics (Fig. 2: $F = 42.4$, $P < 0.0001$). These results indicate that deterrence of abalone feeding by polyphenolics is dosage-dependent, as reported by Steinberg (1988) who worked with polyphenolics extracted from kelp, *Eisenia arborea*, and the littoral gastropod, *Tegula funebris*.

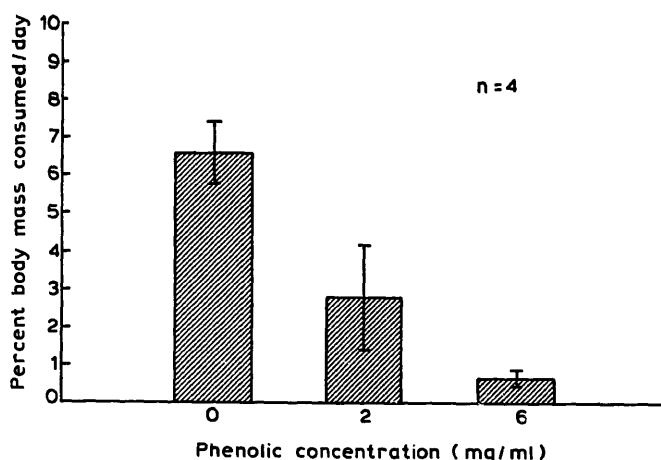


Fig. 2. Consumption rates ($\bar{x} \pm SE$) of agar discs containing varying concentrations of polyphenolics extracted from *D. californicum*. All treatments differ significantly ($P < 0.0001$).

GROWTH EXPERIMENT

For kelp polyphenolics to be effective defensive compounds over evolutionary time, they must reduce the fitness of their herbivores, or mimic compounds that do. Otherwise there is no mechanism to maintain behavioral deterrence. The growth experiment was an initial effort to test this premise. Abalone raised on phenolic-poor and phenolic-rich diets consumed 6.73 and 2.79% of their body mass · day⁻¹, respectively, and this difference is statistically significant ($t = -7.13$, $df = 6$, $P < 0.01$).

Red abalone grown for 88 days on the phenolic-poor diet increased in length at a greater rate than did animals grown on the phenolic-rich diet (Fig. 3). However, both treatment groups of abalone lost mass over the duration of the experiment thus indicating that the animals could not be sustained on agar-based diets, even when particles of *M. pyrifera* were added to the discs. Nonetheless, animals grown on the phenolic-rich diet lost mass at a significantly higher rate than those grown on the phenolic-poor diet ($t = 2.63$, $df = 6$, $P < 0.05$).

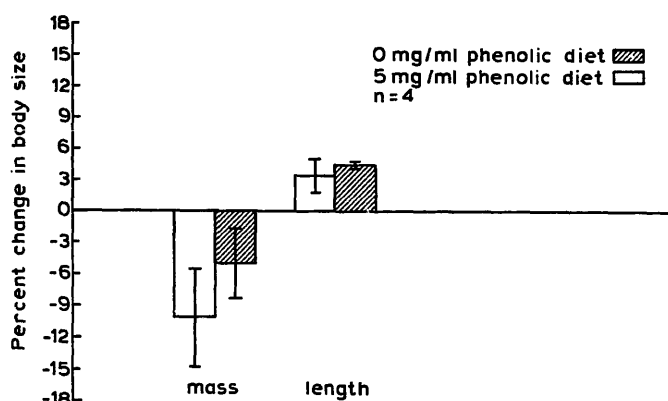


Fig. 3. Percent change in body size of red abalone ($\bar{x} \pm \text{SE}$) grown for 88 days on agar discs containing macerated *M. pyrifera* and phenolics from *D. californicum* added at 0 and 5 mg \cdot ml $^{-1}$. Changes in mass differed significantly between treatments ($P < 0.0001$).

DISCUSSION

Despite the fact that kelp is the major source of plant biomass (and probably production) in kelp forest communities (Foster & Schiel, 1985), most of the evidence that kelp polyphenolics are deterrent to herbivores has been correlative (Anderson & Velimirov, 1982; Steinberg, 1985; Johnson & Mann, 1986). Most kelp species do not contain high concentrations of polyphenolics (Steinberg, 1985, 1989), and because they are often preferred foods (Steinberg, 1985), they appear undefended. This apparent vulnerability is intriguing because kelps are commonly encountered by herbivores in the field.

Previous studies (Geiselman & McConnell, 1981; Steinberg, 1985, 1988; Van Alstyne, 1988) of the effects of polyphenolics on marine herbivores indicate that degree of deterrence is related to phenolic concentration, and phenolics deter many species of gastropods and echinoids. Our finding that polyphenolic compounds in *D. californicum* have a dosage-dependent deterrence effect on red abalone is consistent with the results of Geiselman & McConnell (1981) for *Littorina littorea* and Steinberg (1988) for *Tegula funebris*. Hay and co-workers (Hay et al., 1987a,b, 1988) have shown that terpenoids are also dosage-dependent against a variety of marine herbivores. The fact that these structurally dissimilar groups of compounds all act in a dosage-dependent fashion is inconsistent with predictions of the plant apparency model as originally formulated by Feeny (1976) and Rhoades & Cates (1976). This point, combined with the decreasing distinction between digestibility reducers and toxins (Zucker, 1983; Robbins et al., 1987; Bernays et al., 1989) and the data indicating phenolics are not generally acting (Zucker, 1983; Bernays et al., 1989) support the contention by Hay & Fenical (1988) that a simple distinction between quantitative and qualitative defenses in the marine environment needs re-evaluation.

Our use of a haliotid species in these experiments adds to a growing body of evidence

that algal polyphenolics deter a diverse array of marine herbivores. The haliotids diverged from primitive trochacean gastropods (e.g., *Tegula* spp.) in late Paleozoic/early Mesozoic times (Moore, 1960; Sepkowski, 1982), long before the probable Cenozoic radiations of modern cool-water biotas (Durham & MacNeil, 1967) and the proposed late Cenozoic radiation of kelps (Estes & Steinberg, 1988). The deterrence of trochaceans and haliotids (and other even more distantly related groups, e.g., echinoids) by phenolics is only explainable as: (1) a homologous (parallel) function, derived in a common paleozoic ancestor and maintained through continued natural selection for more than 250 Myr; (2) analogous (convergent) functions that have evolved independently much more recently; or (3) a general deterrence by polyphenolics of herbivore functions which is broadly manifested in marine herbivores independent of co-evolutionary mechanisms. The fact that haliotids worldwide typically are sedentary drift-feeders argues against a co-evolutionary scenario because these animals seem to impart little or no cost in the form of tissue loss to living plants. This indicates that polyphenolic compounds deter an array of marine herbivores regardless of those herbivores' evolutionary relationship with the defended algae. However, because most seaweeds are probably affected by a wide variety of generalists (Gaines & Lutchenko, 1982), most algal defense compounds probably deter numerous species of herbivores.

PLURALISTIC APPROACH

Two approaches have been used to evaluate the effects of polyphenolics on herbivore feeding. In one, the compounds are extracted from plants, incorporated at varying concentrations into agar discs, and tested for deterrence by measuring consumption rates of the discs by herbivores. Two studies (Geiselman & McConnell, 1981; Steinberg, 1988) which used this approach demonstrated that polyphenolics extracted from phaeophytes deterred grazing. A second approach relates herbivore feeding rate on, or preference for, living algae to phenolic content. Several studies (Anderson & Velimirov, 1982; Steinberg, 1984, 1985; Johnson & Mann, 1986; Van Alstyne, 1988) present evidence of this kind to suggest that polyphenolics deter herbivory. Because chemical manipulations and correlative evidence have been used separately, the exact mechanisms of deterrence and their ecological significance remain uncertain in most specific cases.

Although chemical manipulations have isolated phenolics as the cause of deterrence, the ecological significance of these experiments is questionable for two reasons. One is that phenolic concentrations in agar discs and natural algae are difficult to equate. The compounds are equally distributed in the agar matrix and may be sensed by the herbivore upon contact with the discs. However, polyphenolics in living plants are contained in subcellular physodes (Ragan, 1976) and are probably not sensed by an herbivore until gustation when they may be sensed strongly. Second, the physiological effects of polyphenolics in agar may differ from their effects in natural algae. The early literature (Feeney, 1976; Rhoades & Cates, 1976) postulated that phenolic compounds

hindered digestion by binding proteins, a view also embraced in studies of the effects of algal polyphenolics on marine herbivores (Steinberg, 1985; Ragan & Glombitza, 1986; Van Alstyne, 1988). However, phenolics from terrestrial plants may act as toxins rather than digestibility reducers (Zucker, 1983; Robbins et al., 1987; Bernays et al., 1989). Regardless of the mechanism, herbivores evidently may counteract the deterrent effects of phenolics with surfactants and salivary protein and by altering gut pH (Bernays, 1981; Hagerman & Butler, 1981; Mehansho et al., 1983; Martin & Martin, 1984). Polyphenolics bind to agar (Steinberg, 1988). These phenolic-agar complexes may have different behavioral and physiological effects than the unbound phenolics, thus making phenolics in agar-based diets more or less a deterrent than they are at equal concentrations in natural algae.

The correlations of algal phenolic concentration with herbivore preference or consumption rate are ecologically relevant (because the herbivores are eating natural foods), although such evidence does not exclude the possibility that other algal characteristics are responsible for the inverse relationship between phenolic content and feeding preferences. Nonphenolic secondary compounds (Hay et al., 1987a,b) and morphology (Littler & Littler, 1980; Steneck & Watling, 1982; Watson & Norton, 1985) also have been associated with feeding rates and preferences of marine herbivores.

We have combined both approaches here to demonstrate that feeding rates on natural algae are correlated with algal phenolic concentrations, and also that phenolics deter abalone feeding. In the only previous work of this kind, feeding preferences of the gastropod *T. funebris* were shown to be negatively correlated with phenolic content of the rockweed *F. distichus* (Steinberg, 1985) and polyphenolics extracted from *F. vesiculosus* were found to deter grazing by *T. funebris* when incorporated into an agar diet (Steinberg, 1988).

CHEMICAL VS. MORPHOLOGICAL DEFENSES

Most prior studies of marine plant-herbivore interactions have focused on a single mode of plant defense, usually structure or secondary chemistry. Evidence for chemical defenses is reviewed by Hay & Fenical (1988) and for structural defenses by Littler & Littler (1980), Steneck & Watling (1982), and Watson & Norton (1985). However, studies that simultaneously investigate multiple defense tactics (e.g., chemical vs. structural defenses) in a particular plant-herbivore system are generally lacking. Our study provides evidence that structural and chemical defenses in some kelp species may interact or act in concert. Steneck & Watling (1982) proposed that algal form was a determinant factor in herbivore feeding preference, and based in part on this finding, that some herbivorous molluscs were incapable of grazing large, leathery macrophytes as effectively as membranous forms. A related prediction is that herbivorous molluscs prefer algae large enough to crawl on. *Cystoseira osmundacea* was the only algal species in our study whose blades had a different general form from blades of the other species in that abalone cannot crawl on them. Because *C. osmundacea* is phenolic-rich, it is not

surprising that eliminating its gross structural differences with the other species had no effect on relative consumption rates (algal extract grazing experiment). However, removal of structural characteristics from the phenolic-poor species (*L. sinclarii*, *M. pyrifera*, and *N. luetkeana*) also significantly altered their relative consumption rates. This is especially interesting because these species all have large, leathery blades. These findings support Steinberg's (1985) suggestion that structural defenses in the kelps may be most pronounced in species that are poorly defended chemically; they also represent an apparent departure from Steneck & Watling's (1982) model inasmuch as the blades of these species are of similar functional form.

Although the different results of the algal grazing and algal extract grazing experiments suggest morphologically-based defenses among phenolic-poor kelps, there are other explanations that were not investigated. One is that the phenolic-poor species could include attractant or repellant non-polar compounds that were not incorporated into the agar discs of the algal extract grazing experiment. Another is that nutritional value or some other non-morphological attribute of the algae was altered by homogenization or incorporation into agar. These particular explanations seem unlikely because: (1) few nonpolar metabolites have been identified in kelps (Faulkner, 1984; Ragan & Glombitza, 1986); and (2) kelp nutritional values have been found to be uncorrelated with feeding preferences of their herbivores (Steinberg, 1985). Nonetheless, further study is needed to establish reasons for the feeding preference hierarchy among phenolic-poor kelps and why this hierarchy was diminished in the algal extract grazing experiment.

POLYPHENOLICS AND ABALONE GROWTH

Terpenoids have been shown to inhibit growth in an omnivorous fish (Hay et al., 1987b) and to cause mortality in juvenile gastropods (Paul & Fenical, 1986). Vadas (1977) also found that echinoid growth rates correlated with feeding preference among species of phaeophytes and several of Vadas' low preference phaeophytes are now known to be phenolic-rich (Steinberg, 1985). The effects of polyphenolic compounds on growth rate of kelp herbivores otherwise have been unstudied.

Red abalone experience a decline in body mass and thus probably cannot be maintained for long periods on the agar diets commonly used to test the effects of polyphenolics. This may hold for other marine herbivores also. Because polyphenolics are water soluble, finding a diet which will maintain the desired concentrations of the compounds in water is difficult. One study (Steinberg, 1985) showed that the terrestrial tannin, polygallic acid, could be absorbed to blades of *M. pyrifera* for several days. This type of diet may be required to properly study the fitness effects of polyphenolics on marine herbivores.

Tests that measure the effects of plant secondary compounds on growth, reproduction, and mortality are important because for a defensive compound to be effective over evolutionary time it must reduce the herbivore's fitness compared with undefended

species. Grazing deterrence, a behavioral effect, should be lost by the herbivore unless accompanied by a reduction in fitness. Our results show that polyphenolics inhibit growth of red abalone. However the mechanism of reduced growth remains unclear. Our data suggest that a likely mechanism is through reduced feeding rate although phenolic compounds also could act by physiological mechanisms.

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