Marine Biology (2001) 138: 1213–1224
DOI 10.1007/s002270100544

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Feeding rates of *Littorina striata* and *Osilinus atratus* in relation to nutritional quality and chemical defenses of seaweeds

Received: 5 June 1998 / Accepted: 22 January 2001 / Published online: 24 March 2001

Abstract Algae frequently suffer attack by herbivores and in some cases complete destruction has been observed. Despite the role of herbivores in structuring marine algal communities, herbivore food choice and the algal traits that herbivores use to choose foods are poorly documented. There has been much discussion and disagreement regarding the best method of assessing food preference. Experiments designed to detect preferential feeding involve offering a selection of food types to one or more individual consumers. Ideally, the potential foods are presented simultaneously to the consumer within a single experimental arena so that the consumer has the opportunity to express a dietary choice. However, some experimental designs allow individual consumers to feed for a constant time on individually presented alternative foods, which permits comparisons of feeding rates on these different foods. Such an experiment is not a true feeding preference experiment because no food choice is provided, but the results can reflect differences in attractiveness or palatability of various potential foods that may also be expressed when choices are offered. In this work, "discrimination" in selection of algae was tested in two invertebrate species common on intertidal rocks in Gran Canaria (*Littorina striata* and *Osilinus atratus*) by measuring their feeding rates on 20 common algal species in no-choice laboratory experiments. The algae represented the major floral elements of the local intertidal community. *L. striata* and *O. atratus* exhibited high feeding rates on *Enteromorpha muscoides* and *Ulva rigida*, whereas *Alsidium corallinum* and *Laurencia corrallopsis* were refused. The majority of algal species were readily consumed by these two generalist herbivores. No clearly defined trends in the concentrations of ash, nitrogen, carbon, soluble carbohydrates, soluble proteins, and caloric content in the seaweeds were found to account for these differences in feeding rates. In contrast, feeding rates varied according to the presence of secondary metabolites. The low susceptibility to grazing by snails in the least-consumed algae seems to be associated with the presence of secondary metabolites. We suggest that feeding rates of *L. striata* and *O. atratus* on algae are primarily due to avoidance of algal chemical defenses, whereas positive aspects of food quality probably play a lesser role in determining consumption.

Communicated by S. A. Poulet, Roscoff

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Introduction

Food preferences in marine herbivores are a complex and poorly understood subject. For example, it is often unclear why herbivorous fishes and invertebrates consume only a fraction of the variety of seaweeds available to them as potential foods. Several studies on different herbivores have shown that they do not eat many of the most common macroalgae in their habitat (Horn et al. 1982; Horn 1983; Hay 1986; Duffy and Hay 1991, 1994).

Marine seaweeds are known to differ in nutrient and caloric content, that is, in nutritional quality (Paine and Vadas 1969; Montgomery and Gerking 1980; Horn et al. 1986). Therefore, differences in nutritional quality among the available seaweeds could be a major reason for the apparent selectivity observed in herbivores (Himmelman and Carefoot 1975; Mattson 1980; Neighbors and Horn 1991), on the assumption that animals choose that food from which they may derive the most "value" (Larson et al. 1980). In fact, in some marine herbivores the selection of food has been explained in terms of the nutritional quality of the algae (Nicotri 1980; Arrontes 1990). To test this possibility in our algae, we determined the proximate chemical composition (ash, carbon, nitrogen, and soluble carbohydrates and proteins) and energy content of the 20 algal species used in the feeding experiments.

However, herbivore preference can correlate poorly with the quality of the algae as food. Relatively low feeding rates on certain algae by some marine herbivores have been attributed to chemical defenses in the algae. The importance of plant secondary metabolites in reducing herbivory in terrestrial communities is well documented and generally accepted as one of the most effective means of defense against herbivores (Rhoades and Cates 1976; Coley et al. 1985; Rosenthal and Berenbaum 1992). Several authors have shown that many secondary metabolites isolated from seaweeds inhibit grazing by a variety of marine herbivores (Hay and Fenical 1988, 1996; Hay 1991, 1996; Hay and Steinberg 1992; Paul 1992; Paul et al. 1993; Duffy and Hay 1994; Cronin and Hay 1996).

Studies of feeding biology of gastropods (*Aplysia* spp., *Fissurella* spp., *Haliotis* spp., *Littorina* spp, *Tegula* spp.) reveal that some species can discriminate among macroalgal species (Steinberg 1985; Watson and Norton 1985; Barker and Chapman 1990; Franz 1990; Pennings 1990; Granado and Caballero 1991; McShane et al. 1994), and their feeding is sometimes deterred by the presence of a number of compounds (Steinberg 1988, 1989; Granado and Caballero 1991; Shepherd and Steinberg 1992; Steinberg and van Altena 1992). On the other hand, herbivores can sometimes tolerate higher loads of chemical deterrents when the food is high in nutritional value. Alternatively, herbivores can be more susceptible to deterrents when the nutritional content is lower. Thus, Duffy and Paul (1992) presented evidence from field experiments that variation in nutritional quality within the natural range found among reef species is of comparable importance to chemical defenses in affecting an organism's susceptibility to predators. Also, Hay et al. (1994) have shown the synergistic interaction between low nutritional quality and chemical deterrents; they found that the synchronous combination of $CaCO_3$ (low-quality) and secondary metabolites acted synergistically and deterred feeding significantly more than the sum of the effects of each tested separately.

This article reports on whether selection of food by the generalist herbivores, the gastropods *Littorina striata* and *Osilinus atratus*, is driven by secondary metabolites or the nutritional quality of the food. We selected several species of macroalgae that are common to the coast of Gran Canaria Island to investigate their relative palatability to herbivores. To span the major taxa we chose five green, five brown, and ten red seaweeds that represented the major floral elements of the local intertidal community. Two distinct components of food preference can be recognized: one relating to the selection of a potential prey item (attractiveness) and the other concerning the rate at which that prey is ingested (edibility) (Nicotri 1980). Techniques designed to measure attractiveness necessarily involve a choice between two or more possible foods (Lawrence 1975; Vadas 1977). Measurement of edibility, on the other hand, assumes that, under equivalent hunger conditions, herbivores consume preferred foods faster (Leighton and Boolootian 1963; Carefoot 1967). We separately measured this last component of food preference, the edibility of the different algal species offered to snails, using an adaptation of a method developed by Geiselman (1980) that involves suspending potential food sources in agar and measuring feeding on the suspension (Valiela et al. 1979). The susceptibility of an alga to an herbivorous mollusc depends, in part, upon thallus morphology, size, and toughness relative to feeding ability of the mollusc (Steneck and Watling 1982). When we grind and suspend algal material in agar, we destroy some of the morphological traits that may affect feeding rates (Littler and Littler 1980; Hay 1981a; Steneck and Watling 1982; Padilla 1985, 1989). However, because *L. striata* and *O. atratus* have similar feeding apparatus and modes of feeding (Steneck and Watling 1982), we focused on chemical characteristics (secondary metabolites and nutritional content) and chose to test primarily chemical aspects of the seaweeds we studied. Thus we destroyed any morphological differences between plants by drying and grinding them to a fine powder before they were embedded in an agar matrix and presented to the herbivores. Processing algae into agar-based foods limited the variables potentially differing among algal species to algal chemistry and food value.

Materials and methods

Studied species

Algae were collected intertidally by hand at low tide, between 6:30 and 9:30 a.m., from various sites along the coast of Gran Canaria. The following 20 species of marine algae were employed in the feeding experiments, representing a variety of taxonomic groups available to grazers: *Caulerpa racemosa* (Forsk.) J. Ag., *Codium adhaerens* (Cabr.) C. Ag., *Enteromorpha ramulosa* (Clemente) Cremades, *Halimeda discoidea* Decaisne and *Ulva rigida* C. Ag. (Chlorophyta); *Colpomenia sinuosa* (Roth) Derb. et Sol., *Cystoseira abies-marina* (S. Gmel.) C. Ag., *Fucus spiralis* Linneaus, *Padina pavonica* (L.) Thivy in W. Taylor, and *Stypocaulon scoparium* (L.) Kützing (Phaeophyta); *Alsidium corallinum* C. Ag., *Asparagopsis armata* Harvey, *Galaxaura rugosa* (Ellis et Sol.) Lamour., *Gelidium canariensis* (Grunow) Seoane-Camba, *Gracilaria cervicornis* (Turner) J. Ag., *Grateloupia doryphora* Montagne (Howe), *Halopithys incurvus* (Huds.) Batt., *Hypnea spinella* (C. Ag.) Kütz., *Laurencia corrallopsis* (Montagne) Howe, and *Schizymenia dubyi* (Chauv ex Duby) J. Ag. (Rhodophyta). The algae were transported to the laboratory in a cooler. After epiphytes and animals were removed from their surfaces by gentle rubbing of the algal tissues between thumb and forefinger, the fronds were rinsed briefly with distilled water to remove sediment and salts and oven dried to constant weight at 60°C. Dried plant material was ground through a Wiley mill to a particle size of 1 mm in diameter and stored in a desiccator containing silica gel until the start of the trials.

The herbivores used in the feeding experiments were the gastropods *Littorina striata* King and Broderip, and *Osilinus atratus* Wood. The snails were collected in the upper and lower intertidal zone, respectively, nearby where the algae were taken. Once in the laboratory, snails were kept in aquaria and starved for 24 h prior to the initiation of feeding trials to obtain uniformity of response in feeding experiments and to minimize dietary preconditioning (Larson et al. 1980; Watson and Norton 1985). Our previous experience has shown that 24 h is a good starvation period for these gastropods (Granado and Caballero 1991, 1995).

Laboratory feeding experiments

To assess the relative palatability of algae we used an adaptation of the method developed by Geiselman (1980) that involves suspending ground algae in agar and measuring feeding on the suspension (Valiela et al. 1979; Rietsma et al. 1982). This method eliminates the effect of toughness or frond architecture on the feeding rates of grazers. Petri dishes containing an agar suspension of the ground particles were prepared separately for each algal species in the same way. We suspended 1 g of dry algal mass in 100 ml of 1.8% agar-seawater (w/v). Previously, the seawater had been filtered through 0.45 μm Millipore filters and autoclaved. The media were then poured into petri dishes (8.5 cm diameter) to solidify. Controls consisted of agar without ground algae added. To each of these dishes, we introduced two *O. atratus* or four *L. striata*, which were allowed to graze for 30 min. The dishes with snails were not submerged in seawater because when we did it in this way, the animals escaped from the dishes. Each of these tests was replicated at least eight times. Laboratory feeding experiments for all algal species were run in springtime.

Feeding was assessed by counting the number of feeding marks left on the surface of the agar suspension as described in Valiela et al. (1979) and Rietsma et al. (1982). Marks were easily counted under a dissecting microscope. We suppose that there is a close relationship between the number of bites left on the surface of the agar suspension and the volume of food consumed by the animal, regardless of the chemoreceptive mechanisms (smell, taste, etc.) used by the herbivores (see Frazier 1992 for a discussion of how animals perceive secondary metabolites).

Data from this experiment were statistically analyzed using a nonparametric procedure, the Kruskal–Wallis test (Zar 1984). This method was used rather than a one-way analysis of variance (ANOVA) because data sets were not normally distributed, Bartlett's test indicated unequal variances between treatments, and attempts to remedy this via arcsine (and other) transformations failed.

Algal chemical characteristics

Measures of nutritional content of the seaweeds (ash, carbon, nitrogen, energy content, soluble carbohydrates, and soluble proteins) and the presence or absence of secondary metabolites in the algal extracts were determined for each species of alga used in the laboratory feeding experiments. Samples were not from separate specimens but from a pooled sample of many specimens, the same oven-dried ground algae used in feeding assays. All determinations were made in triplicate except measures of energy content, which were duplicated.

Ash-free dry weight (AFDW) was determined by weight loss after ashing at 500°C for 24 h in a muffle furnace. These data were used to compare samples on the basis of ash-free caloric content and to calculate organic matter. Total carbon and nitrogen content of each algal species was measured by combustion. Samples (2–4 mg each) were combusted in a Perkin-Elmer 2400 elemental analyzer using acetanilide as a standard. Total and available caloric content of foods were determined by combusting pellets of samples in an IKA-Calorimeter system (model C700T), calibrated using benzoic acid. A correction for endothermy was not applied. If all algal ash were $CaCO_3$, the correction for our samples with the highest ash content would only amount to about 2% of the measured calories g^{-1} and would not affect our predictions (Montgomery and Gerking 1980). Available caloric content was defined according to Tenore (1981) and calculated by determining the caloric loss after weak acid hydrolysis (in 1 N HCl for 8 h at room temperature). Initial and final weights and absolute caloric content were used to calculate available calories per AFDW. Soluble carbohydrates were extracted by heating subsamples (125 mg each) in 5 ml of 80% methanol at 70°C for 5 min. Then, subsamples were centrifuged at 3,000 rpm for 10 min, the methanol extracts were decanted, and the residues were re-extracted one more time. The carbohydrate content in the methanol extracts was determined by the anthrone method using glucose as a standard. Soluble protein concentrations in each algal species were measured colorimetrically with the bicinchoninic acid method. Samples (250 mg each) of the oven-dried ground algae prepared for the agar suspension assay were further ground in a mortar and pestle, with 5 ml of phosphate buffer and 1 g of sterile sand as abrasive. Following grinding, samples were centrifuged at 3,000 rpm for 5 min to remove particulate matter, and 500 μl of the diluted supernatant was added to 500 μl cupric sulfate in bicinchoninic acid solution. After the dye-binding reaction had proceeded for 1 h, the concentration of protein was estimated by a Shimadzu UV-160A spectrophotometer at 562 nm relative to a bovine serum albumin standard curve.

Oven-dried ground algae were homogenized in a blender in a 1:1 mixture of dichloromethane and methanol. The extracts were filtered with a coffee filter, and solvents were evaporated under reduced pressure to yield the organic algal extract (usually a viscous oil). To test whether secondary metabolites existed in the algal extracts, all extracts were analyzed by thin-layer chromatography (TLC). TLC is an effective and rapid method to determine if an alga contains secondary metabolites, and it has previously been used to detect the presence or absence of secondary metabolites in tropical algae (Norris and Fenical 1986; Paul and Hay 1986; Wylie and Paul 1988). Commercially available plastic-backed TLC plates were used in this study (Schleicher & Schuell, type F 1500/LS 254). Each TLC plate (one per algal extract) was developed in a two-solvent system, first in 100% dichloromethane to screen for nonpolar metabolites, and then in 100% diethyl ether to screen for more polar metabolites. After the solvent front reached the top of the plate, the TLC plates were viewed under an ultraviolet (UV) lamp to observe any UV activity. The plate was then sprayed with 50% sulfuric acid and heated to show color reactions indicative of secondary metabolites. This method is ideal only for the detection of lipid-soluble compounds. Highly polar metabolites are not readily detected by this technique and water-soluble metabolites are not detected at all. Highly unstable metabolites may not be detected by TLC because of their rapid degradation (Paul and Hay 1986). Also, oven drying plant material can degrade some of these compounds, but we did it by this means to test the same material used in the feeding assay. TLC also tells little about the chemical structures of compounds in the extracts unless the compounds can be related to known standards.

Since in the brown algae (division Phaeophyta) the most common of the secondary metabolites are the phlorotannins, which are polymers of phloroglucinol (1,3,5-trihydroxybenzene) (Ragan and Glombitza 1986; Steinberg 1992), total phenol concentration was determined in the five species of brown algae. For each species, duplicate subsamples (250 mg each) of the oven-dried ground algae were extracted in 25 ml of 85% methanol. After 15 min extraction at 65°C, the methanol subsamples were centrifuged at 3,000 rpm for 10 min. Then the methanol extracts were decanted and the residues were re-extracted three more times; the supernatants from the four extractions were collected and combined. Volumes of extracts were measured, and the extracts were assayed for phenols. Total phenol levels were measured using an adaptation of the Folin–Denis colorimetric method (Folin and Denis 1915), which has been used extensively to assay phenolic levels in marine algae (Geiselman 1980; Johnson and Mann 1986; Jennings and Steinberg 1994, 1997). Phloroglucinol was used as a standard (Ragan and Jensen 1977).

To assess statistically the association between feeding rates and the chemical characteristics of the algae (ash, carbon, nitrogen, energy, soluble carbohydrates, and soluble proteins), a nonparametric test was used by ranking the variables and calculating a coefficient of rank correlation, Spearman's coefficient (Sokal and Rohlf 1981). This test was used rather than a simple correlation coefficient because data from feeding experiments were not normally distributed.

Results

Laboratory feeding experiments

The results of the laboratory feeding experiments clearly show that both *Littorina striata* and *Osilinus atratus* preferentially feed on certain algae and eat others in only small amounts, even when no other food is available (Figs. 1, 2). Analysis of the data from this experiment using the nonparametric procedure, the Kruskal–Wallis test (Zar 1984), shows that these values are significantly different ($H=129.8$, $P<0.0001$ for *L. striata* and $H=146.3$, $P<0.0001$ for *O. atratus*).

A multiple-comparison nonparametric test parallel of the Student–Newman–Keuls (SNK) procedure (Sokal and Rolhf 1981; Martín-Andrés and Luna del Castillo 1994) (Figs. 1, 2) indicates that although the compositions of the subgroups lightly change depending on the herbivore species, there are some remarkable similarities: *Enteromorpha muscoides* is one of the most consumed species for both gastropods, whereas *Laurencia corrallopsis*, *Alsidum corallinum*, and *Gracilaria cervicornis* belong to the least-consumed subgroup for both snails. These results indicate that the herbivore uses a plant trait other than toughness to distinguish among foods and suggest that a chemical factor could be involved.

Algal chemical characteristics

Table 1 details some nutritional characteristics of algae that seem relevant to questions of preference. The ash content differed significantly among the species of macroalgae tested, reflecting a difference in organic content ($F_{19,40}=58.5$, $P<0.0001$, ANOVA). Ash content ranged from about 17% dry wt in *Gelidium canariensis* to 79% dry wt in the heavily calcified species, *Halimeda discoidea*. The quality of organic material will vary depending on its chemical composition.

Carbon content varied significantly among the species of macroalgae tested ($F_{19,40}=26.2$, $P<0.0001$, ANOVA). Carbon content ranged from 15% dry wt in *H. discoidea* to 40% dry wt in *G. canariensis*. Studies of the macroalgae's nitrogen content revealed that red macroalgae had a higher nitrogen content (4.1% dry wt ± 0.09) than did brown and green macroalgae (means of nitrogen content for brown and green macroalgae were similar, 3.7% ± 0.13 and 3.3 ± 0.16, respectively) ($F_{2,57}=13.6$, $P<0.0001$, ANOVA; SNK test $P<0.05$). Nitrogen content significantly varied between 2% dry wt

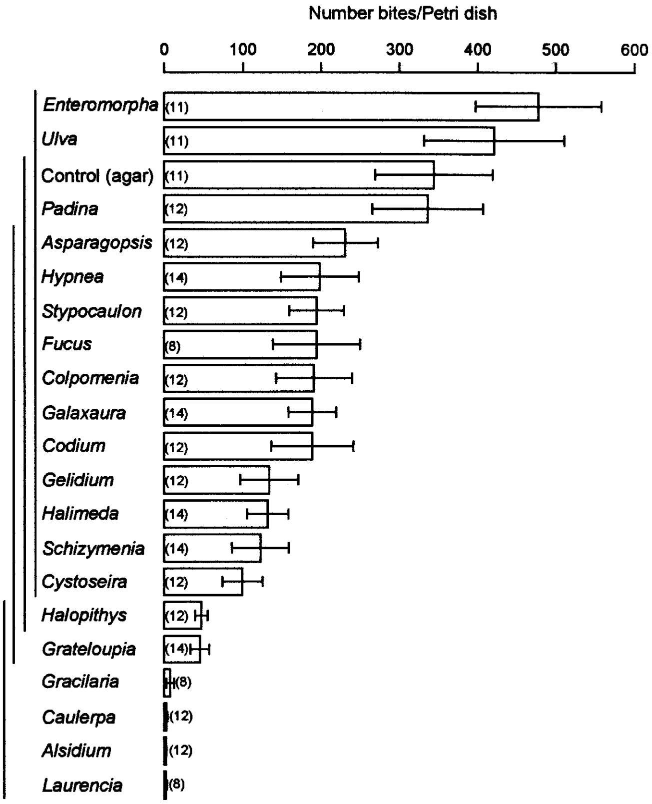

Fig. 1 Feeding rates of *Littorina striata*. *Bars* represent means ± SE. Means that are not significantly different (Kruskal–Wallis followed by a multiple-comparison nonparametric test; $P<0.05$) are joined with *vertical lines*. *Numbers in parentheses* indicate sample size

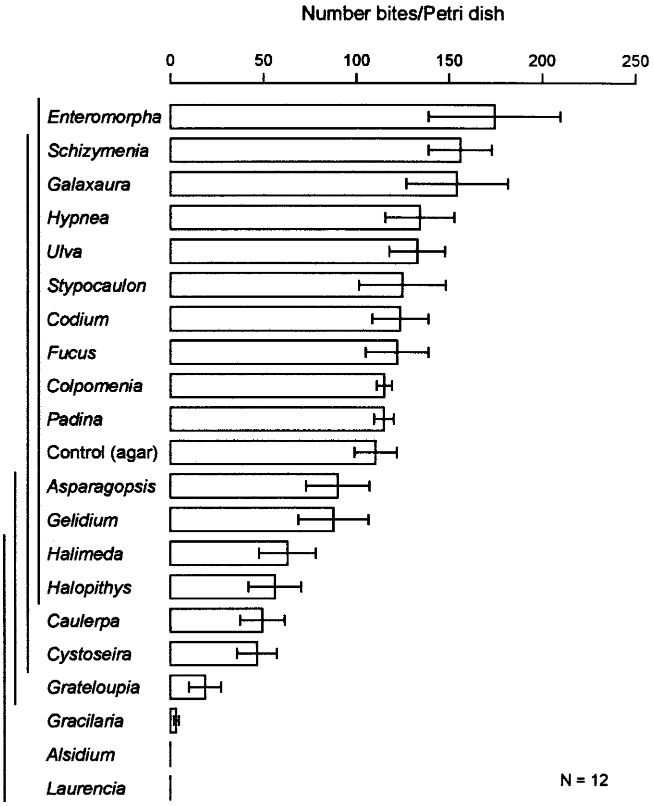

Fig. 2 Feeding rates of *Osilinus atratus*. *Bars* represent means ± SE. Means that are not significantly different (Kruskal–Wallis followed by a multiple-comparison nonparametric test; $P<0.05$) are joined with *vertical lines*. *N* Sample size

Table 1 Mean chemical composition (± SE) of the 20 species of algae used in laboratory feeding experiments. *n.d.* Not determined

Algal species	Ash (%)	Carbon (%)	Nitrogen (%)	Available kilocalories (per g AFDW)	Soluble carbohydrates (mg g^{-1} dry wt)	Soluble protein (mg g^{-1} dry wt)
Chlorophyta						
Caulerpa racemosa	64.6 (0.6)	15.7 (0.5)	2.4 (0.12)	1.6 (0.03)	3.4 (0.2)	16.1 (0.7)
Codium adhaerens	52.2 (0.7)	20.5 (0.6)	2.7 (0.03)	1.3 (0.01)	6.0 (0.3)	16.5 (0.5)
Enteromorpha muscoides	30.0 (1.0)	25.6 (0.1)	3.3 (0.01)	1.3 (0.09)	15.4 (0.4)	19.2 (0.3)
Halimeda discoidea	79.0 (0.5)	15.3 (0.4)	2.1 (0.10)	0.9 (0.02)	2.4 (0.1)	9.8 (0.2)
Ulva rigida	30.9 (1.7)	29.0 (0.1)	3.9 (0.05)	1.1 (0.10)	3.1 (0.1)	9.1 (0.4)
Phaeophyta						
Colpomenia sinuosa	51.0 (0.5)	24.7 (1.1)	3.3 (0.09)	n.d.	3.1 (0.1)	13.9 (0.4)
Cystoseira abies-marina	30.6 (0.4)	36.1 (0.7)	4.1 (0.07)	0.6 (0.04)	3.2 (0.1)	25.7 (0.3)
Fucus spiralis	29.1 (0.3)	30.7 (0.2)	4.4 (0.08)	1.6 (0.01)	15.8 (0.5)	24.1 (0.2)
Padina pavonica	56.0 (0.3)	23.1 (0.3)	3.1 (0.04)	0.8 (0.04)	4.3 (0.2)	16.8 (0.6)
Stypocaulon scoparium	45.5 (0.3)	29.8 (0.3)	3.3 (0.03)	0.9 (0.01)	4.7 (0.3)	52.1 (0.4)
Rhodophyta						
Alsidium corallinum	18.5 (0.2)	39.2 (0.2)	4.9 (0.03)	1.4 (0.09)	52.5 (0.7)	12.2 (0.6)
Asparagopsis armata	35.0 (0.3)	27.9 (0.9)	3.9 (0.11)	0.9 (0.08)	4.9 (0.2)	15.2 (0.4)
Galaxaura rugosa	61.5 (0.4)	21.3 (0.2)	2.8 (0.01)	1.1 (0.01)	10.7 (0.5)	11.5 (0.6)
Gelidium canariensis	16.9 (0.8)	39.9 (0.8)	5.4 (0.09)	1.2 (0.08)	15.6 (0.8)	14.1 (0.4)
Gracilaria cervicornis	36.7 (1.2)	30.8 (0.7)	3.9 (0.10)	1.5 (0.17)	22.7 (0.3)	20.3 (0.5)
Grateloupia doryphora	23.0 (0.2)	32.4 (0.1)	4.3 (0.02)	2.0 (0.01)	7.1 (0.2)	12.3 (0.6)
Halopithys incurvus	35.4 (0.3)	30.0 (0.2)	4.4 (0.04)	1.1 (0.05)	11.0 (0.1)	21.6 (0.4)
Hypnea spinella	29.3 (0.5)	29.1 (0.1)	4.2 (0.06)	0.8 (0.01)	6.5 (0.3)	20.1 (0.2)
Laurencia corrallopsis	29.1 (0.5)	35.8 (0.7)	4.9 (0.05)	1.7 (0.04)	13.3 (0.6)	20.5 (0.3)
Schizymenia dubyi	20.9 (0.1)	32.3 (0.1)	4.4 (0.01)	1.1 (0.03)	12.0 (0.7)	24.8 (0.4)

in *H. discoidea* and 5.4% dry wt in *G. canariensis* ($F_{19,40}=20$, $P<0.0001$, ANOVA).

The available caloric content significantly varied from 0.2 kcal g^{-1} dry wt in *H. discoidea* to 1.6 kcal g^{-1} dry wt in *Grateloupia doryphora* ($F_{18,19}=20.7$, $P<0.0001$, ANOVA). Based on organic content (AFDW), the available caloric content significantly ranged from 0.6 kcal g^{-1} AFDW in *Cystoseira abies-marina* to 2 kcal g^{-1} AFDW in *G. doryphora* ($F_{18,19}=10.2$, $P<0.0001$, ANOVA). Red algae had a higher available caloric content than either green or brown algae (0.9 kcal g^{-1} dry wt compared with 0.7 and 0.6, respectively; $F_{2,35}=3.9$, $P=0.0275$, ANOVA; SNK test $P<0.05$). However, on an AFDW basis, the available caloric content was similar among the three algal divisions ($F_{2,35}=2.6$, $P=0.0859$, ANOVA).

Soluble carbohydrate content significantly varied from 2.4 mg g^{-1} dry wt in *H. discoidea* to 52.5 mg g^{-1} dry wt in *A. corallinum* ($F_{19,40}=841.9$, $P<0.0001$, ANOVA). Red algae had a higher soluble carbohydrate content than either brown or green algae (15.6 mg g^{-1} dry wt compared with 6.2 and 6.1 mg g^{-1}, respectively; $F_{2,57}=6.5$, $P=0.003$, ANOVA; SNK test $P<0.05$).

Soluble protein content significantly varied from about 9 mg g^{-1} dry wt in *Ulva rigida* to 52 mg g^{-1} dry wt in *Stypocaulon scoparium* ($F_{19,40}=453.2$, $P<0.0001$, ANOVA). Brown algal protein (26.5 mg g^{-1} dry wt) was higher than either red (17.3 mg g^{-1} dry wt) or green algae (14.1 mg g^{-1} dry wt) ($F_{2,57}=10.2$, $P=0.0002$, ANOVA; SNK test $P<0.05$).

Spearman rank correlation coefficients were calculated to assess the relation between feeding rates and the chemical characteristics of the algae (ash, carbon, nitrogen, available energy, and soluble carbohydrates and proteins) (Table 2). For *Littorina striata*, ash, carbon, nitrogen, soluble carbohydrate, and soluble protein contents showed no significant relation to feeding rates (see statistics in Table 2). Only available caloric content, on an AFDW basis, showed significant correlation with feeding rates, but this correlation was negative ($r_s=-0.52$, $P=0.022$). For *O. atratus*, none of these quantities correlate significantly (Table 2). This means that these gastropods are evidently not making a food choice based on nutritive value.

Total phenolic levels varied significantly among the five brown algae (Fig. 3 A; $F_{4,5}=443.2$, $P<0.0001$, ANOVA), ranging between 0.2 and 1.4% of the dry weight of the algae. However, mean levels of phenolics in all brown algae were consistently quite low (all <2% by dry wt), comparable to levels contained in many of the palatable brown algae in other studies (Steinberg 1985; Steinberg and Paul 1990). There was no correlation between algal phenolic and feeding rates of the

Table 2 Spearman rank correlation coefficients (r_s) and P values

	Feeding rates			
	Littorina striata		*Osilinus atratus*	
	r_s	P	r_s	P
Percent ash	0.17	0.480	0.14	0.558
Percent carbon	–0.42	0.067	–0.43	0.066
Percent nitrogen	–0.32	0.169	–0.30	0.217
Available kcal g^{-1} AFDW	–0.52	0.022	–0.41	0.093
Soluble carbohydrates	–0.30	0.198	–0.10	0.670
Soluble proteins	–0.08	0.753	0.15	0.540

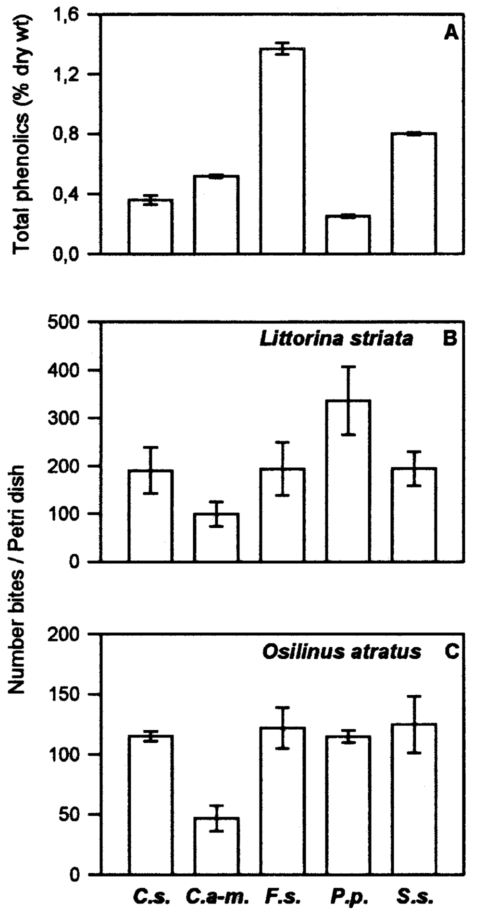

Fig. 3 Total phenolic content in brown algae (**A**), and feeding rates of *Littorina striata* (**B**) and *Osilinus atratus* (**C**). Algae used were *Colpomenia sinuosa* (*C.s.*), *Cystoseira abies-marina* (*C.a-m.*), *Fucus spiralis* (*F.s.*), *Padina pavonica* (*P.p.*), and *Stypocaulon scoparium* (*S.s.*). Data are means ± SE

gastropods (Spearman rank correlation coefficients: $r_s = -0.2$, $P = 0.747$ for *L. striata* and $r_s = 0.6$, $P = 0.285$ for *O. atratus*).

The results of the assays examining feeding rates of *L. striata* and *O. atratus*, expressed as percentage relative to the most consumed alga (*E. muscoides*), are shown in Fig. 4. On this basis, the 20 species of algae were divided into three arbitrary groups: low consumption (< 20% relative to *E. muscoides*), medium consumption (20–60%) or high consumption (> 60%). The presence (+) or absence (–) of secondary metabolites detected by TLC in each species of algae are also indicated. The presence of secondary metabolites in the algae is correlated with low susceptibility to herbivory. For example, all species of low-consumption algae (*A. corallinum*, *Gracilaria cervicornis*, *Grateloupia doryhora*, and *Laurencia corrallopsis*) contained secondary metabolites. In contrast, the highly consumed algae (*E. muscoides*, *U. rigida*, and *Padina pavonica*) did not produce secondary metabolites. Patterns of grazing susceptibility for medium-consumption algae were similar to those discussed above. For *Littorina striata*, none of 11 species of medium-consumption algae (*Asparagopsis armata*, *Hypnea spinella*, *Stypocaulon scoparium*, *Fucus spiralis*, *Colpomenia sinuosa*, *Galaxaura rugosa*, *Codium adhaerens*, *Gelidium canariensis*, *Halimeda discoidea*, *Schizymenia dubyi*, and *Cystoseira abies-marina*) produced secondary metabolites. For *O. atratus*, only 2 species of medium-consumption algae (*Halopithys incurvus* and *Caulerpa racemosa*) produced secondary metabolites, whereas 4 species (*A. armata*, *G. canariensis*, *Halimeda discoidea*, and *Cystoseira abies-marina*) did not. These differences between algal-consumption groups are significant using a 3×2 contingency table analysis and the *G* test ($G = 22.21$, $P < 0.001$ for *L. striata*, and $G = 15.27$, $P < 0.001$ for *O. atratus*).

When the data of the laboratory feeding experiments are classified in two ways, by algal division (Chlorophyta vs Phaeophyta vs Rhodophyta) and by presence of secondary metabolites (absence vs presence) (Table 3), and they are analyzed by two-way ANOVA, we find that the amount of algae consumed by *L. striata* (number of bites per petri dish) does not differ significantly among the three algal divisions ($F_s = 1.74$, $P = 0.178$); and for *O. atratus*, it is close to being not significant ($F_s = 3.08$, $P = 0.048$; Table 4). However, the amount of algae consumed by both gastropods differs significantly between algae with and without secondary metabolites ($F_s = 51.14$, $P < 0.0001$ for *L. striata*, and $F_s = 64.62$, $P < 0.0001$ for *O. atratus*; Table 4). Therefore, both gastropods are preferably eating algae that do not produce secondary metabolites.

Discussion

Our experimental results clearly demonstrate that *Littorina striata* and *Osilinus atratus* feed selectively on tested algae. Feeding preference has been studied in a number of marine herbivores, including molluscs (Pennings et al. 1993; McShane et al. 1994; Wilhelmsen and Reise 1994), crustaceans (Duffy and Hay 1991; Barry and Ehret 1993; Morán and Arrontes 1994), sea urchins (Shunula and Ndibalema 1986; Prince and LeBlanc 1992), and fishes (Lewis 1985; Barry and Ehret 1993).

Diet and food availability studies have shown that some herbivores take food in proportion to its abundance and availability in the field (Ogden et al. 1973, 1976; Abbott et al. 1974; Ogden and Lobel 1978). However, these studies combined with direct food preference tests have indicated that some algae are avoided by herbivores despite their abundance and availability. For example, the green alga *Penicillus pyriformis* and the red alga *Laurencia obtusa* are significantly avoided by herbivorous fishes and the sea urchin *Diadema antillarum*, in spite of the fact that these algae are very common in their habitats (Ogden 1976). Just as we have in this study, many other authors have observed that different species of the genera *Enteromorpha* and *Ulva* are generally preferred by a large number of marine herbivores (Lubchenco 1978; Geiselman 1980; Nicotri 1980; Watson and

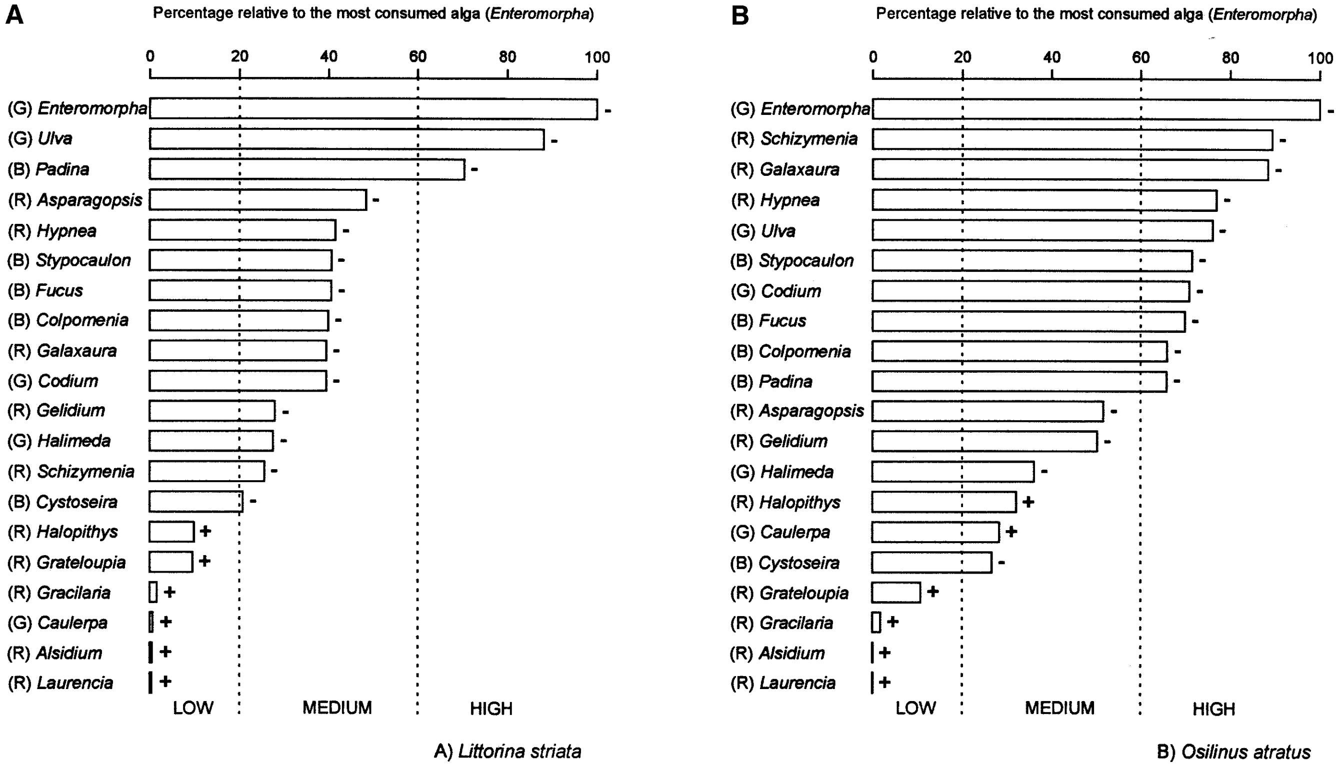

Fig. 4 Percentage of bites relative to the most-consumed algae (*Enteromorpha muscoides*) by **A** *Littorina striata* and **B** *Osilinus atratus*. Symbols: + secondary metabolites detected by TLC; – TLC showed no secondary metabolites; *G* green, *B* brown, and *R* red algae

Norton 1985; Brawley and Fei 1987; Wylie and Paul 1988; Paul et al. 1990; Barry and Ehret 1993).

In contrast to other studies of the genus (Lubchenco 1978; Geiselman 1980; Geiselman and McConnell 1981; van Alstyne 1988), in the present study *Fucus spiralis* did not inhibit feeding by either *L. striata* or *O. atratus*. The genus *Fucus* is thought to exemplify deterrent algae because of its phenolic content. However, in subsequent works, it has been shown that *L. saxatilis* and *L. nigrolineata* eat fucoids, among other available algae

Table 3 Number of bites per petri dish on each algal division according to the absence or presence of secondary metabolites (detected by TLC). The data are means (SE)

Division	*Littorina striata*		*Osilinus atratus*	
	No secondary metabolites	Secondary metabolites	No secondary metabolites	Secondary metabolites
Chlorophyta	291.50 (37.22)	3.00 (0.98)	125.19 (12.08)	49.42 (12.00)
Phaeophyta	203.57 (23.80)	–	104.58 (6.83)	–
Rhodophyta	174.36 (17.93)	24.04 (4.61)	124.42 (9.57)	15.57 (4.25)
Mean	217.06	20.21	117.55	21.21
SE	15.21	3.90	5.45	4.30

Table 4 Two-way ANOVA for the effects of different algal divisions and presence or absence of secondary metabolites on feeding by *Littorina striata* and *Osilinus atratus*. *df* Degrees of freedom; *MS* mean square

Source of variation	*Littorina striata*				*Osilinus atratus*			
	df	*MS*	F_s	*P*	*df*	*MS*	F_s	*P*
Between rows (divisions)	2	47,488.78	1.74	0.178	2	11,814.53	3.08	0.048
Between columns (metabolites)	1	1,397,079.18	51.14	0.0001	1	247,889.83	64.62	0.0001
Interaction (division×metabolites)	1	138,510.92	5.07	0.025	1	7,958.05	2.07	0.151
Within subgroups (error)	231	27,321.11			235	3,836.17		
Total	235				239			

(Hawkins and Hartnoll 1983). More recently, it has been observed that *L. littoralis* feed actively on *F. vesiculosus* (Viejo and Arrontes 1992).

The red alga *Laurencia corrallopsis* deterred grazing by both snails. The genus *Laurencia* is normally avoided by many generalist herbivores (Ogden 1976; Norris and Fenical 1982; Littler et al. 1983; Watson and Norton 1985; Buschmann 1990; Granado and Caballero 1991; Barry and Ehret 1993) and some species of *Laurencia* contain secondary metabolites that deter feeding in field or laboratory assays (Morrison 1986; Hay et al. 1987; Granado and Caballero 1995).

Plant morphology, nutrient content, secondary metabolites, and other chemical or structural properties all contribute to food quality and availability to herbivores (Paine and Vadas 1969; Atsatt and O'Dowd 1976; Nicotri 1980; Rietsma et al. 1982; Horn 1989; Hay et al. 1994; Hay 1996). The differential algal consumption cannot be attributed to the degree of toughness of the algae, since *Littorina striata* and *O. atratus* grazed algae in the agar suspension, where physical consistency of the 20 algal species was identical. A more likely explanation of the observed results would involve selection of algal foods on the basis of chemical factors (Leighton 1966).

In general, the nutritional needs of marine invertebrates are poorly known (Carefoot 1980, 1984, 1987), with the partial exception of economically important species such as some crustaceans (Dall et al. 1990), making it difficult to assess which nutritional quality of algae is most likely to be responsible for selection of food. Differences in chemical composition and energy content among algal species may indicate differences in their value as foods to herbivores. On the basis of nutritional considerations the feeding relationships remain obscure.

A negative correlation could be expected between ash content of the algae and feeding rates of snails: algae with low ash content (high organic content) would be consumed more than algae with high ash content (low organic content). However, feeding rates were not correlated with the ash content of the algae that we tested. In another study Nicotri (1980) showed that feeding preference of the isopod *Idotea baltica* and the amphipod *Ampithoe valida* varied independently of the ash content. Other studies of marine herbivores have revealed that the feeding rate of *Haliotis rubra* is not correlated with the ash content of macroalgae (McShane et al. 1994).

Since nitrogen has been demonstrated to be a critical nutrient for many herbivores (Mattson 1980; White 1984), the gastropods *L. striata* and *O. atratus* might be expected to select algae that are high in nitrogen. However, in this study, as in previous ones (Carefoot 1967; Nicotri 1980; Robertson and Lucas 1983; Duffy and Hay 1991), nitrogen content was unrelated to food preferences or to feeding rates, presumably because crude nitrogen content is not closely related to protein concentration in algae (Horn and Neighbors 1984).

Attempts to explain food choices of herbivores as a function of the protein content of plants have had limited success (Milton 1979; Bryant and Kuropat 1980; Belovsky 1981, 1984; Horn and Neighbors 1984; Horn 1989; Neighbors and Horn 1991). The common failure of protein maximization models to explain food choices of herbivores suggests that herbivores may be concerned with multiple aspects of food quality.

Calorific value of algae is one of the factors potentially contributing to selective feeding behavior in herbivores (Vadas 1977). However, there was no positive correlation between the feeding rates of the gastropods *L. striata* and *O. atratus* and algal caloric content (kcal g dry wt^{-1}, or ash-free^{-1}). Other authors (Carefoot 1967, 1973; Paine and Vadas 1969; Himmelman and Carefoot 1975; Vadas 1977; Nicotri 1980) have found that food preference in a variety of marine herbivores bears little relation to caloric content, which is in agreement with our data.

The avoidance of *Laurencia corrallopsis* is intriguing because snails are refusing to feed on a calorie-rich food (4.7 kcal g dry wt^{-1}). Similar behavior has been reported for the sea urchins *Strongylocentrotus drobachiensis*, *S. pupuratus*, and *S. franciscanus*, (Vadas 1977; Larson et al. 1980).

Some authors have suggested that the lack of a positive correlation between invertebrate food preference and the calorific value of algae is perhaps due to much of the energy content being derived from indigestible structural polysaccharides (Paine and Vadas 1969; Nicotri 1980). Therefore, the caloric content of an algal species may not be a meaningful measure of food value since many of the carbohydrates of an alga are structural and indigestible by herbivores (Lawrence 1975; Lobel and Ogden 1981).

Our results did not provide clear-cut support for the hypothesis that the macrophytes eaten by *Littorina striata* and *O. atratus* are higher in nutritional quality than the macrophytes not consumed by these gastropods. Therefore, selection of food by these snails appeared to be unrelated to the nutritional quality of the algae.

Diet choice in herbivores could be influenced by either attractant or deterrent qualities of potential food items or both. Chemical attractants seem to be little studied, whereas traits, chemical or otherwise, that deter feeding by marine herbivores have received much more attention (Hay and Fenical 1988, 1996; Padilla 1989; Pawlik 1993; Hay and Steinberg 1992; Paul 1992; Hay 1996). The low consumption of some algal species such as *Laurencia corrallopsis* or *Alsidium corallinum* could result from the presence of a chemical defense mechanism. Terrestrial plants are known to produce secondary plant substances that act as repellants to most insects and herbivores, and as attractants to the animals that feed on the plants (Fraenkel 1959; Rhoades and Cates 1976; Swain 1977, 1979; Robbins et al. 1987; Bernays et al. 1989; Rosenthal and Berenbaum 1992). Many secondary metabolites (terpenes, phenolic compounds, etc.) have been found in various species of algae and are thought to affect the palatability, or acceptability, of these algal species to herbivores (Ogden and Lobel 1978; Hay and Fenical 1988). Several brown algae contain phlorotannins (polyphenolics) that inhibit feeding by some invertebrates and fishes (Geiselman and McConnell 1981; Steinberg 1984, 1985, 1988; Hay and Fenical 1988; Winter and Estes 1992).

Phenolic content in Phaeophyta has previously been reported as typically ranging from 0 to 7% dry wt, with occasional reports of levels reaching 15–20% dry wt (Ragan and Jensen 1977; Geiselman 1980; Anderson and Velimirov 1982; Steinberg 1985, 1986; Ragan and Glombitza 1986; Steinberg and van Altena 1992; Steinberg et al. 1995). The levels of phenolics produced by all five species of brown algae examined in this study were generally quite low, comparable to those found in phenolic-poor algae (0–2% dry wt) (Steinberg 1985, 1986; Steinberg and Paul 1990; Targett et al. 1992; Winter and Estes 1992). In strong contrast to others studies, where phenolics have consistent deterrent or inhibitory effects against gastropod and echinoid herbivores (Anderson and Velimirov 1982; Steinberg 1984, 1985, 1988; Johnson and Mann 1986; Winter and Estes 1992), phenolic levels of these algae were not negatively correlated with feeding rates of *Littorina striata* or *O. atratus*. This agrees with the data from Steinberg and van Altena (1992), who reported that no herbivore studied by them consistently preferred phenolic-poor algae over those rich in phenolics.

In accordance with chemical literature, TLC analysis showed the presence of secondary metabolites in several of the algae examined. For example, the genus *Caulerpa* produces caulerpin, caulerpenyne, and caulerpicin, among others (Paul and Fenical 1987). Domoic acid has been isolated from *A. corallinum* (Fattorusso and Piattelli 1980). Several species of *Gracilaria* produce fitols, colestenone, or the prostaglandins PGE_2 and $PGE_{2\alpha}$ (Fenical 1978; Goad 1978; Faulkner 1984). Lividine, grateloupine, and carnosadine have been isolated from several species of *Grateloupia* (Faulkner 1990). *Halopithys incurvus* produces cyclotribromoveratrylene (Faulkner 1984); several species of *Laurencia* contain brominated sesquiterpenes or other halogenated compounds (González et al. 1982; Morrison 1986; Hay et al. 1987, 1988; Caballero and Melián 1988; Granado and Caballero 1995). These secondary algal metabolites are likely to be important determinants of food habits and selectivity for the herbivores studied here.

The results of this study indicate a strong association between resistance to grazing by the herbivorous gastropods *Littorina striata* and *O. atratus* and the presence of secondary metabolites. All of the least-consumed algae (*A. corallinum*, *Caulerpa racemosa*, *Gracilaria cervicornis*, *Grateloupia doryphora*, *H. incurvus* and *Laurencia corrallopsis*) were found to produce secondary metabolites. Our results are consistent with published studies on the feeding preferences of some marine herbivores in relation to chemical defenses of algae (Paul and Hay 1986; Wylie and Paul 1988; Hay 1991; Meyer et al. 1994). These researchers also found that the presence of secondary metabolites in the seaweeds was correlated with low susceptibility to herbivory.

We did not detect secondary metabolites by TLC in extracts from *Asparagopsis armata* and *Halimeda discoidea*, two genera well known for producing secondary compounds. *Asparagopsis* spp. produce a series of very volatile halomethane components, among them $CHCl_3$, $CHBr_3$, CCl_4, and CH_3I, and a number of polyhalogenated acetones (McConnell and Fenical 1977; Fenical 1982; Faulkner 1992; Gribble 1994). *Halimeda* spp. produce diterpenoid metabolites like halimedatrial, 4,9-diacetoxyudoteal, epihalimedatrial, halimedalactone, and halimedin (Paul and Fenical 1987). Oven-drying *Asparagopsis* spp. prior to extraction procedures results in the loss of a great deal of volatile chemical constituents. On the other hand, secondary metabolites from *Halimeda* spp. are very unstable and rapidly degrade unless treated with extreme care. For these reasons, we did not detect these metabolites by TLC and it would explain why these two algae did not inhibit feeding by snails (both algal species were moderately susceptible to herbivory), although extracts of *Asparagopsis* spp. have been reported to be bioactive (Hornsey and Hide 1974) and *Halimeda* spp. are largely avoided by generalist herbivores (Ogden and Lobel 1978; Hay 1981a, b; Norris and Fenical 1982).

The results presented in this study are only correlative, and the need for critical experimental studies is obvious. Not all secondary metabolites produced by algae necessarily function as defenses against herbivores and the testing of the seaweed extracts and isolated metabolites in biologically relevant assays is needed to determine which algal metabolites effectively deter herbivores. We are currently engaged in further work on the specific compounds capable of inhibiting feeding by marine invertebrate herbivores.

Acknowledgements I. Granado thanks the Ministerio de Educación y Ciencia for a “Formación del Personal Investigador” grant. We thank Dr. Viera and E. Soler for the identification of all algae used in this study. Experiments complied with the current laws of Spain. Comments by Dr. Valiela and two anonymous reviewers improved the manuscript.

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