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Feeding preferences by *Littorina striata* King and Broderip on different algal species

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SUMMARY: The palatability of plants as food for herbivores depends on chemical composition. In this study, we tested the relative palatability of selected species of green, brown and red algae to a marine gastropod, *Littorina striata*, using the agar suspension method. When fed to snails (*Littorina striata*), the green algae (*Ulva rigida* and *Enteromorpha ramulosa*) were readily consumed. A brown alga, *Fucus spiralis*, was eaten moderately and two red algae (*Gracilaria ferox* and *Laurencia obtusa*) were avoided. When we offered the snails mixtures of the most and least preferred species, feeding by the snails stopped when the unpreferred alga, *Gracilaria ferox*, was present in greater proportion than the preferred one, *Enteromorpha ramulosa*. Whatever the nature of the specific inhibitory agent, its concentration seaweed to be a more important cue for feeding than the stimulatory one offered by the preferred algal species.

Key words: seaweed, herbivores, invertebrate, chemical defenses, feeding deterrents, palatability, gastropod.

RESUMEN: PREFERENCIAS ALIMENTARIAS DE *Littorina striata* KING Y BRODERIP SOBRE DIFERENTES ESPECIES DE ALGAS. — El consumo de un recurso vegetal por herbívoros depende de su composición química. En este estudio, examinamos el efecto de diferentes macroalgas sobre el pasto de un herbívoro marino, *Littorina striata*. Para ello empleamos el método de suspensión con agar. Una vez determinadas las preferencias alimentarias de este gasterópodo, se estudió si una especie con efecto inhibidor mezclada en diferentes proporciones con una especie normalmente consumida, disminuía el pasto. Los resultados mostraron claramente una preferencia por las algas verdes sobre las rojas, mientras que por el alga parda, *Fucus spiralis*, hubo una preferencia intermedia. Las mezclas de algas fueron rechazadas cuando el alga no preferida se encontraba en mayor proporción que la preferida, demostrando la necesidad de una cierta concentración del agente inhibidor para que sea efectivo.

Palabras clave: macroalgas, herbívoros, invertebrados, defensas químicas, inhibidor, gasterópodos.

INTRODUCTION

Terrestrial plants produce an enormous variety of chemicals that deter herbivores from feeding on them (SWAIN, 1977; HARBORNE, 1978, 1989; ROSENTHAL and JANZEN, 1979; STEINBERG, 1985); however, there is not as much information about algal chemical defenses in marine systems, but many secondary compounds that have been isolated from marine algae exhibit strong biological activities (BAKUS *et al.*, 1986; FAULKNER, 1986; HAY *et al.*, 1987; HAY and FENICAL, 1988).

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In this paper we selected several species of macroalgae that are common in the coast of Gran Canaria Island to study their relative attractiveness to herbivores. To span the major taxa we chose two greens, *Ulva rigida* and *Enteromorpha ramulosa*, one brown, *Fucus spiralis*, and two reds, *Gracilaria ferox* and *Laurencia obtusa*.

Red algae of the genus *Laurencia* are rich sources of secondary metabolites; over 250 compounds have been isolated from this genus. *Laurencia* species produce sesquiterpenoids and non-terpenoids C_{15} acetogenins among other miscellaneous compounds (ERICKSON, 1983). One of the better studied metabolites in red algae is elatol, which is a major compound in

Laurencia obtusa (MARTÍN *et al.*, 1986, 1989). This compound is cytotoxic, icthyotoxic, insecticidal and deters feeding by reef fishes (HAY *et al.*, 1987).

Relatively less is known about bioactive compounds from *Gracilaria* species. Two prostaglandins have been isolated from *Gracilaria lichenoides* (FAULKNER, 1984).

Brown algae contain many bioactive compounds. Brown algae are the only group that produce the polyphenolics that show antibiotic, antifungal, antialgal and antilarval activities (STEINBERG, 1988). *Fucus vesiculosus* produces phloroglucinol polymers that inhibit feeding by the herbivorous snail, *Littorina littorea* (GEISELMAN and MCCONNELL, 1981).

Some green algae produce compounds that stimulate feeding. SAKATA (1989) reported that 1.2-diacylglyceryl-4,-O- (N.N.N-trimethyl) homoserine, isolated from *Ulva pertusa*, is a phagostimulant of the sea hare *Aplysia juliana*. Although we do not have references about stimulant compounds in the green algal genus *Enteromorpha*, but this genus is often preferred by marine herbivores (PAUL and FENICAL, 1987).

Many authors have shown that many of secondary metabolites inhibit grazing by marine herbivores, while other researchers report stimulation feeding by grazers (HAY and FENICAL, 1988; DUFFY and HAY, 1990). Compounds that are toxic to one species may have no effect on another species of grazers (JANZEN, 1979).

In this paper we report studies on feeding by snails on algal species that have not been studied before, to see if they deter or stimulate feeding.

Two different set of experiments were devised, in the first we measured the palatability of different algal species offered to snails separately. In the second of experiments, we investigated the effect of mixtures of two algae (one preferred and another non-preferred species) on feeding by snails.

MATERIAL AND METHODS

Selected species and sites of collection

Algae were collected by hand at low tide from various sites along the north-western coast of Gran Canaria. The five selected species we used in the feeding experiments, represent three divisions of seaweeds. *Ulva rigida* Linneaus (Chlorophyta) is found in tidal pools and on rocks of the intertidal and sometimes in the sublitoral. *Enteromorpha ramulosa* (Smith) Hooker (Chlorophyta) is a common species in pools of the upper intertidal. *Fucus spiralis* Linneaus (Phaeophyta) often grows in small clusters in the high intertidal on sheltered rocky shores. *Laurencia obtusa* (Hudson) Lamouroux (Rhodophyta) grows in the lower intertidal on exposure shores. *Gracilaria ferox* J. Agardh (Rhodophyta) is commonly found in pools of the mid and lower intertidal.

The algae were transported to the laboratory in a cooler where, after epiphytes and animals were removed from their surfaces, the fronds were rinsed with distilled water to remove sediment and salts, and oven-dried at 60 °C for 48 hours (or to constant weight). Dried plant material was ground through a Wiley mill to get a particle size of 0.5 mm and stored in a desiccator until the start of the trials.

The herbivore used in the feeding experiments was *Littorina striata* King and Broderip. This is one of the more common species of intertidal gastropods in Gran Canaria. All snails in the experiments were collected at San Cristóbal beach, on the northeastern coast of Gran Canaria. Only snails between 10 and 15 mm maximum shell diameter were used. In the laboratory, they were kept in covered glass containers that were ventilated through screened tops. The animals were starved for 24 hours before use.

Feeding tests on macroalgal species offered separately

To assess the relative palatability 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 thoughness or frond architecture on grazers, and emphasizes the effect of chemical composition.

Petri dishes containing an agar suspension of the ground particles were prepared separately for each species. We suspended the ground algae in 1.8 ‰ agar-seawater. Previously, the seawater had been filtered through 0.45 um 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 four snails which were allowed to graze for 25 min in the dark. Each of these tests was replicated at least eight times.

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). This agar suspension method of testing palatability is an adaptation of that devised by GEISELMAN (1980).

Feeding on mixed of algal species

The first set of experiments showed that *Enteromorpha ramulosa* was readily accepted as food while *Laurencia obtusa* was not. To test what may happen when both food types are present together, we ran an experiment in which we mixed the preferred and rejected species in different proportion, and meaured feeding by the snails on the mixtures. We chose *E. ramulosa* and *G. ferox*.

The two selected algal species were mixed in different proportions that range from 0 % *Enteromorpha* (100 % *Gracilaria*) to 100 % *Enteromorpha* (0 % *Gracilaria*) by weight, we increased the proportion of the green alga and decreased the red alga, in such a way that the total amount of mixture in the agar suspension was the same in all plates. Feeding tests using *L. striata* were then carried out using the same protocol as above.

RESULTS

Contrast among species

The results of the laboratory experiments show that *Littorina striata* exhibit clear feeding preferences among different species of algae (Fig. 1). Analysis of the data from this experiment using a one-way analysis of variance shows that these values are significantly different (Table 1).

The snails fed primarily on the two green seaweeds, therefore, we can say that there is stimulation of feeding by *Enteromorpha ramulosa* and *Ulva rigida*. The brown alga, *Fucus spiralis* was eaten to a lesser extent, we observed an intermediate preference between *E. ramulosa*, which was eaten the most, and *L. obtusa*, the least one. So, unlike Geiselman's results, *Fucus* does not inhibit feeding by *L. striata*. Finally, media prepared with *Gracilaria ferox* and *Laurencia obtusa* were avoided completely. This two red algae have an evident inhibitory effect.

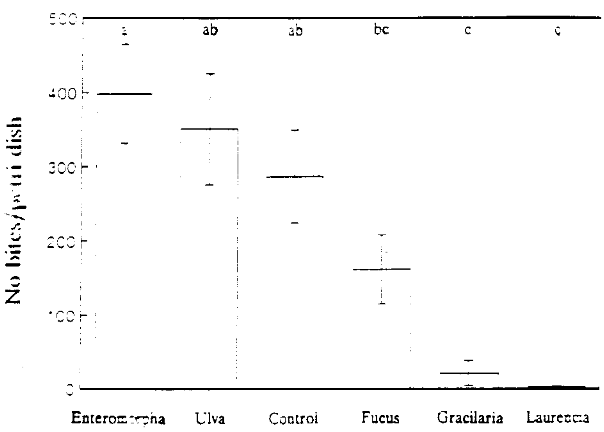

FIG. 1. — Food preferences expressed as number of bites left on the surface of the agar suspension after 25 min of grazing. The vertical lines show standard errors. Means with the same letter do not differ significantly (Student-Newman-Keuls test, P > 0.05).

TABLE 1. — ANOVA table for the effect of different algal species on feeding by *Littorina striata*. df = degrees of freedom. MS = Mean square.

Source of variation	*df*	*MS*	F_s
among treatment (among species)	5	28529.14	8.01***
within treatment (among replicates)	51	28838.73	
total	56		

*** = P ≤ 0.001

Individual means were compared by Student-Newman Keuls procedure (SOKAL and ROLHF, 1981), we got three different groups, overlaped. Means with the same letter (Fig. 1) do not differ significantly (P > 0.05), (a) the two green algae and the control, (b) *Ulva*, control and *Fucus*, and (c) *Fucus* and the two red algae.

Effects of mixtures

This test was repeated in three different days and replicated twice each time. Analysis of the results using a nested ANOVA shows that there is no statistically significant difference among days, but the treatments within days are significantly different (Table 2) (SOKAL and ROLHF, 1981). Therefore, the data were

TABLE 2. — Nested analysis of variance for the effects of time and foods on grazing by snails. df = degrees of freedom. MS = Mean square.

Source of variation	*df*	*MS*	F_s
among groups (among days)	2	34382.17	1.77 ns
among subgroups within groups (among foods within days)	18	19396.56	7.46***
within groups (among replicates)	21	2601.05	
total	41		

ns = not significant. *** = P ≤ 0.001

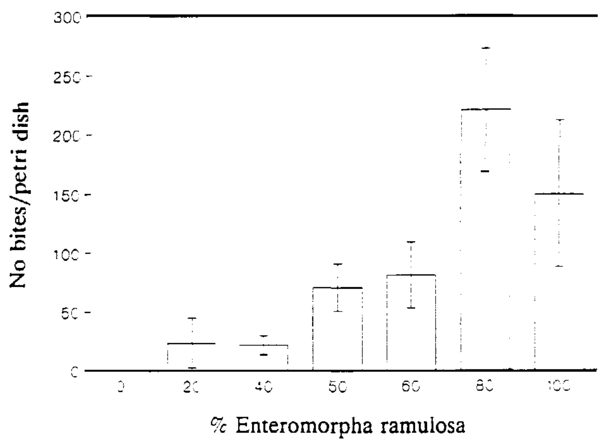

FIG. 2. — Feeding response depending on the relative percentages of *Enteromorpha ramulosa* (% E) and *Gracilaria ferox*, expressed as number of bites left on the surface of the agar suspension after 25 min of grazing.

pooled as if there were six replicates (Fig. 2). Media prepared with more than about 50 % of *Enteromorpha* were preferred over those with less green alga. This corroborates the results of figure 1, as percentage of *E. ramulosa* increases, there is more feeding and, on the contrary, as percentage of *Gracilaria* increases, grazing is inhibited.

DISCUSSION

The two green algae, *E. ramulosa* and *U. rigida*, appeared to stimulate feeding as found GEISELMAN (1980) and SAKATA (1989). *Littorina striata* inhabits the upper intertidal, where these genera are available, but its abundance is variable. Where the snails are not abundant, the ephemeral, fast growing species will proliferate (LUBCHENCO, 1978).

In contrast to other studies of the genus, (GEISELMAN, 1980; GEISELMAN and MCCONNELL, 1981; VAN ALSTYNE, 1988) *Fucus spiralis* did not inhibit feeding by *L. striata*. *Fucus* is thought to examplify deterrent algae because of their high phenolic content. The difference in the results may reflect differences of *Fucus* species. Other authors studied *Fucus vesiculosus*, while we used *F. spiralis*. These two species could have different secondary metabolites. *Fucus spiralis* does not show antimicrobial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* or *Candida albicans* (CABALLERO and MELIAN, 1988). It may also be that there are phytogeographical gradients in chemical defenses. STEINBERG (1986, 1989) reports that tropical species of Fucales have mean levels of phenolics comparable to those found in macroalgae that are preferred foods. The Canary Islands are in a subtropical region, so perhaps *Fucus spiralis*, for whatever reason, lacks the ability to inhibit feeding by *Littorina*.

The red algae tested in this study deterred grazing by snails. Many Rhodophyta produce halogenated terpenoids and acetogenins (FENICAL, 1975, 1982; ERICKSON, 1983), compounds that have antimicrobial activity, cytotoxicity and deter feeding by fish. Few papers have reported effects of red seaweeds on feeding by invertebrate herbivores like gastropods (OHTA, 1978; GEISELMAN, 1980), but *Laurencia obtusa* has obtusol, isoobtusol, and elatol as major metabolites (MARTIN *et al.*, 1986, 1989) and *Gracilaria* produces prostaglandins. These could be the active compounds.

The effects of secondary metabolites on herbivorous gastropods is very much concentration-dependent (GEISELMAN and MCCONNELL, 1981; HAY, 1984; STEINBERG, 1985, 1986). Inhibitory or stimulatory effects depended on the quantity of algae present when both a stimulatory and an inhibitory materials were mixed, the feeding response varied in proportion to the relative amount of the alternative food. The presence or absence of a biologically active compound is not a suitable measure of their potential effects on herbivores (STEINBERG, 1986). Moreover, deterrent compounds seem to be able to overwhelm the effects of attractants.

Further work on the specific compounds capable of stimulating or inhibiting feeding by marine invertebrate herbivores should be done since the behavior is probably cued by specific compounds. It would also be useful to study the phenolic content of *Fucus spiralis* and compare it to other *Fucus* species, as well as examine the quantitative interactions between attractant and inhibitory compounds in mixed diets.

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