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FEATURE ARTICLE

Seaweed–herbivore interactions at a small scale: direct tests of feeding deterrence by filamentous algae

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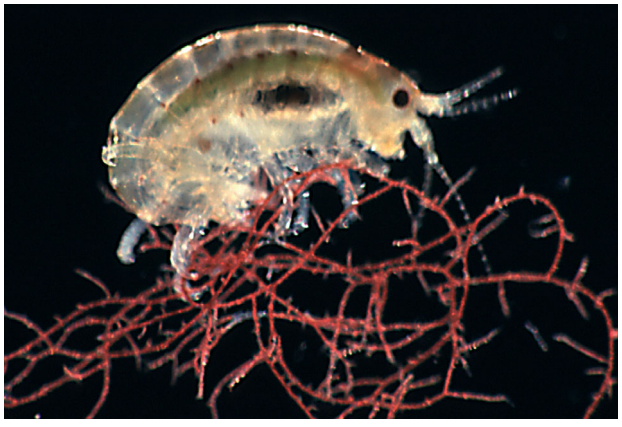
ABSTRACT: High growth rates and temporal or spatial opportunism are considered central to the success of filamentous algae, in particular for escaping or minimising the effects of herbivory. However, the role of chemical defences in filamentous algae has received far less attention. We investigated possible chemical feeding deterrence by filamentous red algae that have conspicuous cellular inclusions (*Asparagopsis armata*, *Anotrichium tenue* and *Balliella amphiglanda*) and 2 others without inclusions (*Callithamnion korfense* and *Ulva* sp.). The 3 algae with cellular inclusions were consumed at lower rates by a generalist amphipod, *Hyale nigra*, than the other 2 algae. To determine the potential role of chemical defences for *A. armata*, we conducted tests against herbivores using algae in which the production of halogenated metabolites was manipulated. This manipulation had no effect on carbon and nitrogen values of the algae, and allowed us to directly test the role of algal secondary metabolites in defence against herbivores without using artificial diets. Bromide (+) algae (with halogenated metabolites) deterred grazing by 2 mesograzers (*Hyale nigra* and juvenile abalone *Haliotis rubra*), which consumed up to 4 times more bromide (–) (metabolite-free) algae than bromide (+) algae. Juveniles of the sea hare *Aplysia parvula* were not deterred by the chemical defences in bromide (+) *A. armata*. In field assays, artificial diets containing a crude extract of *A. armata* were also active against herbivores. Although functional form models typically predict that tolerance — not resistance — should be the key defensive strategy for marine algae with simple architecture, this study demonstrates that resistance traits may also be important and more broadly utilised in filamentous species.

KEY WORDS: Functional form · Mesograzer · Tolerance · Resistance · Secondary metabolite · *Asparagopsis*



Some species of filamentous algae employ strategies against grazing, such as chemical defence, that are most often ascribed to larger macroalgae. Paul et al. report that small herbivores (mesograzers) such as amphipods can differentiate between chemically defended filamentous algae and those that are not (photo: amphipod *Hyale nigra* on the alga *Asparagopsis armata*).

Photo: Nicholas Paul

INTRODUCTION

Functional form or life form models have been used to provide an explanatory framework for the distribution and success of plants and algae with differing growth strategies (Feeny 1976, Littler & Littler 1980, Steneck & Dethier 1994, Haukioja & Koricheva 2000). In terrestrial systems, comparisons have often been drawn between woody and herbaceous plants, with large, long-lived and apparent trees contrasting to smaller, fast-growing herbs with short, opportunistic life histories (Feeny 1976, Haukioja & Koricheva 2000). Life form models have also been important in the

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development of theory for plant–herbivore interactions (Feeny 1976). For example, tolerance of herbivory is typically associated with fast-growing plants, such as herbs, due to their greater capacity for compensatory growth (Strauss & Agrawal 1999), although chemical defences are still commonly utilised by both herbaceous and woody plants for protection against herbivores (Feeny 1976, Fritz & Simms 1992).

In the marine environment, macroalgae differ substantially in size and architecture, with life forms analogous to trees (kelps) and herbs (filaments). Most kelps are large and notably long-lived yet co-exist with small and often ephemeral filamentous algae that grow at the scale of centimetres and less. These filamentous algae are often a highly preferred food in the marine environment (Littler & Littler 1980, Steneck & Dethier 1994) and are important contributors to primary production in benthic communities (Klumpp & McKinnon 1992). Generally, filamentous algae are thought to reduce the impacts of herbivory by escaping consumers in space, time or through high growth rates (Lubchenco & Gaines 1981). Such traits are similar to those of many herbaceous plants in terrestrial systems.

However, despite herbivore deterrents being common in both woody and herbaceous terrestrial plants (Fritz & Simms 1992), most studies on chemical defence in marine algae have been on relatively large, robust species, in particular kelps or fucoids (Steinberg 1984, Van Alstyne et al. 2001, Amsler & Fairhead 2006) and other foliose or fleshy algae (Paul & Hay 1986, Hay et al. 1987, 1988, Ginsburg & Paul 2001, Cruz-Rivera & Hay 2003, Wright et al. 2004). These macroalgae produce a broad range of chemical defences (Hay & Fenical 1988, Paul & Puglisi 2004, Amsler & Fairhead 2006). Perhaps because of the assumed high susceptibility of filamentous algae to herbivores, examples of chemical defence in filamentous forms are rare and predominantly for cyanobacteria (e.g. Paul & Pennings 1991, Pennings et al. 1997, but see Paul et al. 1990). Even some species of unicellular algae have demonstrated chemical defences (Wichard et al. 2005). However, there is recent evidence that filamentous eukaryote algae can produce secondary metabolites that inhibit bacterial fouling (Nylund et al. 2005, Paul et al. 2006). Further evidence that chemical defence in filamentous algae, including herbivore deterrence, is more widespread than previously believed is that many species have specialised cellular inclusions (Young & West 1979, Paul et al. 2006). Cellular inclusions are typically required for the safe storage of bioactive metabolites and are often conspicuous structures, as a result of the refractile properties of the stored contents (Young & West 1979, Paul et al. 2006).

While a variety of types and sizes of herbivores have been considered in feeding deterrence by macroalgae — from large (e.g. fishes and urchins; Paul & Hay 1986, Hay et al. 1994, Van Alstyne et al. 2001) to small (e.g. mesograzers; Hay et al. 1987, Brawley 1992, Cruz-Rivera & Hay 2003, Amsler et al. 2005) —, the same consideration has not necessarily been given to algal size. Unlike large herbivores that can remove a filamentous individual in a single bite, small herbivores make feeding choices at a fine scale and can be present at high densities (Brawley & Adey 1981, Duffy & Hay 2000). Some mesograzers preferentially graze filamentous algae (Brawley & Adey 1981) and consequently may exert a strong selective pressure on filamentous algal communities. If herbivore deterrence is important for some filamentous algae but not others, then the scale of a herbivore to use in an assay of chemical defences should also be small, comparable to the size of the filamentous individual.

In this study, we identified potential chemical defences against small herbivores in filamentous algae and tested defence explicitly using a novel feeding assay. First, we compared the consumption rates of 5 filamentous algae, 3 with cellular inclusions (that may be associated with chemical defences) and 2 without, using a generalist amphipod *Hyale nigra*. We then quantified herbivore deterrence by one of these algae, *Asparagopsis armata*, as its halogenated secondary metabolites can be manipulated by omitting bromine from the culture medium (Paul et al. 2006). This allowed us to test the role of algal natural products in feeding deterrence whilst maintaining the subtleties of the filamentous morphology, which is particularly important given the predicted high susceptibility of filaments to herbivores (Littler & Littler 1980, Steneck & Dethier 1994). The feeding responses of 3 mesograzers (an amphipod, an abalone and a sea hare) to manipulated (bromide (–)) and unmanipulated (bromide (+)) *A. armata* were then measured using both choice and no-choice assays. We also determine the effects of crude extracts of *A. armata* in artificial diets against herbivores in the field.

MATERIALS AND METHODS

Consumption of filamentous algae. We collected 3 filamentous red algae that form specialised cellular inclusions. These were *Anotrichium tenue* Nägeli (Ceramiaceae), the tetrasporophyte of *Asparagopsis armata* Harvey (Bonnemaisoniaceae) and *Balliella amphiglanda* Huisman & Kraft (Ceramiaceae) (Fig. 1). We also collected the filamentous red alga *Callithamnion korfense* Millar (Ceramiaceae) and green alga *Ulva* sp. (filamentous form previously of the genus *Enteromorpha*; Ulvaceae), neither of which had obvious storage structures or cellular inclusions. Ulvaceae

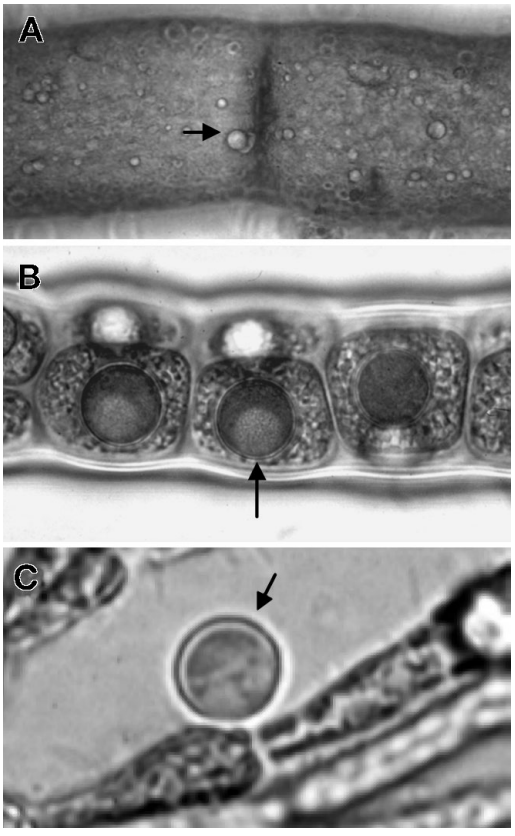

Fig. 1. Unstained living thalli of 3 filamentous red algae showing refractile cellular inclusions (arrows). (A) *Anotrichium tenue*, (B) *Asparagopsis armata*, (C) *Balliella amphiglanda*

have typically been used as control diets in assays of chemical defence (Paul & Hay 1986), even though some algae in this group have activated chemical defences (Van Alstyne & Houser 2003). Algae were collected from Bare Island (33° 59′ 38″ S, 151° 14′ 00″ E), Sydney, Australia.

The consumption rates of the 5 filamentous algae were measured in a no-choice feeding assay using the generalist amphipod *Hyale nigra*. Consumption was determined by changes to area because variations in mass were too small to be quantified. Area consumed was quantified from 'before and after' digital images. Imaging was done by spreading the algae across a slide and flattening it under a glass coverslip, taking care not to damage the algae. Digital images were made using a stereomicroscope at low magnification. Image analysis was performed with Image J (Scion, www.scioncorp.com) public domain software, which determines the projected area of each individual. Treatment (n = 10, with herbivores) and control algae (n = 10, without herbivore) were run for each species for 36 h (over 2 nights) in 30 ml static seawater at 19°C. One amphipod was added to each treatment dish.

Collection and culturing of *Asparagopsis armata*. The red alga *A. armata* was selected to further investigate feeding deterrence by a filamentous alga as it is possible to manipulate the presence of the halogenated metabolites in cultured algae. Culturing *A. armata* in artificial media containing bromine produced algae with halogenated metabolites, bromide (+) algae, and culturing in the absence of bromine produced algae without halogenated metabolites, bromide (–) algae (Paul et al. 2006). This provided a unique opportunity to test of the role of the halogenated metabolites from *A. armata* in defence against herbivores.

Asparagopsis armata was collected from the shallow subtidal zone (to 2 m depth) at Bare Island. Clean, apical sections were cultured in sterile seawater for approximately 4 weeks, after which time those individuals that grew well were selected for subsequent culturing. Algae were chopped finely with a scalpel (to limit the residual amount of metabolites) and then cultured in one of 2 artificial media, identical except for the presence or absence of bromide ions at natural concentrations (details in Paul et al. 2006). Algae were cultured in 500 ml of media in glass dishes under 20 µmol photons m^{-2} s^{-1} (36 W daylight globes) with a 16:8 h light:dark cycle at 19°C. The culture medium was changed every 2 to 3 weeks. Algae were grown until they were of sufficient size for use in feeding assays (~5 mg fresh weight [FW]). Only individuals that grew well in the artificial culture media and were of normal morphology were harvested and subcultured. This ensured that algae used for the feeding assays had similar structure.

Effects of bromine manipulation on *Asparagopsis armata*. Removing bromine from the artificial growth medium prevents the production of the halogenated secondary metabolites in *A. armata* (Paul et al. 2006). The levels of the major halogenated metabolites in bromide (+) algae are 0.3 to 0.5% dry weight for bromoform and 0.07 to 0.1% dry weight for dibromoacetic acid (Paul et al. 2006). These values are towards the lower end of the natural range of concentrations present in the field. No halogenated metabolites (either brominated or chlorinated) are detectable when *A. armata* is cultured without bromine and the production of non-halogenated metabolites is not affected by this manipulation (Paul et al. 2006).

To quantify the effect of bromine removal on components of algal biology other than secondary metabolites, the carbon and nitrogen composition were measured in bromide (+) and bromide (–) algae. Samples were taken from 7 individuals cultured in bromide (+) and bromide (–) media. As single filaments were typically small, numerous filaments were pooled to give ~5 mg dry weight [DW] after freeze-drying for each replicate individual (n = 7). Carbon and nitrogen con-

tent was analysed by the Research School of Biological Sciences, Australian National University, Canberra, Australia. Percent carbon and nitrogen dry masses were measured for both bromide (+) and bromide (–) algae. Comparisons of the carbon and nitrogen content of bromide (+) and bromide (–) plants were made using paired sample *t*-tests, with individual plants paired for each growth medium.

Herbivores. Three herbivores, the amphipod *Hyale nigra* Haswell, the sea hare *Aplysia parvula* Mörch and juvenile black-lip abalone *Haliotis rubra* Leach, were selected to examine feeding deterrence in *A. armata*. *H. nigra* is not common on *A. armata* (N. Paul pers. obs.), and in order to obtain sufficient numbers for feeding assays, animals were collected from *Sargassum linearifolium*, a large brown macroalga at Bare Island. Adult abalone *Haliotis rubra* readily consume the gametophyte of *A. armata* in the field (Shepherd & Steinberg 1992), but the preference of juvenile abalones is not known. Juvenile *H. rubra* were provided by NSW Fisheries, Port Stephens, New South Wales, where they had been cultured until they were capable of feeding on macroalgae (~1 to 3 mo old, 3 to 10 mm in length). The sea hare *Aplysia parvula* is regularly found on chemically defended algae (Rogers et al. 2002), including the gametophyte of *A. armata*, at Bare Island. *A. parvula* (0.1 to 0.9 g FW) were collected from algae on which they aggregate, predominantly from *Asparagopsis armata*, *Delisea pulchra* and *Sargassum linearifolium*. All herbivores were maintained at constant temperature (19°C) and were fed on a diet of *Ulva* sp. for at least 24 h prior to assays, in order to reduce potential biases from recent feeding.

Feeding assays using whole plants. To test if the halogenated metabolites in bromide (+) *Asparagopsis armata* function as herbivore deterrents, we ran feeding experiments with each herbivore in the laboratory. Each assay used a single herbivore in 30 ml of static seawater at 19°C.

Prior to running the feeding assays in natural seawater, we assessed whether new (apical) growth of *Asparagopsis armata* resumed production of halogenated metabolites over the duration of the assays. *A. armata* stores the halogenated metabolites as a refractile inclusion in its gland cells (Paul et al. 2006). This inclusion can be easily viewed with a compound microscope. After 12 h of immersion in seawater, no gland cells were obvious in the apical sections of the bromide (–) filaments. After 36 h, some small gland cells had formed between 0 and 4 cell tiers beneath the apical cell, indicating the synthesis of halogenated metabolites in the new growth. All assays were run for a maximum of 12 h (overnight). If a herbivore consumed a substantial (i.e. visible) portion of the diet, it was removed from the dish prior to 12 h.

We first ran a no-choice feeding assay with *Hyale nigra* using manipulated *Asparagopsis armata* bromide (+) and bromide (–) algae, as well as a third growth medium treatment, seawater, as a control. This allowed for a direct comparison between feeding on algae cultured in bromide (+) and those cultured in natural seawater. Feeding (or loss of area) was measured for each media treatment (n = 10) over 12 h using digital images (see above for details of imaging). Autogenic controls (n = 10) were incorporated into the analysis to adjust for any changes in algal size that did not result from herbivory.

For choice assays with the amphipod, abalone and sea hare, the identity of bromide (+) or bromide (–) algal filaments after feeding was determined by the presence or absence of refractile inclusions using a compound microscope. This ensured that all pieces scattered during feeding were pooled with the correct tissue type for image analysis. Autogenic controls (n = 7 to 10) were also run for each assay.

Feeding assay using artificial diets. Our attempts to test herbivore deterrence against consumers in the field with the manipulated *Asparagopsis armata* were not successful, as cultured algae could not be fastened effectively to a substratum. So in order to test whether the natural products of *A. armata* are active against consumers in the field, we also performed a feeding assay with artificial diets.

Extracts of *Asparagopsis armata* were incorporated into artificial diets at their natural concentration per unit dry weight. Crude extract was obtained by extracting 9.1 g of freeze-dried material exhaustively in dichloromethane (DCM). This yielded 44.2 mg of extract. This extract was incorporated into a diet comprised of freeze-dried *Ulva* sp., agar and ultrapure water in a mass ratio of 1:3:60, respectively. The extract was dissolved in di-ethyl-ether (DEE) and added to finely ground *Ulva* to give a dry weight equivalent to the natural level (i.e. 44.2 mg extract per 9.1 g diet). Agar was added to 115 ml water and heated until boiling in a microwave. The dried *Ulva* was made into a slurry with the addition of 22 ml of water, to which the agar was added after cooling to ~50°C. Control discs were produced in the same way but without the addition of extract to the DEE.

Diets (discs ~5 g) were paired (treatment and control) and secured to coralline turf in areas where *A. armata* was present at Bare Island; 20 replicate pairs were deployed (but see 'Results' for replication in the analysis). Prior to deployment, discs were weighed in the laboratory, along with discs to be used in pairs of autogenic controls (n = 9). Each of the pre-weighed paired discs was attached to a clip using a small piece of twine. As the discs were suspended slightly above the turf, it was likely that fish were the major consumers. Common herbivorous fishes in the area include the damselfish

species *Parma microlepis* and *P. unifasciata*, although these were not quantified. Autogenic controls were kept in seawater collected from the study site for the same length of time as experimental discs. These autogenic controls were not ideal (as they were not caged in the field site), but do provide values for the relative change in treatment versus control discs. This assay was run overnight for approximately 16 h, after which both experimental and autogenic control discs were blot-dried and final weights obtained.

Data analysis. As some autogenic controls increased in size during the course of the feeding assays, the figures for each assay display consumption as a change in area over time calculated using a correction coefficient for initial size. For each experimental replicate (plus herbivore) this was calculated by the equation:

$$\text{Consumption} = \{[\text{Area}_{\text{initial}} \times (\text{Mean of the Autogenic Control Area}_{\text{final}} / \text{Autogenic Control Area}_{\text{intial}})] - \text{Area}_{\text{final}}\}/\text{time (h)}$$

However, the actual analyses (outlined below) incorporate this variance in before and after measurements of autogenic controls (those minus herbivores). In all feeding assays with the small herbivores, the consumption rates were generally low relative to changes in autogenic controls.

Feeding assays were either analysed by 2-factor ANOVA (no-choice assays) or *t*-tests (choice assays), as per Peterson & Renaud (1989). For no-choice assays with *Hyale nigra*, consumption ($mm^2\ h^{-1}$) was expressed as a mean rate for each treatment alga. A significant interaction between herbivore (presence/absence) and treatment algae indicated an effect of algal treatment on feeding. If an interaction was detected, then Tukey's HSD multiple comparison was used to compare the mean feeding rates on treatment algae after raw data were adjusted using the correction coefficient (see previous paragraph). For choice assays (*Hyale nigra*, *Haliotis rubra*, *Aplysia parvula*), significant preferences for either bromide (+) or bromide (–) algae were determined by *t*-tests (Peterson & Renaud 1989). The *t*-statistic is calculated from the difference between the mean difference in area consumed for paired treatment replicates (bromide (+)/(–) with herbivores) and the mean difference in area for paired autogenic controls (bromide (+)/(–) without herbivores). As it was difficult to visually assess whether small animals had fed sufficiently, we set a minimum value for consumption of at least 1 SD of the mean change in area of the corresponding autogenic controls. This ensured that only those paired replicates in which feeding had definitely occurred were analysed.

All analyses were run using SYSTAT 10 (SPSS). Assumptions of homogeneity of variance and normality were assessed by examining the scatterplots and distribution of residuals (Quinn & Keough 2002). If variances were heterogenous for choice assays, then a Mann-Whitney *U*-test was employed.

RESULTS

Differential feeding on filamentous algae

There were significant differences in consumption rates of the different filamentous species by the amphipod *Hyale nigra* (2-factor ANOVA: Alga × Herbivore, $F_{4,90} = 4.20$, $p < 0.001$) (Fig. 2). Consumption of the 3 algae with specialised storage structures, *Anotrichium tenue* (small refractile bodies, Fig. 1A) *Asparagopsis armata* (gland cells, Fig. 1B) and *Balliella amphiglanda* (vesicle cells, Fig. 1C) was less than consumption of the 2 algae without specialised structures, *Callithamnion korfense* and *Ulva* sp. (Tukey's HSD multiple comparison, $p < 0.05$) (Fig. 2). Although *H. nigra* is a small herbivore, the algae on which it fed are correspondingly small (Fig. 2, inset).

Effects of artificial media on *Asparagopsis armata*

Growing *Asparagopsis armata* in media with and without bromine produced algae with and without halogenated compounds, respectively. The absence of the characteristic refractile inclusion in the gland cells of *A. armata* in bromide (–) cultured algae indicated the

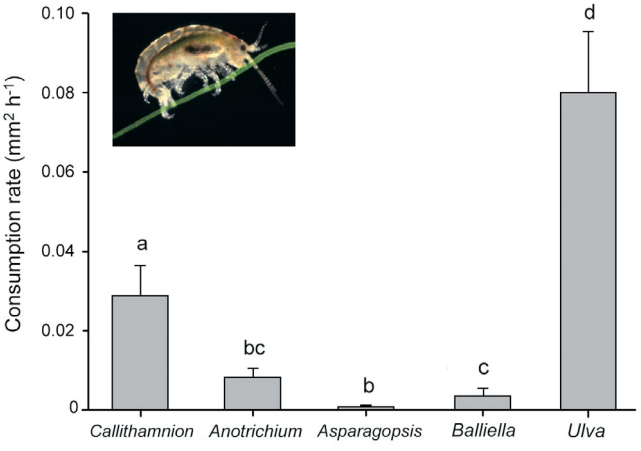

Fig. 2. *Hyale nigra*. No-choice feeding assays with 5 filamentous algae. Mean (±SE) consumption rates (n = 10) after corrections had been made for changes to autogenic controls (n = 10). Algae having conspicuous cellular inclusions (as determined by light microscopy, see Fig. 1) were consumed at lower rates than those without (ANOVA; $F_{4,90} = 4.20$, $p < 0.001$). Bars sharing the same letter do not differ significantly at $p = 0.05$ (Tukey's HSD). Inset: *H. nigra* is a generalist amphipod, seen here feeding on *Ulva* sp.

cessation of halogenated metabolite production (see Paul et al. 2006 for more details). There were no significant differences between bromide (–) and bromide (+) algae in percent carbon DW (paired *t*-test, p = 0.65; mean ± SE: Br(–) 32% ± 1, Br(+) 31.5% ± 1), percent nitrogen DW (paired *t*-test, p = 0.32; mean ±SE: Br(–) 2.75% ± 0.15, Br(+) 2.9% ± 0.1) or C:N ratio (paired *t*-test, p = 0.26; mean ±SE: Br(–) 11.8% ± 0.6, Br(+) 11.2% ± 0.4).

Table 1. *Hyale nigra*, *Haliotis rubra* and *Aplysia parvula.* Whole-plant feeding assay with the alga *Asparagopsis armata.* Pairwise-choice feeding assay showing consumption rates (mean ± SE) of bromide (–) and bromide (+) algae (i.e. manipulated algae either without or with halogenated metabolites, cf. Fig. 3). *Significant difference at p < 0.05 (*t*-test). ns: not significant (p = 0.05; *t*-test)

Species	n	Consumption rate ($mm^2\ h^{-1}$) Br (–)	Br (+)
Amphipod *Hyale nigra*	9	0.03 ± 0.006*	0.007 ± 0.0027
Abalone *Haliotis rubra*	16	0.19 ± 0.04*	0.06 ± 0.01
Sea hare *Aplysia parvula*	17	0.30 ± 0.12ns	0.25 ± 0.10

Whole-plant feeding assays

The amphipod *Hyale nigra* consumed bromide (–) algae at a significantly higher rate than both bromide (+) and seawater-cultured algae in the no-choice experiment (2-factor ANOVA: Media × Herbivore, $F_{2,102} = 9.25$, p < 0.001) (Fig. 3). Importantly, no difference in consumption was observed between algae grown in bromide (+) medium and those grown in the seawater control (Tukey's HSD multiple comparison), (Fig. 3).

The manipulation of the halogenated metabolites in *Asparagopsis armata* had a strong effect on feeding choice by 2 of the 3 herbivores. The amphipod *Hyale nigra* strongly preferred bromide (–) algae over bromide (+) algae in the pairwise-choice experiment (*t*-test, p = 0.003, $n_{experimental}$ [$n_{exp.}$] = 9, $n_{autogenic\ control}$ [$n_{auto.}$] = 9, Table 1). The abalone *Haliotis rubra* also preferred bromide (–) algae over bromide (+) algae (*t*-test, p = 0.036, $n_{exp.}$ = 16, $n_{auto.}$ = 10) (Table 1). The sea hare *Aplysia parvula* showed no preference for either alga (*t*-test, p = 0.812, $n_{exp.}$ = 17, $n_{auto.}$ = 7) (Table 1).

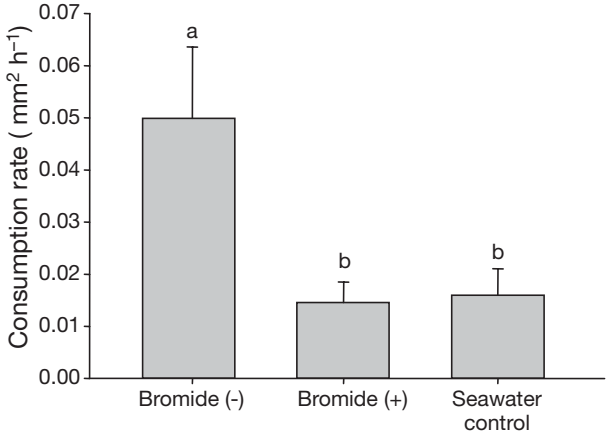

Fig. 3. *Hyale nigra*. Whole-plant feeding assays with the alga *Asparagopsis armata*. No-choice feeding assay showing the differences (ANOVA; $F_{2,102} = 9.25$, p < 0.001) in consumption rate (mean ± SE, n = 24–26) on bromide (–) *A. armata* (grown in artificial medium made with sterile water, no bromide added) and bromide (+) *A. armata* (grown in artificial medium made with sterile water, bromide added), compared to a seawater control (grown in sterile seawater). Algae grown on bromide (+) and seawater control media contain halogenated secondary metabolites. Bars sharing the same letter do not differ at p = 0.05 (Tukey's HSD)

Field assay with artificial diets

When DCM extracts of *Asparagopsis armata* were incorporated into agar-based artificial diets, those diets that contained extracts deterred feeding more than diets without extract in the field (Mann-Whitney *U*-test; p = 0.013, $n_{exp.}$ = 7, $n_{auto.}$ = 9) (Fig. 4).

DISCUSSION

Filamentous algae in marine systems are small and typically have high production rates (Klumpp & McKinnon 1992). They are considered to be highly susceptible to herbivores (Littler & Littler 1980, Steneck & Dethier 1994) and thus to occupy spatial and temporal

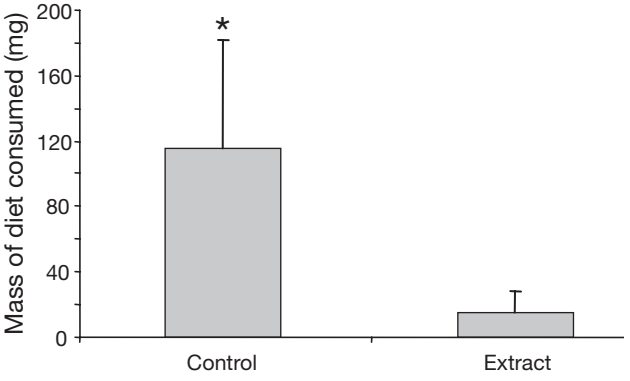

Fig. 4. Effect of crude extract from alga *Asparagopsis armata* on feeding in field experiments. Mean (±SE) total consumption of artificial diets, treatment (with extract) and control (no extract), are shown for the pairwise-choice (n = 7). *Significant at p < 0.05 (Mann-Whitney *U*-test)

niches in environments with low to moderate herbivory (Lubchenco & Gaines 1981). However, numerous exceptions to the functional form model (Littler & Littler 1980) have been discussed (Padilla & Allen 2000). Here we propose another, that the different rates of herbivore consumption among the 5 filamentous algae examined may relate to chemical defences, as indicated by the presence of specialised cellular structures in those algae that were least consumed. For one of these algae, the filamentous red alga *Asparagopsis armata*, we have demonstrated that its halogenated natural products deterred feeding by 2 relevant herbivores. This combination of chemical defence (resistance) and the high growth rates characteristic of filamentous forms (tolerance) may be an important strategy for some filamentous algae.

Filamentous turfing communities of benthic reefs are comprised of many different species. We have shown that some filamentous algae were consumed at lower rates than others by the amphipod *Hyale nigra*. A unifying feature of those algae that were least consumed was the presence of conspicuous cellular inclusions in *Asparagopsis armata*, *Balliella amphiglanda* and *Anotrichium tenue*. Although structural and nutritional differences may have influenced the outcome of this experiment, the storage of chemical defences in these specialised structures provides an alternative explanation. *A. armata* produces numerous bioactive halogenated metabolites that are stored in refractile inclusions (McConnell & Fenical 1977, Paul et al. 2006). While there have been no reports on the natural product chemistry of either *B. amphiglanda* or *A. tenue* (Marinlit 2005), the widespread occurrence of similar storage structures, in particular in the red algae of the Ceramiaceae (Young & West 1979), suggests that chemical defence may be more common in filamentous algae than predicted by functional form models (Littler & Littler 1980, Steneck & Dethier 1994).

The filamentous form represents a highly successful growth strategy, present in all 3 macroalgal divisions. However, large macroalgae have been the traditional focus for marine chemical ecology (Steinberg 1984, Hay & Fenical 1988, Paul & Puglisi 2004, Amsler & Fairhead 2006). Ecological tests of chemical defence are for the most part absent for filamentous algae (but see Paul et al. 1990 for an example of chemical defence by a filamentous green alga). More is known of the chemical defence of cyanobacteria (Paul & Pennings 1991, Pennings et al. 1997) and of diatoms (Wichard et al. 2005) than of filamentous eukaryote algae. This lack of documentation of defensive chemicals in filamentous algae is perhaps a combined product of the assumed dependence by these algae on tolerance strategies (Littler & Littler 1980, Lubchenco & Gaines 1981, Paine 1990), the difficulty in collecting sufficient biomass to test chemical defence, and, if doing so, the difficulty in maintaining any interactive effects of defence and the filamentous form.

The technique of manipulating halogenated metabolites in *Asparagopsis armata* enabled us to separate the specific roles of these metabolites from potential effects of other aspects of algal biology on herbivore response. Manipulating bromine in the culture media had no effect on the carbon and nitrogen values of the algae and the halogenated metabolites remained localised *in situ*, as they were not extracted. This meant that potential complications resulting from differences in metabolite concentration or nutrition between test algae and artificial diets were avoided (Duffy & Paul 1992, Cruz-Rivera & Hay 2003), any synergistic or additive effects of the metabolites were preserved (Hay et al. 1994), and the structural integrity of the filamentous form was maintained (Littler & Littler 1980, Steneck & Dethier 1994). Furthermore, the consumption rates of bromide (+) algae and control algae cultured in natural seawater did not differ in the no-choice feeding assay with the amphipod *Hyale nigra*, indicating that the deterrent effect of the halogenated natural products in bromide (+) algae is similar to that of the natural products of *A. armata* in seawater.

Feeding deterrence by bromide (+) algae against 2 of the 3 mesograzers (the amphipod and juvenile abalone) can be directly attributed to the halogenated metabolites of *Asparagopsis armata*. Our rationale for focussing on chemical defence against herbivory in filamentous algae at a small scale, using small herbivores (mesograzers), was that these herbivores can reach high densities and have a substantial impact on algal biomass (Brawley 1992, Duffy & Hay 2000). Mesograzers may even exert a greater selective force on the filamentous algal community than larger herbivores, because large herbivores can remove an entire filamentous alga in a single bite (i.e. a single choice) whereas small herbivores will make their choice at a much finer scale. This choice could be at the scale of single branches or possibly the scale of cells within algal filaments. Considering these issues of scale and the selection of metabolite-free algae by the smallest herbivore *Hyale nigra*, generalist amphipods could be important in structuring filamentous algal communities and selecting for resistance.

Mesograzers not only include animals that spend their entire lives as mesograzers (e.g. amphipods) but also many juvenile versions of large benthic herbivores (e.g. abalone and urchins). Juveniles of the abalone *Haliotis rubra* selected bromide (–) algae over bromide (+) algae in a choice assay. Considering that algal defensive compounds can negatively effect abalone growth (Winter & Estes 1992), avoiding chemical defence in *Asparagopsis armata* may be important for

small *H. rubra*. Interestingly, adults of *H. rubra* are not deterred from eating the gametophyte of *A. armata* in the field (Shepherd & Steinberg 1992). As the halogen chemistry is similar for gametophytes and tetrasporophytes of *Asparagopsis armata* (Paul et al. 2006), these results suggest that the difference in choice between adult and juvenile abalone is not solely a function of the presence or absence of chemical defence.

Many soft-bodied ascoglossans, including sea hares, are often considered specialists as they consume chemically defended prey that are unpalatable to other consumers (Hay 1992). As a consequence, sea hares have the ability to either process or sequester metabolites that are otherwise harmful (Pennings 1990, Paul & Pennings 1991, Ginsburg & Paul 2001, Rogers et al. 2002). For this reason, it may be no surprise that the sea hare *Aplysia parvula* was not deterred from feeding on bromide (+) *Asparagopsis armata*. But considering that *A. parvula* is common on *Asparagopsis armata* and other red algae that produce halogenated metabolites (Rogers et al. 2002), it is of greater interest that bromide (+) algae was not a preferred food, i.e. that feeding was not stimulated by chemical defence. However, other sea hare species have also shown a lack of preference for seaweeds that have high concentrations of active metabolites (Ginsburg & Paul 2001). These data suggest that either sea hares cue to aspects of the host algae other than secondary metabolites or perhaps do not cue at all and survive by an associational resistance, similar to other mesograzers (Hay et al. 1987).

Although many plants exist in environments with intense grazing, the traits that facilitate persistence may not be always clear, especially if the plant exhibits a combination of resistance and tolerance strategies (Mauricio 2000, Koricheva et al. 2004). For marine algae, chemical defence and strategic growth can be combined features (Hay et al. 1988). Other marine organisms employ multiple resistance traits, for instance a combination of chemical and structural defences (Paul & Hay 1986) or one of chemical and nutritional defences (Duffy & Paul 1992, Cruz-Rivera & Hay 2003). However, the combination of chemical defence and the filamentous form represents a largely unexplored facet of algal chemical ecology. If the requirement for defence is low and resources are adequate, then algal communities may be dominated by high producers (Lubchenco & Gaines 1981). However, as no environment is completely free of herbivores (Paine 1990), a more successful competitive strategy for filamentous algae may be a combination of high relative growth rates and defence. This combination of resistance and tolerance traits could arise despite the assumed costs of secondary metabolite production (Mauricio 2000, Dworjanyn et al. 2006), especially if the costs of defence for marine algae are not large or are only expressed at certain times in the growth cycle.

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