

# Effects of secondary metabolites and CaCO<sub>3</sub> on feeding by surgeonfishes and parrotfishes: within-plant comparisons

Steven C. Pennings\*, Melany P. Puglisi, Todd J. Pitlik, Amalia C. Himaya, Valerie J. Paul

Marine Laboratory, University of Guam, Mangilao, Guam 96923, USA

**ABSTRACT:** Tropical seaweeds and 'plant-like' animals often contain both secondary metabolites and high concentrations of minerals. Typically, secondary metabolites are most concentrated in the tip of the organism and minerals in the base. We used within-individual variation in defenses in the alga *Neomeris annulata* to test the hypothesis that surgeonfishes are deterred from feeding by calcium carbonate but not by chemical defenses, whereas parrotfishes are deterred from feeding by chemical defenses but not by calcium carbonate. Our results supported this hypothesis. The parrotfish *Scarus sordidus* preferred to feed on an artificial food containing CaCO<sub>3</sub> compared to control food, but preferred control food over food containing a sesquiterpene-rich organic extract of *N. annulata*. Two species of parrotfishes fed primarily on the metabolite-poor, CaCO<sub>3</sub>-rich basal regions of *N. annulata*, whereas 2 species of surgeonfishes fed primarily on the metabolite-rich, CaCO<sub>3</sub>-poor tips. Variation in grazing location had important consequences for *N. annulata*. Individuals grazed on the tips grew in length more slowly than individuals grazed around the base. Individuals grazed on the tips were temporarily rich in CaCO<sub>3</sub>; individuals grazed around the base were temporarily rich in sesquiterpenes. These patterns were probably not a result of induction of defenses, but rather a consequence of selective removal of CaCO<sub>3</sub>-poor and sesquiterpene-poor material respectively.

**KEY WORDS:** Algae · CaCO<sub>3</sub> · Chemical defense · Herbivory · Mineral defense

## INTRODUCTION

A fundamental question in plant-herbivore interactions is 'what determines the feeding choices of herbivores?' The 2 most common approaches to this question have been to compare feeding on different plant species and to compare feeding on experimental foods that vary in only 1 parameter, such as protein or secondary metabolite levels. These approaches, both of which have proven very profitable, represent extremes on a continuum of trade-offs between realism and experimental control.

A third approach is to compare feeding on different parts of a single food species. This approach has proven valuable in both terrestrial (e.g. Raupp 1985,

Damman 1987, Reavey 1991) and marine (e.g. Steinberg 1984, Paul & Van Alstyne 1988, Van Alstyne 1989) systems because it is more realistic than using artificial foods, but holds constant a number of factors that vary in between-species comparisons. However, many parameters important to herbivores may simultaneously vary between parts of a single plant. For example, old and new leaves of trees vary in toughness, water content, protein content and secondary metabolite levels, all of which may be important to herbivores (Mattson 1980, Coley 1983, Reavey 1991, Stamp & Casey 1993; see Johnson & Mann 1986 for a marine example). However, the problem of covarying parameters can be minimized by studying structurally very simple plants with strong gradients in a few factors of known importance to herbivores. We use this approach to test the hypothesis that secondary metabolites and calcium carbonate in marine algae have different effects on different types of tropical herbivorous fishes.

\*Present address: University of Georgia Marine Institute, Sapelo Island, Georgia 31327, USA.  
E-mail: scpenn@uga.cc.uga.edu

Many marine algae and 'plant-like' animals such as soft corals are defended both by secondary metabolites and by high concentrations of minerals such as  $\text{CaCO}_3$  (Paul & Hay 1986, Hay & Fenical 1988, Paul 1992, Pennings & Paul 1992, Pennings & Svedberg 1993, Hay et al. 1994, Schupp & Paul 1994). The concentration of these defenses is usually not constant throughout the organism. Secondary metabolites are typically more concentrated near the tips, and minerals are more concentrated near the base where they can also provide structural support (Koehl 1982, Hay et al. 1988, Harvell & Fenical 1989, Wylie & Paul 1989, Van Alstyne et al. 1992). We suggest that variation within an organism in defense type will lead to different groups of consumers feeding upon that organism in qualitatively different ways. Different types of consumer damage may then have different consequences for the damaged organism.

Consumers often vary widely in their sensitivity to particular chemical (Hay & Steinberg 1992) or mineral (Schupp & Paul 1994) defenses. For example, experiments using artificial foods suggested that many surgeonfishes are relatively tolerant of chemical defenses but deterred from feeding by  $\text{CaCO}_3$ , whereas many parrotfishes are deterred from feeding by chemical defenses but are tolerant of or stimulated to feed by  $\text{CaCO}_3$  (Schupp & Paul 1994). Schupp & Paul (1994) did not experimentally address the important implications of their work for individual living plants: if consumers vary in their sensitivity to different defenses, and if these defenses are not evenly distributed throughout individual prey organisms, different consumers should tend to feed on different parts of their prey. Additionally, if consumers vary in their distribution in the field, both the intensity and the nature of feeding damage may vary spatially (Gaines 1985). Finally, different parts of all but the simplest organisms are specialized for different functions, such as support versus resource collection. Thus, if different consumers remove parts of an organism with different functions, damage by different consumers should have different consequences.

In this paper we explore these issues using the green alga *Neomeris annulata* (hereafter called *Neomeris*). The thallus of this small alga is a structurally simple, erect cylinder. Bases of individual thalli are heavily calcified, whereas tips contain high concentrations of brominated sesquiterpene secondary metabolites (Meyer & Paul 1995). We address 4 hypotheses. First, we test the hypothesis that chemical defenses of *Neomeris* will deter feeding by parrotfishes, but that  $\text{CaCO}_3$  will not. Second, we test the hypothesis that, because of intra-thalli variation in the location of  $\text{CaCO}_3$  and secondary metabolites, and because of the differing susceptibilities of parrotfishes and surgeon-

fishes to these defenses, parrotfishes will tend to feed near the bases of *Neomeris* thalli and surgeonfish near the tips. Third, we conduct a preliminary experiment to test the hypothesis that the intensity and nature of grazing damage in the field vary spatially. Fourth, we test the hypothesis that different types of damage (grazing at the base vs at the tip) have different consequences for *Neomeris* thalli. In particular, we examine growth and secondary metabolite concentrations of thalli following herbivory.

## METHODS

**Study site and species.** Research was conducted on the island of Guam in the western Pacific Ocean (13° 25' N, 144° 55' E). Field work was conducted in Agat Bay and laboratory experiments were conducted at the Marine Laboratory of the University of Guam. Agat Bay is guarded by a fringing coral reef. The reef flat is shallow (0 to 1 m water depth at low tide), several hundred meters wide, and covered with limestone rubble and sand. The fore-reef slope drops quickly to ca 6 to 8 m of water depth where it grades into a gently sloping bottom of sand and rubble.

The calcified green alga *Neomeris* (Fig. 1) is distributed throughout the Indo-Pacific and Caribbean, and contains a suite of structurally similar brominated sesquiterpenes (Barnekow et al. 1989, Paul et al. 1993). *Neomeris* is common on reef flats around Guam, and also occurs at low densities on reef slopes to a depth of >10 m. Individual thalli are erect cylinders up to ca 7 cm tall and 0.5 cm in diameter; thalli often occur in dense aggregations giving the appearance of a thick green carpet. The distal third of each thallus is bright green, and contains relatively high concentrations of secondary metabolites and relatively low levels of  $\text{CaCO}_3$ ; basal parts are white, low in secondary metabolites, and high in  $\text{CaCO}_3$ . *Neomeris* typically ranks low in preference to herbivorous fishes (Wylie & Paul 1988, Paul et al. 1993). Grazing damage in the field takes 2 distinct forms: (1) the tip of the thallus is removed, or (2) the sides of the thalli are grazed near the base (authors' pers. obs.; Fig. 1).

The parrotfishes *Scarus sordidus* and *S. schlegeli* and the surgeonfishes *Naso unicornis* and *N. lituratus* are common on reefs and reef-flats around Guam. We collected small individuals (8 to 14 cm) with dip and barrier nets from reefs and reef-flats for use in laboratory experiments.

**Effect of *Neomeris* characteristics on grazing by parrotfish.** To test the hypothesis that parrotfish, *Scarus sordidus*, are deterred from feeding by secondary metabolites from *Neomeris* but not by  $\text{CaCO}_3$ , we conducted a series of 3 laboratory experiments.

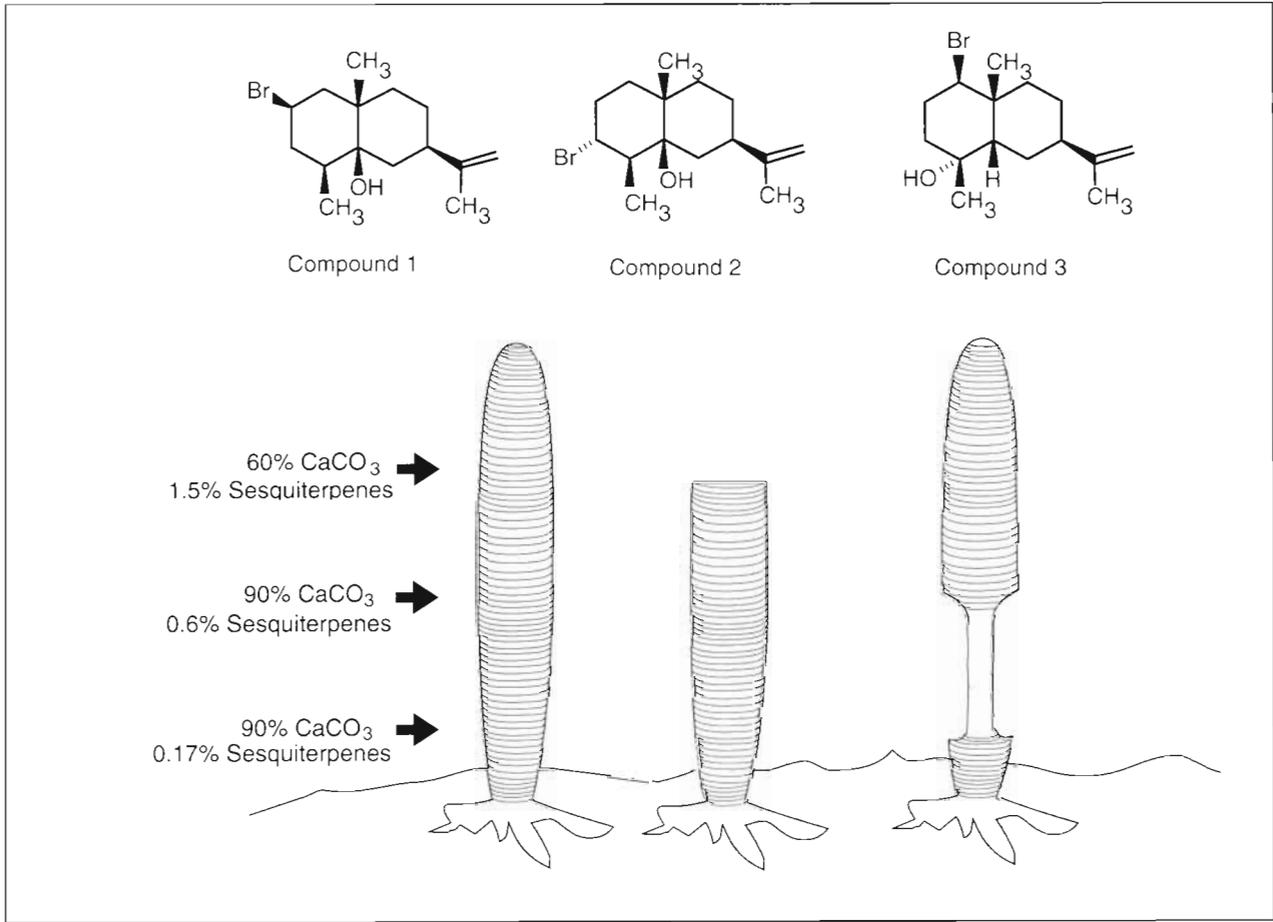


Fig. 1. *Neomeris annulata*. Drawing of typical thallus, showing average concentrations of sesquiterpenes (all 3 compounds combined) and  $\text{CaCO}_3$  in each part of the thallus (from Meyer & Paul 1995). Typical patterns of grazing damage are shown (not grazed, grazed at the tip, grazed at the base), as are structures of the 3 sesquiterpenes found in thalli from Guam

Pairs of fish (parrotfish behaved abnormally if isolated) were held in 20 l aquaria with running seawater. Fish were offered a choice between 2 cubes (ca 1 cm<sup>3</sup>) of artificial food placed 1 cm apart on metal pins. Control food was made with 5 g agar dissolved in 100 ml water. The water and agar were heated, 9 g of powdered, freeze-dried *Enteromorpha* sp. (a green alga that is highly palatable to most marine herbivores) was stirred in, and the mixture was poured into a shallow plastic dish to gel. Control food was paired with experimental food which contained one of the following additional sets of ingredients added with *Enteromorpha* sp.: (1) 89.7 g  $\text{CaCO}_3$  (calcite, 86.5% of dry mass of recipe), (2) 0.24 g *Neomeris* crude extract (1.7% of dry mass), or (3) both  $\text{CaCO}_3$  and *Neomeris* extract.  $\text{CaCO}_3$  and crude extract concentrations are whole-thalli averages (Pitlik unpubl. data) and so are lower concentrations of each than would occur in the bases and tips respectively. We utilized calcite for our  $\text{CaCO}_3$  treatments since it was commercially available in reagent

grade. Crude extract of *Neomeris* was obtained by extracting *Neomeris* thalli 3 times in 1:1 methanol:dichloromethane and evaporating the organic-soluble material to dryness. Individual replicates ( $n = 17$  to 20 per experiment) were terminated when approximately half of the total food offered to fish was consumed (1 to 30 h). No-herbivore control foods were placed in each aquarium inside beakers capped with mesh to allow water movement; these cubes controlled for possible differences in autogenic weight gain or loss of the control and experimental diets. No-herbivore control foods were immersed for the same length of time as were foods exposed to the fish. Data were analyzed following Peterson & Renaud (1989): the change in mass of experimental foods minus the change in mass of control foods was compared for paired herbivore and no-herbivore treatments with a paired  $t$ -test.

**Variation in grazing behavior among fish species.** To test the hypothesis that parrotfishes and surgeonfishes would graze at different locations on the

*Neomeris* thallus, we offered *Neomeris* to 4 species of fish in the laboratory. Single surgeonfish or groups of 2 parrotfish were held in 20 l aquaria with running seawater. Each fish or pair of fish was presented with a piece of limestone rubble bearing ca 20 to 40 *Neomeris* thalli. Starting at 2 d, and continuing daily until 10 d (parrotfish grazed quickly, but surgeonfish sometimes took longer to feed), we terminated replicates in which >10 thalli had been grazed, and scored the number of thalli that had been grazed on the tips versus the bases. Initial observations suggested that the parrotfish would graze all the *Neomeris* thalli extremely heavily during the first 2 d; consequently, we included another set of treatments with parrotfish in which we removed the rocks after 3 h of exposure to the fish. We ran 7 to 11 replicates each with *Naso unicornis*, *N. lituratus*, *Scarus sordidus* and *S. schlegeli*. Data (proportion of total grazing on tips vs bases) were analyzed with ANOVA. Since the data were proportions, they were arcsine-square-root transformed to improve normality (Sokal & Rohlf 1981).

#### Variation in grazing among locations in the field.

To test the hypothesis that the nature of grazing would vary among locations in the field, we placed rocks bearing *Neomeris* in 4 locations and monitored grazing damage. Rocks bearing approximately 20 to 200 *Neomeris* thalli were located on the reef in Agat Bay and tagged with flagging tape. Eight to 11 rocks were placed at each of 4 locations: (1) on the reef flat with no adjacent structure (covered by ca 1 m of water at low tide), (2) on the reef flat within 1 m of structure (boulders on the reef flat commonly shelter groups of small fish; at this site a discarded engine block was easier for us to access and appeared to have the same effect), (3) on the upper fore-reef slope (ca 2.3 m depth at low tide) and (4) on the lower edge of the reef slope where the reef graded into a sand/rubble slope (ca 6 m depth at low tide). Each group of rocks was placed within a ca 5 × 5 m area to facilitate easy recovery. These locations were chosen to represent a wide range of conditions under which *Neomeris* naturally grows. Because we did not replicate groups of rocks within each type of condition, we cannot statistically test whether the variation in herbivory that we observed was a function of the traits that we have chosen to describe each location (reef zone, water depth, cover) or rather of other unknown factors that might have varied between locations. However, our interest was merely in exploring whether variation in the nature of herbivory might occur among different types of habitats.

The site was visited at intervals over the next 19 d, and individual rocks removed when >10 thalli/rock were grazed or at 19 d. For each rock, we calculated (1) the proportion of thalli that were grazed per day and (2) the proportion of thalli that were grazed on the

tips versus the bases. For the latter variable we combined data from multiple rocks if fewer than 10 thalli were grazed per rock so that each replicate estimate was based on a sample of at least 10 grazed thalli. Because so few thalli were grazed at the reef flat location without adjacent structure, all the grazed thalli from all the rocks at this location were pooled to yield a single replicate. This estimate is presented in the results for qualitative comparison but was not included in the formal statistical analysis. Data were arcsine-square-root transformed and analyzed with ANOVA.

**Effect of grazing on *Neomeris* growth.** To test the hypothesis that *Neomeris* growth would vary as a function of the type of grazing damage, we followed the growth of naturally grazed thalli in the laboratory in 2 experiments. In the first, we utilized thalli that had been grazed in the field (see above, 'Variation in grazing among locations in the field'). Individual thalli (1 to 4 per rock) that had not been grazed or had been grazed on the tip or side were measured to the nearest mm and surrounding thalli removed. Rocks were submerged in a large outdoor aquarium with running seawater and re-measured after 2 wk. The second experiment was identical in design and execution to the first except that it utilized thalli that had been grazed in laboratory experiments (see above, 'Variation in grazing behavior among fish species'). Data (mm growth d<sup>-1</sup>, n = 2 to 13 treatment<sup>-1</sup> experiment<sup>-1</sup>) were analyzed with ANOVA.

#### Effect of grazing on *Neomeris* secondary chemistry.

To test the hypothesis that grazing would induce production of secondary metabolites, we conducted a series of grazing experiments using both artificial and natural grazing. In the artificial grazing experiments, we collected rocks bearing dense groups of *Neomeris* thalli and placed them in a large outdoor aquarium with running seawater. Using scissors, we clipped the extreme tips of thalli (distal 10 to 20%) to simulate light grazing on the tips. Thalli were either clipped once and allowed to regenerate for 3 d, or were clipped once a day for 5 successive days, and allowed to regenerate for 24 h. In each case, additional rocks bearing thalli that were not clipped served as controls.

In natural grazing experiments with parrotfish, we collected rocks bearing dense groups of *Neomeris* thalli and allowed them to be grazed by parrotfish, *Scarus sordidus*, in the laboratory. Pairs of fish were housed in eight 40 l aquaria with running seawater. Each pair of fish was allowed to graze 2 successive rocks. In each case, a control rock was placed in the aquarium behind a mesh barrier to prevent access by the fish. Parrotfish were observed to graze primarily at the bases of thalli. Once most of the thalli on a rock had suffered some grazing damage, the replicate was terminated immediately.

To conduct natural grazing experiments in the field, we collected 14 rocks bearing dense groups of *Neomeris* thalli. Half of these rocks were placed on the reef flat near a discarded engine block that sheltered a number of herbivorous fishes; earlier work had indicated to us that thalli at this location would be heavily grazed (see 'Results: Variation in grazing among locations in the field'). Control rocks were placed approximately 8 m away on the reef flat at a similar water depth but in a location lacking large physical structure; earlier work had indicated that thalli at this location would suffer very low rates of grazing (see 'Results: Variation in grazing among locations in the field'). After 7 d, rocks near the engine block were moved to the control location. At this time we observed that thalli on these rocks had been heavily grazed at the tips but that very few of the thalli at the control location had been grazed. Thalli were allowed to grow at the control location for an additional 6 d before collection (rocks were individually tagged to allow identification of treatment). Although this experiment potentially confounds grazing effects with other effects due to location, we observed no striking differences between locations other than the presence of structure and, hence, of herbivorous fish.

At the conclusion of each of the induction experiments we removed *Neomeris* thalli from rocks and extracted them 3 times in 1:1 methanol:dichloromethane. The organic-soluble material was collected and evaporated to dryness using a rotary evaporator. Remaining algal solids were dried at 60°C and weighed to calculate the yield of crude extract per dry mass of alga. The concentration of the 2 major terpenes in the extracts was determined using quantitative high-performance liquid chromatography (HPLC) (Beckman 110B solvent pump, 250 × 4.6 mm 5 µm Si column, ethyl acetate/hexane mobile phase, RI detector, electronic integrator) with injections of pure metabolites as external standards. We did not quantify the concentration of compound 3 (Fig. 1), which is present in only trace amounts. In every case, the unit of replication was the rock, and all thalli from each individual rock were pooled. Data were analyzed with *t*-tests or non-parametric analogs.

## RESULTS

### Effect of *Neomeris* characteristics on grazing by parrotfish

*Scarus sordidus* ate significantly more of the food containing CaCO<sub>3</sub> than of control food (Fig. 2; *p* < 0.0001). However, food containing crude extract of *Neomeris* was eaten significantly less than control food

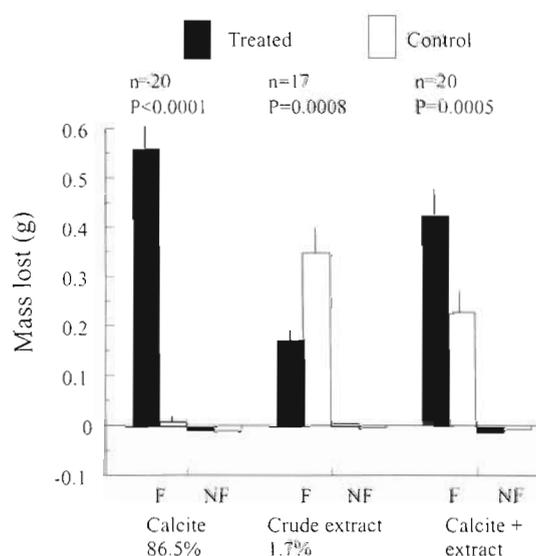


Fig. 2. Effect of calcite, crude *Neomeris annulata* extract and calcite + extract on consumption of artificial diets by the parrotfish *Scarus sordidus*. Data [mass loss (g) of artificial foods] are means + 1 SE. Sample sizes and p-values are given above groups of bars. F: fish; NF: no fish

(Fig. 2; *p* = 0.0008). When both CaCO<sub>3</sub> and crude extract were presented together, the parrotfish preferred the treated over the control food (Fig. 2, *p* = 0.0005), but far less dramatically than when CaCO<sub>3</sub> was presented alone. These results supported our first hypothesis that chemical defenses of *Neomeris* would deter feeding by parrotfishes, but that CaCO<sub>3</sub> would not.

### Variation in grazing behavior among fish species

Fishes varied significantly in whether they grazed the tips or the bases of *Neomeris* thalli (Fig. 3; *p* < 0.0001). As predicted by our second hypothesis, *Naso unicornis* and *N. lituratus* did >80% of their grazing on tips. In contrast, *Scarus sordidus* and *S. schlegeli* grazed primarily at the bases of thalli in 3 h experiments. When experiments were run for 2 d, both parrotfish species totally consumed individual *Neomeris* thalli (probably the fish initially grazed bases but then went on to graze the rest of the plants since no alternative foods were available). These heavily grazed thalli create the impression that parrotfishes grazed significantly more towards the tips of thalli in the longer-term experiments (Fig. 3; ANOVA with Tukey comparisons, *p* < 0.05). However, the reason for this was an increase in the number of thalli that were totally consumed rather than a change in preference from bases to tips. Overall, *S. schlegeli* showed a significantly greater preference for bases than did *S. sordidus* (Fig. 3).

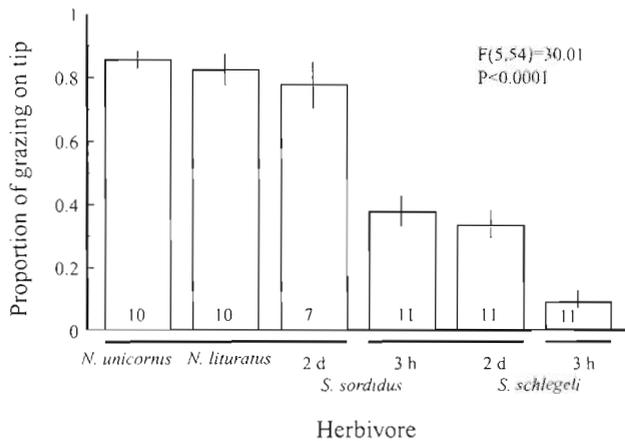


Fig. 3. Variation in grazing behavior among 4 fish species consuming *Neomeris annulata*. Data (proportion of grazing on tip vs base) are means  $\pm$  1 SE; sample sizes are given inside bars. Means that are not significantly different (ANOVA with Tukey comparisons) are joined with a horizontal line. Assays with parrotfishes were run for both 2 d and 3 h; surgeonfishes fed more slowly and assays were run for up to 10 d

**Variation in grazing among locations in the field**

Grazing rates varied significantly among the 4 locations, as predicted by our third hypothesis, with thalli located on the reef flat near structure being grazed much more heavily than thalli in any of the other locations (Fig. 4;  $p < 0.0001$ ). Thalli on the reef flat away from any structure (reef flat 1 m) were grazed the least, and thalli on the reef slope experienced intermediate levels of grazing. Despite strong differences in grazing intensity, and contrary to our hypothesis, no differences were found in the type of grazing. Thalli in all locations tended to be grazed primarily at the tip

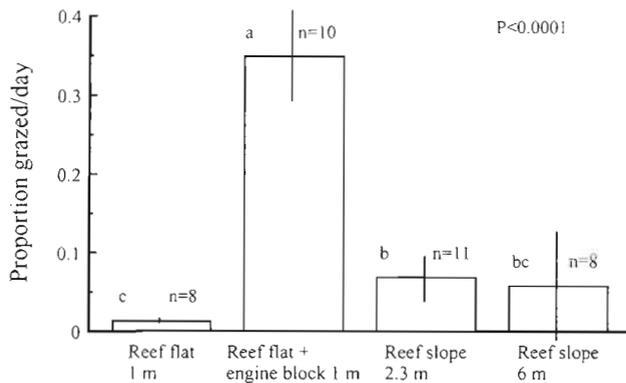


Fig. 4. Variation in rates of grazing on *Neomeris annulata* among 4 locations in the field. Data (proportion of thalli grazed  $d^{-1}$ ) are means  $\pm$  1 SE; sample sizes are given above bars. Means with the same letter above the bar are not significantly different (ANOVA with Tukey comparisons)

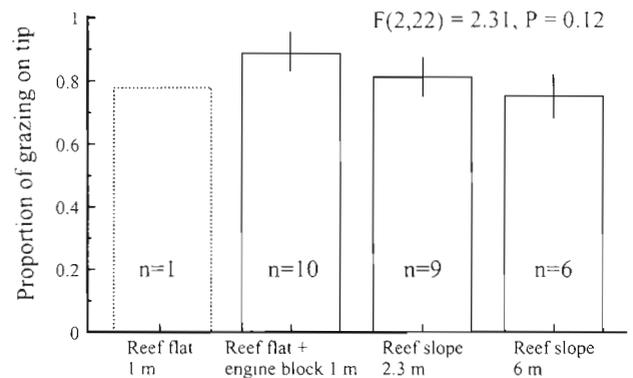


Fig. 5. Variation in type of grazing on *Neomeris annulata* among 4 locations in the field. Data (proportion of grazing on tip) are means  $\pm$  1 SE; sample sizes are given in bars. Dotted bar for 'Reef flat 1 m' treatment indicates that this treatment was not included in the ANOVA because grazing was so light at this site that all replicates had to be pooled to obtain a single sample with at least 10 grazed thalli

(Fig. 5), and the proportion of grazing on the tip of thalli did not differ significantly among locations ( $p = 0.12$ ).

**Effect of grazing on Neomeris growth**

Variation in grazing had a significant effect on subsequent growth as predicted by our fourth hypothesis (Fig. 6; ANOVA, treatment effect:  $p = 0.001$ ). In general, thalli grazed on the tip grew the slowest and control thalli grew the fastest. However, this pattern was far more pronounced in the first experiment which used thalli grazed in the field than in the second experiment which used thalli grazed by fish in the laboratory

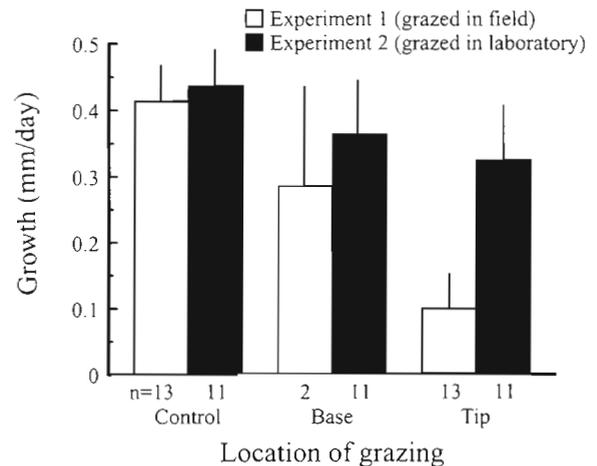


Fig. 6. *Neomeris annulata*. Growth ( $mm\ d^{-1}$ ) of control thalli and thalli grazed on the bases or tips. Data are means  $\pm$  1 SE, sample sizes are given under each bar

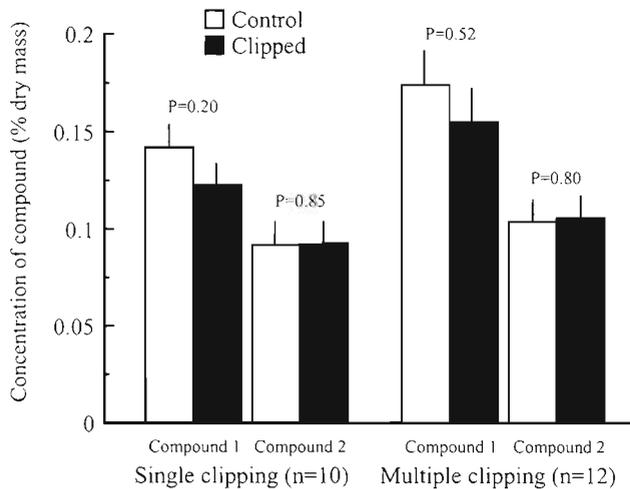


Fig. 7 Artificial grazing experiments. Concentration (% of algal dry mass) of compounds 1 and 2 in groups of *Neomeris annulata* thalli artificially grazed in the laboratory. Data are means + 1 SE; sample sizes and p-values are given in figure

(ANOVA, experiment effect:  $p = 0.051$ ; treatment  $\times$  experiment interaction:  $p = 0.048$ ).

#### Effect of grazing on *Neomeris* secondary chemistry

In contrast to the predictions of our fourth hypothesis, artificial grazing had no effect on the concentration of secondary metabolites in either the single or multiple clipping experiments (Fig. 7). Grazing in the laboratory by the parrotfish *Scarus sordidus* did appear to induce production of secondary metabolites (Fig. 8,

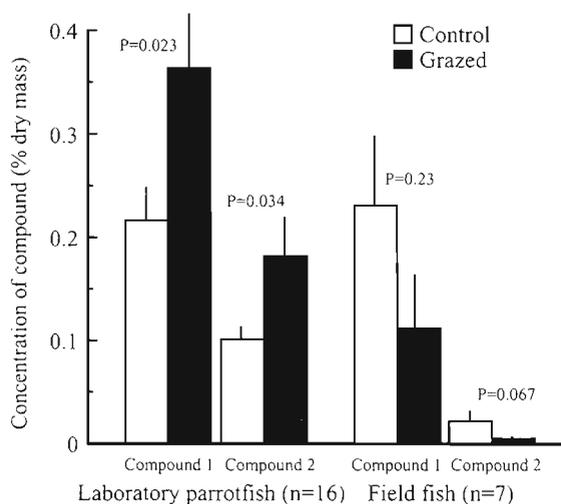


Fig. 8. Natural grazing experiments. Concentration (% of algal dry mass) of compounds 1 and 2 in groups of *Neomeris annulata* thalli grazed by parrotfish *Scarus sordidus* in the laboratory or by reef fish in the field. Data are means + 1 SE; sample sizes and p-values are given in figure

'Laboratory parrotfish', compound 1:  $p = 0.023$ ; compound 2:  $p = 0.034$ ); however, inspection of the thalli suggested that these results were a consequence of the fishes' preference for thalli bases. By removing  $\text{CaCO}_3$ -rich, metabolite-poor material from the bases, the fish left behind only the metabolite-rich parts of the thalli for analysis. When thalli were grazed by fish in the field (primarily at the tips) and then allowed to regenerate, no evidence of induction was found (Fig. 8, 'Field fish'); rather, both metabolites show a trend towards lower concentrations in recently grazed thalli.

## DISCUSSION

Our results support the hypothesis that surgeonfishes and parrotfishes respond to chemical and mineral defenses of algae in different ways. This hypothesis was initially based on artificial diet studies with secondary metabolites from *Halimeda* spp. (Schupp & Paul 1994). Here, we utilized secondary metabolites from a different alga (*Neomeris*) and a very different experimental approach exploiting individual variation in whole live plants. Our results suggest that Schupp & Paul's (1994) result is robust to variation in both algal species and experimental approach. Moreover, observations of diet choices by fish in the field indicate that parrotfishes often consume calcified algae but most surgeonfishes rarely do (reviewed in Schupp & Paul 1994). This variation in herbivore sensitivity to different defenses may be an important factor selecting for multiple lines of defense (Schupp & Paul 1994). It is important to note, however, that considerable variation in diet and dentition occurs within these large groups of fishes—we do not expect that all parrotfish or all surgeonfish species will behave identically.

Hay et al. (1994) suggested that  $\text{CaCO}_3$  and chemical defenses may co-occur because they interact synergistically to enhance their effectiveness. However, the opposite phenomenon may also occur: the presence of  $\text{CaCO}_3$  may cancel out the effect of chemical defenses, making the algae *more* attractive to some consumers than if the algae only possessed chemical defenses (Schupp & Paul 1994, this paper). Fish that normally consume  $\text{CaCO}_3$  in the field may use it as a feeding cue or even have a physiological need for it in their diet (reviewed in Schupp & Paul 1994). Consequently, the usefulness of  $\text{CaCO}_3$  as a defense may depend upon the types of herbivores present in a given environment.

#### Patterns of feeding on *Neomeris*

The 2 surgeonfishes and the 2 parrotfishes that we studied fed on *Neomeris* in different ways. The sur-

geonfishes removed the lightly calcified, chemically defended tip; the parrotfishes grazed on the heavily calcified, metabolite-poor base. These differences in feeding behavior are consistent with differences in the sensitivity of these fishes to different types of algal defenses. The 2 surgeonfishes are strongly deterred from feeding by  $\text{CaCO}_3$  (Schupp & Paul 1994), but are relatively tolerant of algal chemical defenses (Meyer et al. 1994). In contrast, the 2 parrotfishes are not deterred from feeding by  $\text{CaCO}_3$ , but are more sensitive to chemical defenses (Schupp & Paul 1994; this paper, Fig. 2). Thus, the fact that different types of defenses are not distributed homogeneously throughout the thallus, coupled with the fact that different herbivores are differentially sensitive to these defenses, leads to different herbivores feeding on *Neomeris* in different ways.

It is a common observation that different parts of terrestrial plants are eaten by different species of herbivores (e.g. Lawton 1983, Maddox & Root 1990, Gaston et al. 1991, Stamp & Casey 1993). Similar feeding-site specialization can also occur on large marine seaweeds like kelps (Hay & Steinberg 1992). However, the structural complexity of these plants makes it difficult to unambiguously determine the cause of herbivore preferences because so many factors vary between different plant parts. Moreover, because these examples all involve herbivores that are relatively small compared to their food plants, and therefore use the plants as a residence as well as a food, it is difficult to separate the effects of plant quality from the effects of physical factors and predation risk (Hay & Fenical 1988, Schultz 1992, Stamp & Casey 1993). The structural simplicity of *Neomeris* and its small size relative to the fishes involved made it an ideal system with which to demonstrate a clear link between patterns of plant defenses, differential herbivore sensitivity to these defenses, and patterns of herbivore feeding on whole thalli.

#### Consequences of variation in feeding location

Grazing in different locations on *Neomeris* has different consequences for the thalli. Most of the photosynthesis of *Neomeris* likely takes place in the green, actively growing tip, rather than in the heavily calcified base. Not surprisingly, we found that experimental thalli grazed at the tip grew more slowly than plants grazed at the base. In nature, where *Neomeris* thalli often grow in dense aggregations, thalli grazed at the tip might suffer even more because they would be shaded by their neighbors. In contrast, thalli grazed at the base might be more likely to be lost in heavy wave action (Padilla 1993). If herbivores are distributed non-randomly across the reef (Hay 1981a, b, 1984, 1985,

Lewis & Wainwright 1985), thalli in different areas might experience consistently different types of grazing damage. Although we found no evidence of this in our experiments, it should be noted that the work was conducted at only 4 locations, all of which were relatively close to each other, and which may have contained similar herbivore assemblages.

Because surgeonfishes and parrotfishes feed non-randomly with respect to thalli defenses, the defensive armament of *Neomeris* is changed after it has been grazed. On a whole-thallus level, individuals that have been grazed by parrotfishes are relatively rich in secondary metabolites because much of the metabolite-poor part of the thallus has been removed. Similarly, thalli grazed at the tips tend to be low in secondary metabolites because most of the metabolite-rich part of each thallus has been removed. We suggest that these patterns are not the result of increased or decreased production of secondary metabolites by *Neomeris*, but simply reflect the fact that different herbivores selectively removed parts of the thallus that were either poor or rich in secondary metabolites. Although regeneration by the plant can restore the original defenses, a temporary imbalance in defenses must be an inevitable consequence of herbivory in nature. It seems reasonable to speculate that, until corrected, this imbalance would affect the vulnerability of the thallus to subsequent herbivory.

#### Induction of chemical defenses in algae

Our artificial grazing experiments found no evidence that *Neomeris* could induce production of chemical defenses. In general, most studies of algae have found that they do not induce production of secondary metabolites following grazing damage (Paul 1992, Paul & Van Alstyne 1992, Steinberg 1994). The only exceptions are studies on 2 species in the genus *Fucus* from temperate latitudes (Van Alstyne 1988, Yates & Peckol 1993). To date, relatively few studies of chemical induction have been done with algae. However, it is possible that the high rates of spatially predictable but temporally unpredictable herbivory on tropical seaweeds select for either constitutive (Steinberg 1994) or activated (Paul 1992, Paul & Van Alstyne 1992) defenses rather than for the ability to induce defenses.

*Acknowledgements.* We thank the NSF (OCE-9116307 and GER-9023311) and NIH/MBRS (GM41796) for financial support. HPLC instrumentation was funded by grants from the Research Corporation and NSF (BSR-8605299). We thank Karen Meyer for assistance with the analytical chemistry and Darrell Casey for drawing Fig. 1. We thank the reviewers for helpful comments. This is contribution number 370 from the Marine Laboratory of the University of Guam.

## LITERATURE CITED

- Barnekow DE, Cardellina JH II, Zektzer AS, Martin GE (1989) Novel cytotoxic and phytotoxic halogenated sesquiterpenes from the green alga *Neomeris annulata*. J Am Chem Soc 111:3511–3517
- Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol Monogr 53: 209–233
- Damman H (1987) Leaf quality and enemy avoidance by the larvae of a pyralid moth. Ecology 68:88–97
- Gaines SD (1985) Herbivory and between-habitat diversity: the differential effectiveness of defenses in a marine plant. Ecology 66:473–485
- Gaston KJ, Reavey D, Valladares GR (1991) Changes in feeding habit as caterpillars grow. Ecol Entomol 16:339–344
- Harvell CD, Fenical W (1989) Chemical and structural defenses of Caribbean gorgonians (*Pseudopterogorgia* spp.): intracolony localization of defense. Limnol Oceanogr 34: 382–389
- Hay ME (1981a) Spatial patterns of grazing intensity on a Caribbean barrier reef: herbivory and algal distribution. Aquat Bot 11:97–109
- Hay ME (1981b) Herbivory, algal distribution, and the maintenance of between habitat diversity on a tropical fringing reef. Am Nat 118:530–540
- Hay ME (1984) Predictable spatial escapes from herbivory: how do these affect the evolution of herbivore resistance in tropical marine communities? Oecologia 64:396–407
- Hay ME (1985) Spatial patterns of herbivore impact and their importance in maintaining algal species richness. Proc 5th Int Coral Reef Symp 4:29–34
- Hay ME, Fenical W (1988) Marine plant-herbivore interactions: the ecology of chemical defense. A Rev Ecol Syst 19: 111–145
- Hay ME, Kappel QE, Fenical W (1994) Synergisms in plant defenses against herbivores: interactions of chemistry, calcification, and plant quality. Ecology 75:1714–1726
- Hay ME, Paul VJ, Lewis SM, Gustafson K, Tucker J, Trindell RN (1988) Does the tropical seaweed *Halimeda* reduce herbivory by growing at night?: diel patterns of growth, nitrogen content, herbivory, and chemical versus morphological defenses. Oecologia 75:233–245
- Hay ME, Steinberg PD (1992) The chemical ecology of plant-herbivore interactions in marine versus terrestrial communities. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary plant metabolites, 2nd edn, Vol II: evolutionary and ecological processes. Academic Press, New York, p 371–413
- Johnson CR, Mann KH (1986) The importance of plant defence abilities to the structure of subtidal seaweed communities: the kelp *Laminaria longicruris* de la Pylaie survives grazing by the snail *Lacuna vincta* (Montague) at high population densities. J Exp Mar Biol Ecol 97: 231–267
- Koehl MAR (1982) Mechanical design of spicule-reinforced connective tissue: stiffness. J Exp Biol 98:239–267
- Lawton JH (1983) Plant architecture and the diversity of phytophagous insects. A Rev Entomol 28:23–39
- Lewis SM, Wainwright PC (1985) Herbivore abundance and grazing intensity on a Caribbean coral reef. J Exp Mar Biol Ecol 87:215–228
- Maddox GD, Root RB (1990) Structure of the encounter between goldenrod (*Solidago altissima*) and its diverse insect fauna. Ecology 71:2115–2124
- Mattson WJ Jr (1980) Herbivory in relation to plant nitrogen content. A Rev Ecol Syst 11:119–161
- Meyer KD, Paul VJ (1995) Variation in secondary metabolite and aragonite concentrations in the tropical green seaweed *Neomeris annulata*: effects on herbivory by fishes. Mar Biol 122:537–545
- Meyer KD, Paul VJ, Sanger HR, Nelson SG (1994) Effects of seaweed extracts and secondary metabolites on feeding by the herbivorous surgeonfish *Naso lituratus*. Coral Reefs 13:105–112
- Padilla DK (1993) Rip stop in marine algae: minimizing the consequences of herbivore damage. Evol Ecol 7:634–644
- Paul VJ (1992) Ecological roles of marine natural products. In: Eisner T, Meinwald J (eds) Explorations in chemical ecology. Comstock Publishing Associates, Ithaca
- Paul VJ, Cronan JM Jr, Cardellina JH II (1993) Isolation of new brominated sesquiterpene feeding deterrents from tropical green alga *Neomeris annulata* (Dasycladaceae: Chlorophyta). J Chem Ecol 19:1847–1860
- Paul VJ, Hay ME (1986) Seaweed susceptibility to herbivory: chemical and morphological correlates. Mar Ecol Prog Ser 33:255–264
- Paul VJ, Van Alstyne KL (1988) Chemical defense and chemical variation in some tropical Pacific species of *Halimeda* (Halimedaceae; Chlorophyta). Coral Reefs 6:263–269
- Paul VJ, Van Alstyne KL (1992) Activation of chemical defenses in the tropical green algae *Halimeda* spp. J Exp Mar Biol Ecol 160:191–203
- Pennings SC, Paul VJ (1992) Effect of plant toughness, calcification, and chemistry on herbivory by *Dolabella auricularia*. Ecology 73:1606–1619
- Pennings SC, Svedberg JM (1993) Does CaCO<sub>3</sub> in food deter feeding by sea urchins? Mar Ecol Prog Ser 101:163–167
- Peterson CH, Renaud PE (1989) Analysis of feeding preference experiments. Oecologia 80:82–86
- Raup MJ (1985) Effects of leaf toughness on mandibular wear of the leaf beetle, *Plagioderma versicolora*. Ecol Entomol 10:73–79
- Reavey D (1991) Do birch-feeding caterpillars make the right feeding choices? Oecologia 87:257–264
- Schultz JC (1992) Factoring natural enemies into plant tissue availability to herbivores. In: Hunter MD, Ohgushi T, Price PW (eds) Effects of resource distribution on animal-plant interactions. Academic Press, San Diego, p 175–197
- Schupp PJ, Paul VJ (1994) Calcium carbonate and secondary metabolites in tropical seaweeds: variable effects on herbivorous fishes. Ecology 75:1172–1185
- Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. W. H. Freeman and Company, New York
- Stamp NE, Casey TM (1993) Caterpillars: ecological and evolutionary constraints on foraging. Chapman & Hall, New York
- Steinberg PD (1984) Algal chemical defense against herbivores: allocation of phenolic compounds in the kelp *Alaria marginata*. Science 223:405–407
- Steinberg PD (1994) Lack of short-term induction of phlorotannins in the Australasian brown algae *Ecklonia radiata* and *Sargassum vestitum*. Mar Ecol Prog Ser 112: 129–133
- Van Alstyne KL (1988) Herbivore grazing increases polyphenolic defenses in the intertidal brown alga *Fucus distichus*. Ecology 69:655–663
- Van Alstyne KL (1989) Adventitious branching as a herbivore-induced defense in the intertidal brown alga *Fucus distichus*. Mar Ecol Prog Ser 56:169–176
- Van Alstyne KL, Wylie CR, Paul VJ, Meyer K (1992) Antipredator defenses in tropical Pacific soft corals (Coelelerata: Alcyonacea). I. Sclerites as defenses against generalist carnivorous fishes. Biol Bull 182:231–240

Wylie CR, Paul VJ (1988) Feeding preferences of the surgeonfish *Zebrosoma flavescens* in relation to chemical defenses of tropical algae. *Mar Ecol Prog Ser* 45:23–32

Wylie CR, Paul VJ (1989) Chemical defenses in three species of *Sinularia* (Coelenterata, Alcyonacea): effects against

generalist predators and the butterflyfish *Chaetodon unimaculatus* Bloch. *J Exp Mar Biol Ecol* 129:141–160

Yates JL, Peckol P (1993) Effects of nutrient availability and herbivory on polyphenolics in the seaweed *Fucus vesiculosus*. *Ecology* 74:1757–1766

*This article was submitted to the editor*

*Manuscript first received: July 14, 1995*

*Revised version accepted: November 9, 1995*