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AN ABSTRACT OF THE THESIS presented by Jo Nita Quenga Kerr for the Degree of Master of Science in Biology, May 25, 1994.

Title: Animal-plant defense association: the soft coral *Sinularia* sp. (Cnidaria, Alcyonacea) protects algae from herbivory

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A defense association occurs when an organism escapes predation by being closely associated with another organism. I describe a marine defense association provided by the soft coral *Sinularia* sp. for algae growing at its bases. Total algal biomass was significantly greater in the 0-10 cm zone than in zones 10-20 and 20-30 cm around individual soft coral colonies. Total algal biomass was also significantly greater within clusters of colonies than in zones 0-10, 10-20 and 20-30 cm around the perimeter of the clusters. The calcified green algae *Halimeda incrassata* and *H. opuntia* comprised the bulk of the total algal biomass and were the only species that exhibited a pattern of growth similar to the total biomass.

Fishes consistently grazed less algae and seagrass strands placed next to soft corals than farther away. Significantly less *H. incrassata* was grazed next to soft corals than in the open. Thalli of the palatable red alga *Acanthophora spicifera* were grazed significantly less at 2 cm than 30 cm distant from the bases of soft corals. Thus, a pattern of differential grazing occurred around soft corals.

I also tested the following: (1) if objects, other than soft corals, produced the same pattern of grazing, (2) if removing soft corals disrupted the pattern, and (3) if the

shape of *Sinularia* sp. produced the pattern. Fishes grazed indiscriminately around objects (rocks or outcrops of dead hard coral), whereas the same pattern of grazing occurred around soft corals. This suggested that the presence of an object alone is not responsible for reduced grazing next to the soft coral. When soft corals were removed, the pattern of grazing was disrupted, whereas around intact colonies the pattern persisted. Subsequently, funnel-shaped carrageenan models, that resembled the arborescent shape of *Sinularia* sp., produced a similar grazing pattern.

Removing soft corals did not appear to encourage grazing of exposed algae by fishes. This may be explained by the chemically deterrent or heavily calcified nature of *Halimeda* spp. (the dominant algae around soft corals) which would prohibit vigorous consumption by herbivorous fishes. Algal species richness did not appear to be enhanced by the presence of *Sinularia* sp.

I found no evidence for chemical defense in experiments with soft coral extracts, carrageenan models, and rehydrated *Sinularia* sp. colonies. Just as much seagrass was grazed around models and colonies with added extracts, as around controls. However, a significant effect of distance was consistently obtained in experiments with funnel-shaped models and rehydrated soft corals, whereas, no effect of distance occurred with beaker-shaped models.

These results suggest that the arborescent shape of *Sinularia* sp. provides a refuge for young recruits of *Halimeda* spp., and allows the algae to mature until they are not preferred by herbivores. This study is the first to directly test whether morphological features or chemical properties of a benthic marine organism are responsible for protecting closely associated algae.

ANIMAL-PLANT DEFENSE ASSOCIATION: THE SOFT CORAL *SINULARIA* SP. (CNIDARIA, ALCYONACEA) PROTECTS ALGAE FROM HERBIVORY

BY

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A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

IN

BIOLOGY

UNIVERSITY OF GUAM

MAY 1994

ACKNOWLEDGEMENTS

I thank the members of my committee, Charles Birkeland, Gary Denton, and Robert Richmond for their guidance and tutelage, and my advisor, Valerie Paul, for assisting me in the field above and beyond the call of duty. Steven Pennings provided valuable advice for field experiments and statistical analyses. Comments from Ernest Matson and Stephen Nelson greatly improved and shortened the manuscript. The following provided their time and energy to assist me in the field: A.M. Kerr, P. Schupp, B. Davidson, T. Pitlik, M. Puglisi, C. Dayton, J. Gallo, and A. Himaya. The Marine Laboratory technicians, K. Sakamoto, B. Irish, and C. Bassler, and F. Cushing helped with equipment and transportation to the field. S. Wilkins helped with seaweed identification. A. Duenas, M. Peredo, and A. Espiritu provided editorial assistance and always made sure I filled out a timesheet. The Marine Lab Class of 1994 offered much empathy and moral support. Dangkulu na si Yu'us ma'ase' to my parents, Sergio and Jovita Quenga, for their encouragement, support, and countless babysitting hours. Finally, A.M. Kerr, A. I. Kerr, A.C. Kerr, and R.A. Kerr provided much of the concentration and strength when I needed it the most. This study was funded by the NIH MBRS program GM 44796, and a grant from the Lerner-Gray Fund for Marine Research.

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INTRODUCTION

A defense association is defined as a relationship in which an organism gains protection from predators by being closely associated with another organism (Hay 1986, Littler *et al.* 1987). Similar relationships have primarily been observed in terrestrial ecosystems. For example, in the context of plant defense guilds, Atsatt and O'Dowd (1976) and O'Dowd and Williamson (1979) described three ways by which guild members act as antiherbivore resources. First, guild members may be insectary plants (plants which harbor and maintain herbivore predators and parasites). Second, chemical or physical properties of guild members may repel herbivores and prevent them from locating prey. Third, guild members may serve as decoys and lure herbivores away from normal prey. In all three cases, plants targeted by herbivores benefit by growing in an environment of reduced susceptibility to herbivory.

Of primary interest to this study are associations based on the second description. For example, forage grasses, such as *Agrostis* and *Festuca*, growing near the noxious buttercup *Ranunculus bulbosus*, are protected from grazing by cattle (Fisher 1890 cited in Atsatt and O'Dowd 1976) because *R. bulbosus* produces ranunculin which irritates the skin and mucus membranes of cattle (Fisher 1922 cited in Atsatt and O'Dowd 1976). Another example is the resistance to herbivory gained by the palatable African savanna grass *Themeda triandra* Forsk (McNaughton 1978). Buffalo and wildebeest tend to graze less *T. triandra* when it grows in stands containing a relatively higher percentage of unpalatable plants such as *Cymbogogon excavatus* (Hochst.) Stapfg. and *Loudetia kagerensis* (K. Schum) Hutch.

In marine communities, defense associations occur between two or more algal species as well as between algae and invertebrates (Hay 1986, Littler *et al.* 1986, Littler *et al.* 1987, Pfister and Hay 1988). For example, palatable algae, such as *Hypnea musciformis* (Wulfen in Jacquin) Lamx. and *Spyridia hypnoides* (Bory in Belanger) Papenfuss, are afforded an escape from herbivores when they grow as epiphytes on less palatable brown algae *Sargassum filipendula* C. Agardh and *Padina vickersiae* Hoyt (Hay 1986). The red alga *Gracilaria tikvahiae* McLachlan is protected from grazing by sea urchins when it grows under a canopy of *S. filipendula* (Pfister and Hay 1988). In these cases, it is not clear whether chemical or structural characteristics of the less palatable alga are responsible for deterring predators. However, chemical defense is implied in a study by Littler *et al.* (1986) in which the palatable alga *Acanthophora spicifera* (Vahl) Boergesen is grazed less by herbivores when placed next to the toxic brown alga *Stypopodium zonale* (Lamouroux) Papenfuss, than when placed next to plastic mimics of *S. zonale* (see also Pfister and Hay 1988).

In these studies, the unpalatable seaweeds are the dominant species and higher species richness is observed in these sites as compared to locations in which the unpalatable species are not dominant. Moreover, palatable algae more often assume lower costs (i.e., suppressed growth rates and lowered nutrient availability) of associating with unpalatable species, rather than the cost of increased herbivory by not associating at all (Hay 1986, Littler *et al.* 1987, Pfister and Hay 1988).

Two marine animal-plant defense associations have been documented. The fire coral *Millepora alcicornis* Lamarck and the purple sea fan *Gorgonia ventalina*

Linnaeus provide algae that grow close to either animal with microhabitats of reduced herbivory (Littler *et al.*1987). Also, a significantly higher number of algal species are found next to either animal than in surrounding areas where the animals do not occur. Whether these defense associations are mediated by chemical or physical properties possessed by the animals is not known, but the associations are derived from algal susceptibility to grazing which is contingent not only on the characteristics of the algae, but on the morphology, chemistry, distribution, and abundance of the animals (Littler *et al.* 1987).

In this study, I describe an animal-plant defense association between the soft coral *Sinularia* sp. and algae that grow next to the soft coral. Preliminary data show that *Halimeda incrassata* (Ellis) Lamouroux and *H. opuntia* (Linnaeus) Lamouroux grow in abundance at the bases of the soft corals and that algal biomass decreases significantly with increasing distance from the colonies. This association may originate from chemical defenses possessed by the soft coral or the shape or presence of the soft coral may reduce encounter rates between fishes and algae that grow at the bases. Alternately, the soft coral may offer a favorable chemical or physical microhabitat in which the algae flourish. Nematocysts, or stinging cells, are found in soft corals, however, the cells are generally harmless and ineffective as a defense against other organisms (Sammarco *et al.* 1985, Sammarco and Coll 1988). Therefore, nematocysts are probably not responsible for this association.

Studies of defense associations in marine environments have alluded to a role for defensive chemistry (Littler *et al.* 1986, Littler *et al.* 1987, Pfister and Hay 1988);

however, none have directly tested this possibility. I expected that soft coral chemical defenses might play an important role in creating the observed patterns of algal distribution since many species of soft corals are known to be chemically well defended (La Barre *et al.* 1986a). A variety of diterpenes and sesquiterpenes have been isolated from soft corals (Coll *et al.* 1982, Coll *et al.* 1985, Ireland *et al.* 1988, Maida *et al.* 1993). These compounds have been shown to possess ichthyotoxic, antifeedant, allelopathic, antiinflammatory, and anti-leukemic properties (Sammarco *et al.* 1985, La Barre *et al.* 1986a, Sammarco and Coll 1988). Wylie and Paul (1989) tested relative chemical deterrence of the three main *Sinularia* species found at the present study site: *Sinularia* sp., *S. maxima* Verseveldt, and *S. polydactyla* Ehrenberg. They demonstrated that the lipid-soluble extracts were deterrent toward generalist predators at concentrations equal to or less than that of whole colonies. Chemical analysis of the extracts yielded a mixture of six to seven terpenoid compounds in *Sinularia* sp. (Wylie and Paul 1989).

Preliminary work showed that (1) algal biomass is higher close to soft coral colonies than farther away, and (2) differential grazing by herbivorous fishes may produce these patterns of algal growth. Subsequently, I asked the following questions: (1) are herbivores deterred by the presence of an object (other than a soft coral), (2) does removal of *Sinularia* sp. colonies encourage more grazing around removal sites than around intact colonies, (3) are morphological features, or (4) the chemical properties of *Sinularia* sp. responsible for reduced grazing by herbivores of algae that grow next to soft corals? Finally, similar studies have shown that significantly more

algal species are found in such microhabitats than in areas without associated species (Hay 1986, Hay 1992, Littler *et al.* 1987). Thus, I also examined whether algal species richness was enhanced by the presence of *Sinularia* sp.

MATERIALS AND METHODS

Study Site

All field work was conducted on a patch reef (Val's Reef) in Cocos Lagoon, Guam (described in Wylie and Paul 1989). Soft corals are one of the most conspicuous invertebrates on the reef, with *Sinularia* sp. being the most abundant soft coral species. Major algal species are *Dictyota bartayresii* Lamouroux, *Halimeda incrassata*, *H. opuntia*, and cyanobacteria (Wylie and Paul 1989). Much of the soft coral and algal growth occurs on dead *Acropora* spp. which comprises most of the substrate of the reef. Data collection began in June 1991 and concluded in May 1994.

Statistical Analyses

To meet analysis of variance (ANOVA) requirements, the data were checked for normality, homoscedasticity, and additivity with the following tests: Wilk-Shapiro, Largest Variance/Smallest Variance (F-max), and Tukey's 1 Degree of Freedom test for Nonadditivity, respectively, on *Statistix 4.0*® (Analytical Software, St. Paul, Minnesota, U.S.A.). When heteroscedasticity could not be reduced by transforming

the data, I used a Kruskal-Wallis Test followed by a nonparametric comparison of means test (Zar 1984). I used three types of analysis of variance (ANOVA) tests.

(1) Two-way ANOVAs without replication were used to determine the distribution of algae around individual soft corals and clusters of soft corals. I was interested in the amount of algal biomass found in three zones around individual colonies, and in zones within and around clusters of colonies. The following variables were used: biomass was the dependent variable, colony was the random factor, and zone was fixed. The error term was colony × zone. Tukey's Honestly Significant Difference (HSD) pairwise comparison of means test was used to determine which means were significantly different (Sokal and Rolf 1981). I also used this type of ANOVA for grazing experiments and will describe the variable substitutions in the appropriate sections.

(2) Two-way nested ANOVAs were used for grazing experiments with two treatments. I compared the lengths of seagrass strands grazed when placed around two treatments (soft corals or objects) at three distances around each treatment. The following variables were used: length (of seagrass grazed) was the dependent variable, treatment and distance were fixed, and colony was the random factor nested within treatments. Colony × treatment and colony × treatment × distance were error terms, and treatment × distance was the interaction term. If the terms, distance or treatment × distance, were significant I examined the treatments separately by performing a two-way ANOVA without replication, and used Tukey's HSD test to determine which means were significantly different. I also used this ANOVA model for soft coral

removal experiments and will describe the variable substitutions in the appropriate sections.

(3) Three-way ANOVAs were used to analyze data from experiments with soft coral extracts and models. I was interested in the effect of two treatments (models containing extract or controls) on the length of seagrass grazed at three distances from soft coral models. Length was the dependent variable, treatment, distance, and location were fixed factors. I added location as a variable because I had paired the treatment models with their respective controls in the field. The error terms were treatment × location and treatment × location × distance. Interaction terms were location × distance and treatment × distance. When factors or interaction terms were significant, I examined the treatments separately in the same manner as above.

Distribution of Algae Around *Sinularia* sp.

Algal samples were collected from zones 0-10, 10-20 and 20-30 cm distant from the bases of isolated individual *Sinularia* sp. All algae were collected from five circular areas (using a ring with a diameter of 5 cm) within each zone, and the samples were pooled. The total area sampled in each zone was 98 cm^2. The algae were taken to the laboratory, sorted, and identified. The wet mass of each species was obtained after removing excess water by spinning tougher algae in a salad spinner and blotting delicate algae between paper towels. *Sinularia* often grow in tight clusters, with little space between the colonies. Clusters of colonies were similarly sampled at 0-10, 10-20, and 20-30 cm around the perimeter of the clusters, as well as from

primary substrata within the clusters. Macroalgal species and wet biomass were determined for each zone. I combined algal turf and epiphytic species under one category and obtained wet biomass for each zone. Total algal biomass (macroalgae + algal turf and epiphytes) was calculated for each zone to see if this varied with distance from the bases of soft corals. All data were transformed (either square root or natural log) to meet ANOVA requirements. The data were analyzed using a two-way ANOVA without replication as described in the Statistical Analyses section.

Grazing Experiments

Halimeda incrassata grazing experiment

To determine whether grazing by herbivorous fishes produced the observed patterns of algal growth, I trimmed strands of young *Halimeda incrassata* to a length of 4 cm and placed 74 strands next to soft corals, and 77 strands in the open, away from soft corals at the study site. The strands were held upright in wooden clothespins that were weighted with steel bolts. Since 0.5 cm of each strand was pinned, this left 3.5 cm available for grazing. To allow for breakage not due to grazing, I placed 50 *H. incrassata* strands, also held upright by clothespins, in a flow-through seawater tank at the U.O.G. Marine Laboratory. I allowed three days to pass before returning to measure the remaining lengths of *H. incrassata* in the field, as well as the controls in the lab.

The data were converted to lengths removed or grazed by subtracting the length remaining from each strand from the length available for grazing (3.5 cm). Because transforming the data did not remove heteroscedasticity, I used a Kruskall-Wallis Test to determine if the treatments were significantly different, and followed with a nonparametric Student Newman-Keuls multiple comparison test (for unequal sample sizes) to determine which treatments were significantly different (Zar 1984).

Acanthophora spicifera grazing experiment

A grazing experiment was performed using *Acanthophora spicifera* (Vahl) Boergesen, a red alga known to be preferred by fish (Lewis 1985). *Acanthophora spicifera* was collected from Ypao Beach, Tumon Bay, taken to the laboratory and kept overnight in a mesh bag in a flow-through seawater system. The thalli were trimmed with a razor blade to a length of 7 cm, and excess branches were removed. The strands were stored in a mesh bag in a flow-through seawater system overnight, and were used in the grazing experiment within 48 hours of collection.

At the experimental site in Cocos Lagoon, six *A. spicifera* strands were arranged in two lines at distances of 2, 10, and 30 cm from the bases of 17 individual soft corals. The strands were held in place by wooden clothespins, as before, with 6.5 cm of algae projecting from each pin. The total length of *A. spicifera* available for grazing at each distance was 13 cm. The sites were intermittently monitored to confirm that fish were grazing the *A. spicifera* and that no losses could be attributed to mechanical damage. After 2.5 h the strands were collected and the length remaining

measured in the field. All replicates showed evidence of grazing. The total length of algae eaten at each distance was calculated and converted to a proportion of the total length offered at each distance (13 cm). The data were arcsine-square root transformed and a two-way ANOVA without replication was used as described in the Statistical Analyses section.

Algal removal - grazing experiment

The sampling procedure indicated that algal biomass was significantly greater closer to *Sinularia* sp. This introduced the possibility that macroalgae that grow next to soft corals prevented fishes from locating and grazing *Halimeda incrassata* and *Acanthophora spicifera* strands placed next to soft corals in the above grazing experiments. To test this, an algal removal experiment was performed. All algae growing within 10 cm of the bases of 12 randomly chosen soft corals were removed. Another 12 colonies were designated controls around which algal growth was left intact. Seagrass (*Halodule uninervis* Ascherson) was offered for grazing because *A. spicifera* was unavailable after a run of juvenile rabbitfish in May 1992 depleted much of the macroalgae around Guam. (A separate experiment showed that fish graze the seagrass just as readily as *A. spicifera*). Paired 5 cm strands of seagrass were placed at distances of 2, 10, and 30 cm around the algal removal and control colonies. In this experiment, the fishes (mostly parrotfish) grazed fairly quickly and waiting periods for each replicate varied from 1 to 15 minutes. The length of seagrass remaining was

measured in the field. I expected to find no difference in the lengths of seagrass grazed around algal removal and control colonies.

The length of seagrass eaten at each distance was calculated and converted to a proportion of the total length available for grazing at each distance (9 cm). The data were arcsine-square root transformed and analyzed using a two-way nested ANOVA as described in the Statistical Analyses section.

Combined grazing - object experiment

To test if the presence of any object deters grazing by herbivorous fish, paired 5-cm strands of seagrass were placed at distances of 2, 10, and 30 cm from the bases of 19 *Sinularia* sp., as well as around 15 objects (rocks or outcrops of dead hard coral branches similar in size to the soft corals). After 3 hours, the remaining seagrass was measured as above. I expected that around the soft corals, less seagrass would be grazed closer to the soft coral than farther away, and that around the objects, seagrass would be grazed indiscriminately.

The data, as length of seagrass eaten, were converted to a proportion of the amount of seagrass available for grazing and then arcsine-square root transformed as before. Data from 4 soft coral replicates were randomly discarded since the *Statistix* program could not analyze uneven sample sizes for this particular model. A two-way nested ANOVA was performed as described in the Statistical Analyses section.

Grazing experiments around *Sinularia* sp. removal and control sites

To test if the grazing pattern would be disrupted or disappear if *Sinularia* sp. were removed, 5 cm strands of seagrass were placed in two lines around 11 intact soft corals and 11 removal sites at distances of 2, 10 and 30 cm from the colonies or removal scars. After 2.5 h, the seagrass strands were collected and measured. The data were transformed and analyzed as above with a two-way nested ANOVA.

Grazing experiment around soft coral models

To test if the shape of the soft corals may be influencing the grazing patterns, 15 carrageenan models were constructed to resemble the arborescent form of the soft corals. Funnels with rubber stoppers placed in the necks were used as molds to cast the models. 400 ml of a carrageenan-water mixture, in the ratio of 5 g carrageenan:100 ml water, were heated to a smooth consistency and poured into each funnel. After 10 min, the models cooled and solidified, and were removed from the molds. Each model was 9.5 cm high with a top diameter of 12 cm and base diameter of 2.5 cm.

The models were speared with aluminum dowels which, when driven into the reef, served to anchor and keep them upright and orientated so that they resembled inverted cones. Strands of seagrass (5 cm) were placed around each model in the same manner as before. After a short waiting period to allow fish to graze, the strands were collected and measured. The data were transformed as before and

analyzed with a two-way ANOVA without replication, as described in the Statistical Analyses section, replacing colony with model as the random variable.

Removal Experiments and Algal Species Richness

Sinularia sp. removal experiments

To determine if removing *Sinularia* sp. would encourage fishes to graze exposed macroalgae, 7 individual colonies were randomly removed, and 7 colonies were left intact as controls. By tossing a coin, the first colony was designated to be a control and the second colony was removed. Thereafter, subsequent colonies were alternately designated controls or removed. I expected that removing soft corals would encourage fishes to graze algae in the removal sites, and that algal biomass in the removal sites would be much less than in control sites. After one week the sites were checked, however, no observable losses had occurred and the experiment was continued for another week. After 2 weeks, although there were no observable differences in algal cover, I decided to sample the sites anyway. Algae were collected from three zones, 0-10, 10-20 and 20-30 cm, around the bases of the soft corals and removal scars in the same manner described in the section on algal distribution. The algae were taken to the laboratory, sorted, identified, and wet mass was obtained after removing excess water. The data were square root-transformed before analysis. A two-way nested ANOVA was performed with biomass replacing length as the dependent variable, zone replacing distance as a fixed factor, and appropriately substituted interaction and error terms as described in the Statistical Analyses section.

A longterm *Sinularia* sp. removal experiment was performed because the sites in the first experiment may have been prematurely sampled. In this experiment 15 soft corals were left intact and 15 soft corals were removed. At the end of the experiment (4 mo), 11 control sites and 13 removal sites were still available for sampling. Algal samples were collected from around removal and control sites as described above. The samples were sorted, identified and wet mass obtained as before. The biomass data were natural log-transformed and a two-way nested ANOVA was performed as described above.

Algal species richness around soft corals and removal sites

Algal samples from the second *Sinularia* sp. removal experiment were sorted by species and weighed as described above. I expected to find more algal species next to soft corals than farther away, as well as more species next to intact colonies relative to removal sites. Previously, turf and epiphytic algae had been grouped under one category. To obtain a more accurate measure of species richness in each zone, these were microscopically examined and identified at least to the level of genus. Algae of the same genera that could not be identified to species were designated as sp.1, sp.2 and so on. I counted the number of algal species in zones, 0-10, 10-20 and 20-30 cm, around 11 sites with intact *Sinularia* sp. colonies and 11 sites from which soft corals had been removed. The data did not require transformation and a two-way nested ANOVA was performed as described in the Statistical Analyses section with

the number of species (in each zone) as the dependent variable, sites (either removal or intact colony) as the random factor nested within treatments, and appropriately substituted interaction and error terms.

Longterm *Sinularia* sp. - algal removal experiment

To determine whether the presence of *Sinularia* sp. influences algal recruitment and establishment around the bases of soft corals, 15 colonies were removed and 15 were left intact. Algae were removed from a radius of 30 cm around each colony and removal scar. Steel wire brushes and scouring pads were used to remove algal turf from the substratum. After 3 mo, I returned to survey algal recovery around the sites. Since only algal turf, *Dictyota* spp, and cyanobacteria (*Schizothrix* spp.) appeared to dominate the sites (*Halimeda* spp. were rare), I decided to perform a non-destructive survey using a quadrat, 9×9 cm^2, with 9 cross-hairs. The quadrat was haphazardly tossed 4 times in each zone (0-10, 10-20 and 20-30 cm) around the colonies and removal sites. Objects under each cross-hair were recorded, including algal species, rock or dead coral.

The data were converted to a proportion of the total number of points (36) available in each zone. All macroalgal species proportions were combined to obtain a total proportion of algal species in each zone. Cyanobacteria and crustose corallines were omitted from the analysis because they could not be collected and quantified with the sampling methods used in the earlier biomass measurements. The proportions were arcsine-square root transformed and a two-way nested ANOVA was performed in

the same manner as above replacing algal species numbers with total algal proportions. The proportions of *Dictyota* spp. within the zones were analyzed in the same way.

Experiments with Soft Coral Extracts

Sinularia sp. model experiments

To test whether the chemical nature or the shape of *Sinularia* sp. is responsible for producing the algal growth patterns around the soft corals, sixteen colonies were individually extracted in 1:1 (vol:vol) methylene chloride:methanol. The organic and aqueous extracts from each colony were separated. Sixteen individual organic extracts were obtained after evaporating the solvent under vacuum in a rotary evaporator, and sixteen individual aqueous extracts were freeze-dried to powder form. The volume of each colony had been determined by water displacement in a graduated cylinder before the initial extraction.

Two types of models of the same volumes as the soft corals were cast. To test for deterrent properties of the extracts alone, the first models were cast from Nalgene® beaker molds which did not resemble the soft corals except in approximate volume or size. To test for synergistic effects of shape and soft coral extracts on grazing patterns, the second model type was cast from funnel molds as described in the previous model experiment. The organic and water layer extracts were tested separately using the two types of models.

Eight beaker models containing organic extracts redissolved in 5 to 10 ml of methylene chloride were made. Control beaker models containing the same volume of methylene chloride as the respective treatment model were also made. A carrageenan-water mixture, in the ratio 5 g carrageenan:100 ml water, was poured into the beaker molds to the respective volumes of the soft corals (volumes ranged from 95-390 ml) and the redissolved organic extracts were individually stirred into the mixtures before the models solidified. Eight beaker models containing *Sinularia* sp. aqueous extracts redissolved in Milli-Q® water were also made, as well as eight control models containing the respective volumes of Milli-Q® water used to redissolve the extracts. The beaker models ranged in height from 3.5 cm (for a volume of 95 ml) to 6 cm (for a volume of 390 ml). The organic and aqueous extracts were tested on different days. The funnel-shaped models were made using the same carrageenan:water ratio and the remaining organic and aqueous extracts were redissolved and individually stirred into each mixture as described above. These models ranged in height from 6 cm (for a volume of 95 ml) to 9 cm (for a volume of 390 ml).

The models were speared with aluminum dowels and anchored to the reef as in the first model experiment. Those containing extract were paired with their respective control models (no extract) of the same volume, and placed in the field more than 60 cm apart so that their experimental zones did not overlap. Paired 5-cm strands of seagrass were placed at distances of 2, 10 and 30 cm from the models. After waiting periods that varied between 1.5 to 2.5 h, the seagrass strands were removed and the length remaining was measured. The data, as length of seagrass eaten, were converted

to a proportion of the total amount of seagrass available, 9 cm per distance. The proportions were either squared or arcsine-square root transformed and a three-way ANOVA was performed as described in the Statistical Analyses section.

To confirm that the models actually exuded extract into the surrounding water for the duration of the experiment, models containing either organic or aqueous extracts, as well as control models, were placed in separate containers of seawater for 2.5 h after the field experiments were completed. Exudates were qualitatively detected by extracting the seawater in ethyl acetate and performing thin layer chromatography (TLC) on the extracts. TLC analysis of seawater extracts from all models containing organic and aqueous compounds indicated the presence of soft coral extracts, and thus, leaching from the models. TLC analysis of seawater extracts from control models showed no presence of soft coral extracts.

I expected that the seagrass around the beaker models without extract (controls) would be grazed indiscriminately and that the seagrass placed around the models containing either organic or aqueous extracts would be grazed in the same pattern as in the previous grazing experiments using *Sinularia* sp. colonies. I expected similar results for the funnel-shaped models, although perhaps even less seagrass might be grazed at 2 cm than farther away for both extract and control models simply because the shape of the model may hinder grazing. If the latter occurred, I still expected that there would be significantly less grazing around models containing extracts than around control models.

Rehydrated *Sinularia* sp. colonies

I attempted to improve my methods of testing for combined effects of soft coral shape and extracts by using actual soft corals. I extracted 30 *Sinularia* sp. roughly following the method described in Alino *et.al.* (1992). The soft corals were freeze-dried for several days to remove all water before beginning the extractions. Dried, whole soft corals were then extracted in 100% methanol (instead of 95% ethanol) for 20 h, the methanol layer was then decanted and replaced with a 3:1 mixture (vol:vol) of methanol:dichloromethane. Over the next several days, the soft corals were sequentially extracted in 1:1 and 1:3 mixtures of methanol:dichloromethane, and finally in 100% dichloromethane.

The solvent was evaporated from each layer under vacuum in a rotary evaporator and the extracts were combined to obtain the total organic extract content of each soft coral. Fifteen individual extracts were redissolved in dichloromethane and applied evenly to the surface of the corresponding colony. Fifteen extracted colonies were used as controls to which the same volumes of dichloromethane (used to redissolve the extracts) were applied. The soft corals were placed in a fume hood overnight to allow the dichloromethane to evaporate.

Seawater was used to rehydrate the soft corals which were then speared with aluminum dowels which served to anchor and keep the colonies upright when driven into the reef. The extract and control colonies were paired and placed more than 60 cm apart so that their experimental zones did not overlap. Six 5-cm strands of seagrass were placed in two lines around the colonies at distances of 2, 10 and 30 cm.

After 1 h, the strands were collected and measured. The data, as length eaten, were converted to a proportion of the total amount available for grazing (9 cm) and did not require transformation. A three-way ANOVA was performed as described in the Statistical Analyses section.

RESULTS

Distribution of Algae Around *Sinularia* sp.

Total algal biomass around individual soft corals was significantly greater in the 0-10 cm zone than in the10-20 and 20-30 cm zones ($p = 0.002$, $n = 16$, Table 1 and Figure 1). Clusters of colonies exhibited a similar pattern with significantly greater total algal biomass within the clusters than in the 0-10, 10-20 and 20-30 cm zones outside the clusters ($p < 0.0001$, $n = 5$, Table 1 and Figure 1).

Halimeda incrassata and *H. opuntia* were the only algal species that demonstrated the same pattern of growth as the total biomass and were also the major species comprising the total. Around individual *Sinularia* sp., the biomass of *Halimeda* spp. in the 0-10 cm zone was significantly greater than in the 10-20 and 20-30 cm zones ($p = 0.002$, $n = 15$, Table 2 and Figure 2). For clusters of colonies, the biomass of *Halimeda* spp. was significantly greater within the clusters than in the 0-10, 10-20 and 20-30 cm zones ($p = 0.005$, $n = 4$, Table 2 and Figure 2). The biomass of *Dictyota* spp. and epiphytic or algal turf did not show similar patterns of growth (Table 3).

Table 1. Results of two-way ANOVAs without replication of mean wet algal biomass in zones (0-10, 10-20 and 20-30 cm) around 16 individual *Sinularia* sp. and within and around 5 clusters of colonies. Colony or cluster are random factors and zones are fixed factors. Colony × zone (or cluster × zone) is the error term.

Source of variation	df	MS	F	p
Individual Colonies				
Colony	15	6.55	9.26	< 0.0001
Zone	2	5.47	7.73	0.002
Colony × Zone	30	0.71		
Clusters of Colonies				
Clusters	4	3.03	16.93	< 0.0001
Zone	3	2.73	15.24	< 0.0001
Clusters × Zone	12	0.18		

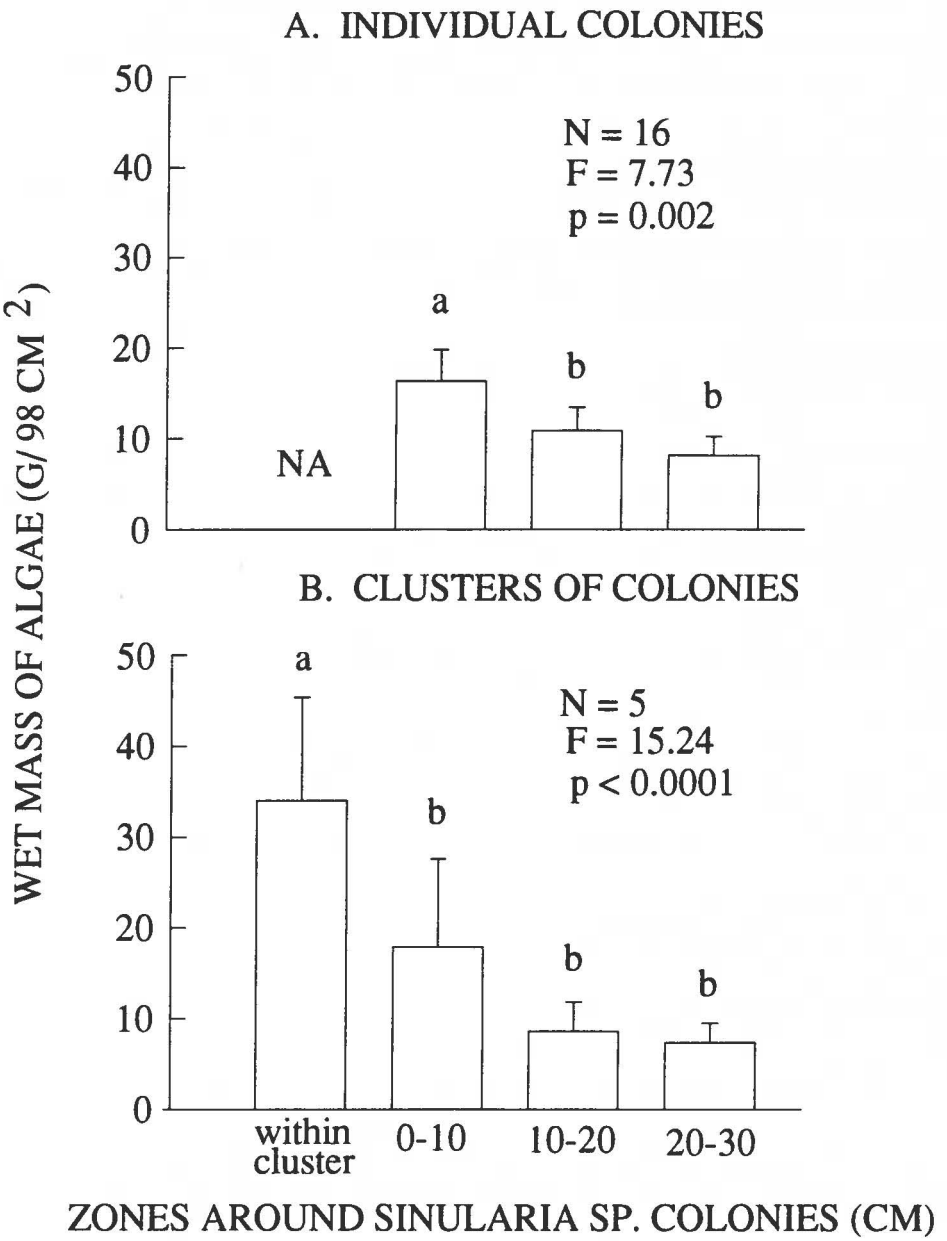

Figure 1. Total algal biomass in zones around 16 individual *Sinularia* sp. colonies (A) and within and around 5 clusters of colonies (B). The data for individual colonies were square root transformed and the data for clusters were natural log transformed before analysis by two-way ANOVA without replication. Untransformed means are presented for clarity. P-values indicate significant difference among zones and vertical bars are +1 SE. Identical letters above bars define means that are not significantly different (Tukey's HSD test, $p < 0.05$).

Table 2. Results of two-way ANOVAs without replication of mean wet biomass of *Halimeda* spp. in zones around 15 individual *Sinularia* sp. and within and around 4 clusters of colonies. The data were square root transformed before analysis. Colony or cluster are random factors and zones are fixed factors. Colony × zone (or cluster × zone) is the error term.

Source of variation	df	MS	F	p
Individual colonies				
Colony	14	6.69	5.90	< 0.0001
Zone	2	9.08	8.00	0.002
Colony × Zone	28	1.13		
Clusters of colonies				
Cluster	3	15.19	11.00	0.002
Zone	3	12.45	9.02	0.005
Cluster × Zone	9	1.38		

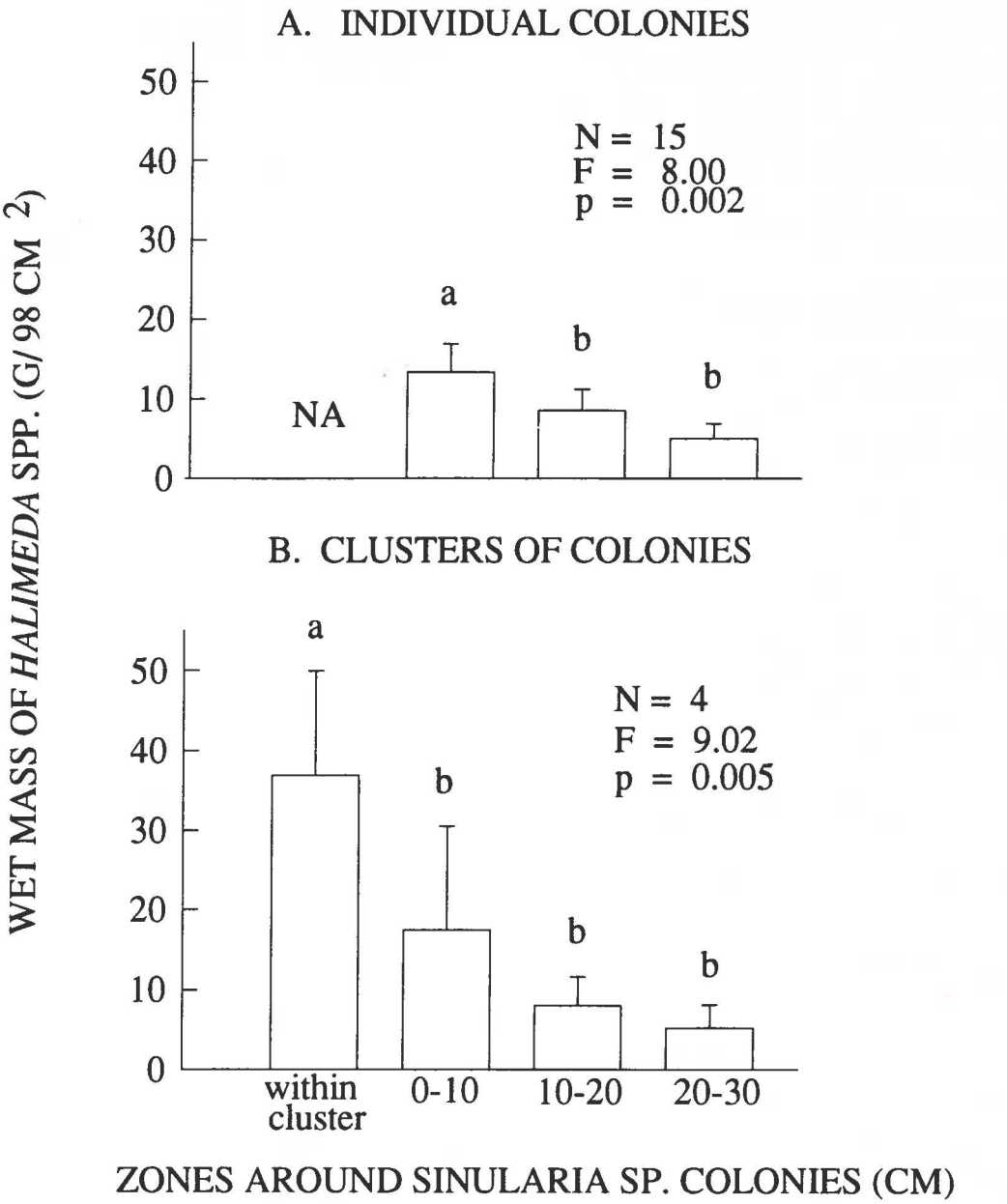

Figure 2. Biomass of *Halimeda* spp. in zones around 15 individual *Sinularia* sp. colonies (A) and within and around 4 clusters of colonies (B). The data were square root transformed before analysis by two-way ANOVA without replication. Untransformed means are presented for clarity. P-values indicate significant differences among zones and vertical bars are +1 SE. Identical letters above bars define means that are not significantly different (Tukey's HSD test, $p < 0.05$).

Table 3. Results of two-way ANOVAs without replication of mean wet biomass of *Dictyota* spp. (D) and algal turf or epiphytes (E) in zones (0-10, 10-20 and 20-30 cm) around individual *Sinularia* sp. and clusters of colonies. Colony or cluster are random factors and zones are fixed factors. Colony × zone (or cluster × zone) is the error term.

Source of variation	df	MS		F		p	
		D	**E**	**D**	**E**	**D**	**E**
Individual colonies							
Colony	15	3.784	2.165	11.53	5.70	< 0.0001	< 0.0001
Zone	2	0.282	0.374	0.86	0.98	0.434	0.385
Colony × Zone	30	0.328	0.380				
Clusters of colonies							
Cluster	4	4.772	2.310	8.49	2.15	0.002	0.137
Zone	3	0.476	0.426	0.85	0.40	0.495	0.758
Cluster × Zone	12	0.562	1.073				

Halimeda incrassata grazing experiment

Halimeda incrassata strands placed in the open away from soft corals (n = 77) had significantly greater losses than either strands placed next to soft corals (n = 74), or control strands (n = 50) ($p < 0.0001$, Figure 3). Since losses to controls were minimal, this suggested that grazing was responsible for most of the amount removed from strands placed in the open. Losses to controls were also significantly different from losses to strands placed next to soft corals, indicating that some grazing did occur next to soft corals (Figure 3).

Acanthophora spicifera grazing experiment

There were significant differences in grazing among distances ($p = 0.002$, n = 17, Table 4). Tukeys HSD test ($p < 0.05$) showed that *A. spicifera* strands placed 30 cm from *Sinularia* sp. bases were grazed significantly more than strands placed 2 cm from the soft corals (Tukey's HSD, Figure 4).

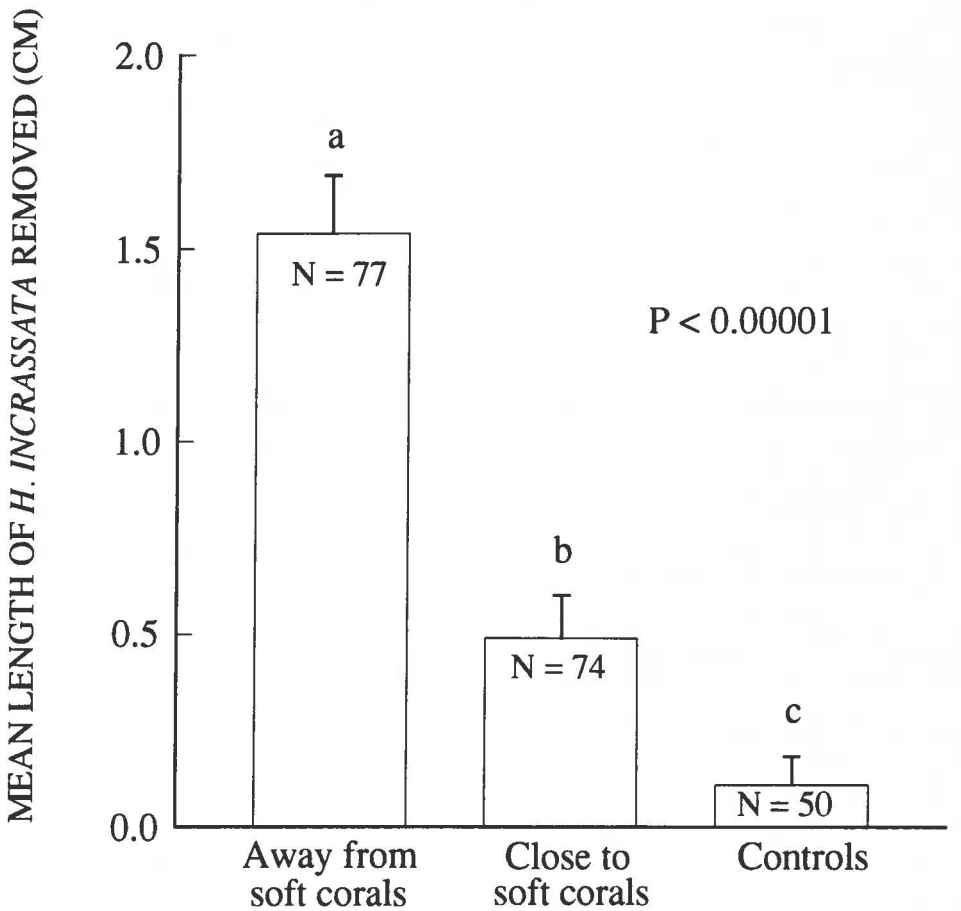

Figure 3. Mean lengths of *Halimeda incrassata* removed by either grazing or breakage (controls). P-value indicates that the means are significantly different (Kruskal-Wallis Test). Different letters above the bars indicate that there are no homogenous means (nonparametric Student Newman-Keuls multiple comparison test). Vertical bars are +1 SE.

Table 4. Results of a two-way ANOVA without replication of lengths of *Acanthophora spicifera* strands eaten when placed at distances 2, 10, and 30 cm from the bases of 17 individual *Sinularia* sp. The data were converted to a proportion of the total amount of algae available for grazing and then arcsine-square root transformed. Colony is the random factor, distance is the fixed factor, and colony × distance is the error term.

Source of variation	df	MS	F	p
Colony	16	0.15	2.69	0.008
Distance	2	0.42	7.54	0.002
Colony × Distance	32	0.06		

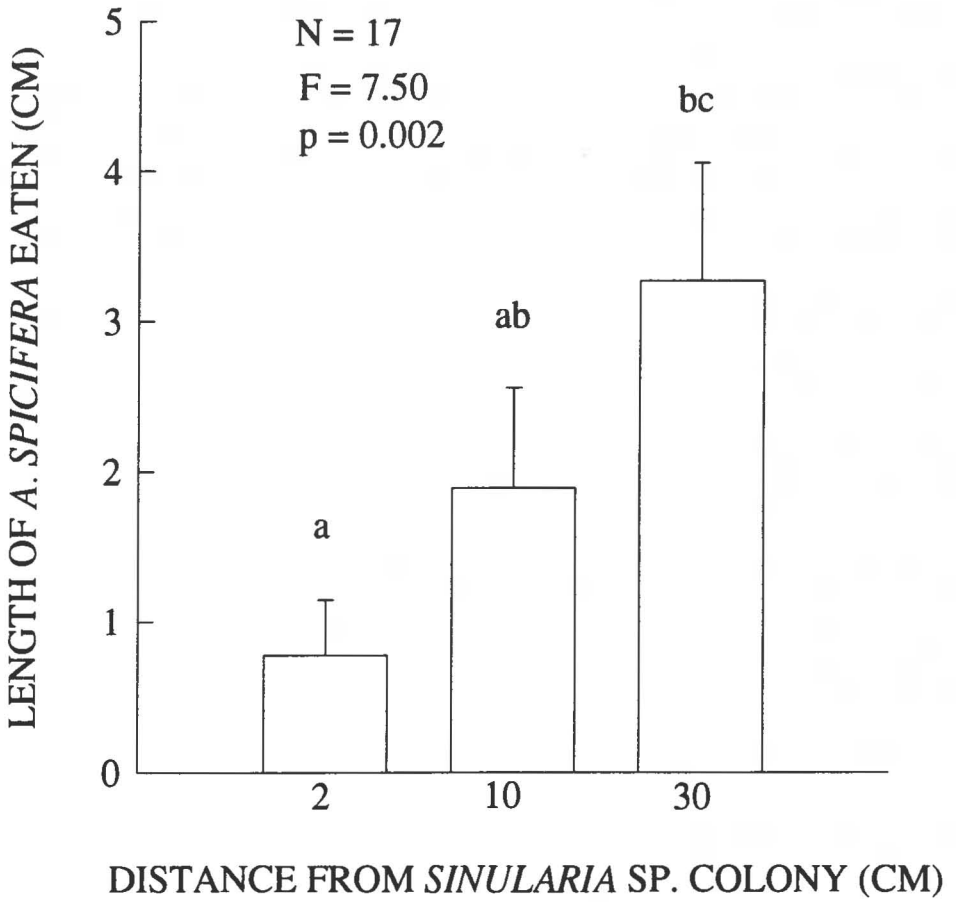

Figure 4. Grazing experiment with *Acanthophora spicifera* strands placed 2, 10, and 30 cm from 17 *Sinularia* sp. colonies. Data were converted to a proportion of the total length available for grazing and were then arcsine-square root transformed. A two-way ANOVA without replication was performed and p-value indicates that length grazed is significantly different among the distances. Untransformed means are presented for clarity and vertical bars are +1 SE. Identical letters above bars define means that are not significantly different (Tukey's HSD, $p < 0.05$).

Algal removal - grazing experiment

Neither treatment ($p = 0.768$) nor the interaction term of treatment × distance ($p = 0.169$) had a significant effect ($n = 12$, Table 5). That is, intact macroalgae at the bases of the soft corals did not prevent fish from locating and grazing seagrass strands placed next to the soft corals. Because there was a significant effect for distance ($p < 0.0001$, Table 5), I examined the treatments separately and found that significantly less seagrass was eaten at 2 cm than 30 cm distant from both algal removal and intact colonies (Tukey's HSD, Figure 5). This is consistent with previously observed patterns of grazing (Figure 4).

Combined grazing - object experiment

There was no significant difference between treatments ($p = 0.114$). However, significant effects were obtained for the terms treatment × distance ($p = 0.003$) and distance ($p = 0.001$, $n = 15$, Table 6). To determine the source of the interaction, I examined the treatments separately. For soft corals ($n = 19$), significantly less seagrass was grazed at 2 cm than 10 and 30 cm away, whereas, grazing occurred indiscriminately around objects ($n = 15$, Tukey's HSD, Figure 6). This indicated that more than the presence of an object alone is needed to produce the grazing pattern observed around soft corals.

Table 5. Results of a two-way nested ANOVA of lengths of *Halodule uninervis* eaten at distances of 2, 10, and 30 cm around 12 individual *Sinularia* sp. from which algae had been removed (from a radius of 10 cm from the bases), and 12 colonies with surrounding algae left intact. The data were first converted to a proportion of the total length of seagrass available for grazing and then arcsine-square root transformed. Treatment and distance are fixed factors, and individual colonies are random factors nested within treatments.

Source of variation	df	MS	F	p
Treatment (Algae removed or intact)	1	0.010	0.09	0.768
Colony	11			
Distance	2	1.495	28.20	< 0.0001
Treatment × Distance	2	0.098	1.85	0.169
Treatment × Colony	22	0.115		
Treatment × Colony × Distance	44	0.053		

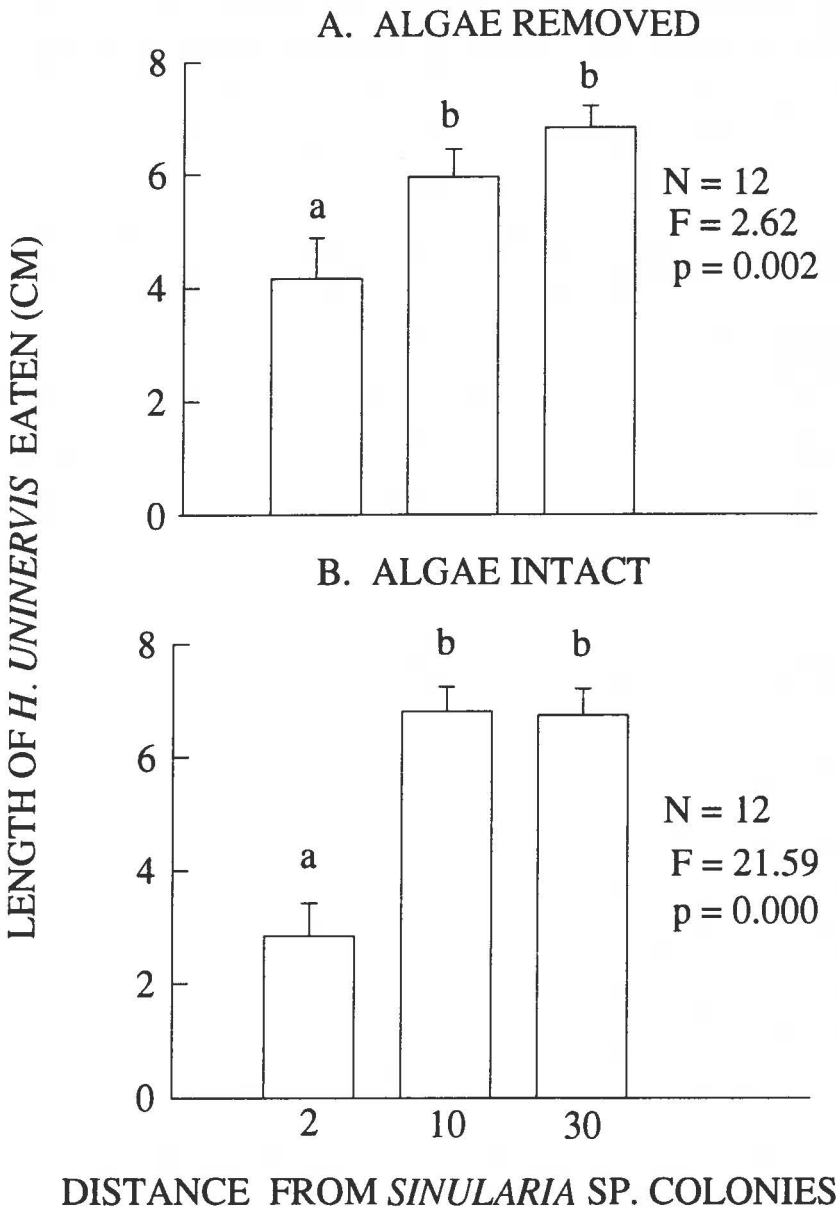

Figure 5. Mean lengths of *Halodule uninervis* grazed around soft corals from which algae had been removed (from a radius of 10 cm around the bases) (A), and colonies around which algae were left intact (B). Data were converted to a proportion of the total length available for grazing and then arcsine-square root transformed. Two-way ANOVAs without replication were performed separately for each treatment. P-values indicate significant difference in length grazed among the distances. Vertical bars are +1 SE. Identical letters above bars define means that are not significantly different (Tukey's HSD, $p < 0.05$). Untransformed means are presented for clarity.

Table 6. Results of a two-way nested ANOVA of lengths of *H. uninervis* strands grazed at distances of 2, 10 and 30 cm around 15 individual *Sinularia* sp. and 15 objects (rocks or outcrops of hard coral). The data were converted to a proportion of the total amount of seagrass available for grazing and then arcsine-square root transformed. Treatment (soft corals or objects) and distance are fixed factors, and sites are random factors nested within treatments.

Source of variation	df	MS	F	p
Treatment (soft corals or objects)	1	0.679	2.66	0.114
Site	14			
Distance	2	0.604	8.72	0.001
Treatment × Site	28	0.255		
Treatment × Distance	2	0.443	6.39	0.003
Treatment × Site × Distance	56	0.069		

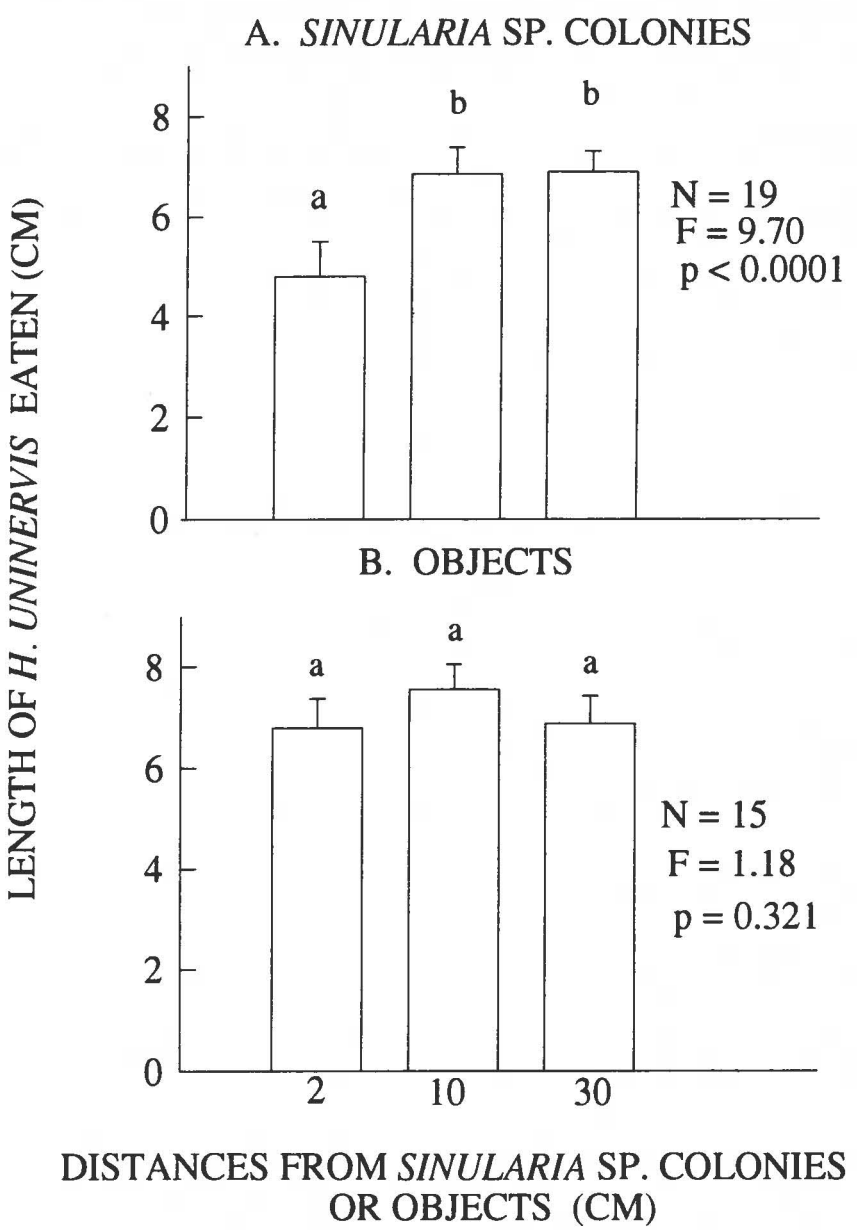

Figure 6. Mean lengths of *H. uninervis* grazed around 19 soft corals (A) and 15 objects (rocks or outcrops of dead hard coral (B). Data were converted to a proportion of the total length available for grazing and then arcsine-square root transformed. A two-way ANOVA without replication was performed separately for each treatment. P-values < 0.05 indicate significant difference in length grazed among distances. Vertical bars are +1 SE. Identical letters above bars define means that are not significantly different (Tukey's HSD test, $p < 0.05$). Untransformed means are presented for clarity.

Grazing experiment around *Sinularia* sp. removal and control sites

There was no significant difference between treatments ($p = 0.993$). However, there was a significant interaction between treatment and zone ($n = 11$, $p = 0.002$, Table 7). To determine the source of the interaction, I examined the treatments separately. For intact colonies ($n = 11$), significantly less seagrass was grazed at 2 cm than at 30 cm (Tukey's HSD, Figure 7). For removal sites ($n = 11$), the amount of seagrass grazed was also significantly different among the distances, however, the pattern had been disrupted and grazing had occurred indiscriminately (Tukey's HSD, Figure 7). This suggested that the presence of *Sinularia* sp. influenced the pattern of grazing around the soft coral.

Grazing experiment around soft coral models

There were significant differences among the distances ($p < 0.0001$, Table 8). Less seagrass was grazed at 2 cm than 10 and 30 cm distant from the models ($n = 15$, Figure 8). This suggested that the shape of *Sinularia* sp. is involved in producing the observed grazing patterns.

Table 7. Results of a two-way nested ANOVA of lengths of seagrass grazed at distances of 2, 10 and 30 cm around 11sites with intact individual *Sinularia* sp. and 11 sites from which colonies had been removed. Data were converted to a proportion of the total length available for grazing and then arcsine-square root transformed before analysis. Treatment (colony intact or removed) and distance are fixed factors, and sites are random factors nested within treatments.

Source of variation	df	MS	F	p
Treatment (soft corals intact or removed)	1	0.000	0.000	0.993
Site	10			
Distance	2	0.232	2.360	0.107
Treatment × Site	20	0.334		
Treatment × Distance	2	0.730	7.420	0.002
Treatment × Site × Distance	40	0.098		

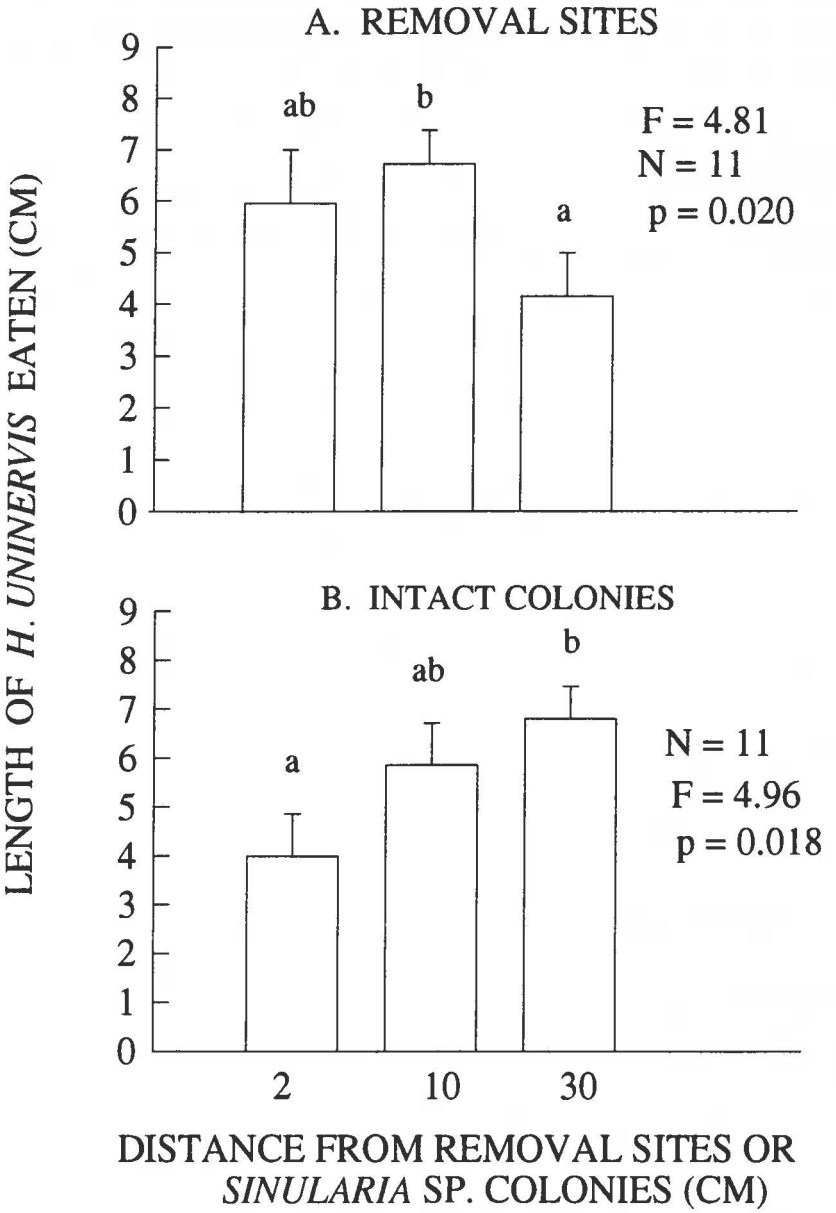

Figure 7. Mean lengths of *H. uninervis* grazed around 11 soft corals (B) and sites from which soft corals had been removed (A). Data were converted to a proportion of the total length available for grazing and then arcsine-square root transformed. Two-way ANOVAs without replication were performed separately for each treatment. P-values indicate significant differences in lengths grazed among the distances. Vertical bars are +1 SE. Identical letters above bars define means that are not significantly different (Tukey's HSD test, $p < 0.05$). Untransformed means are presented for clarity.

Table 8. Results of a two-way ANOVA without replication of lengths of *H. uninervis* grazed at distances of 2, 10 and 30 cm from 15 funnel-shaped carrageenan models. The data were converted to a proportion of the total length available for grazing and then arcsine-square root transformed before analysis. Model is random and distances are fixed.

Source of variation	df	MS	F	p
Model	14	0.095	2.330	0.028
Distance	2	0.560	13.780	< 0.0001
Model × Distance	28	0.041		

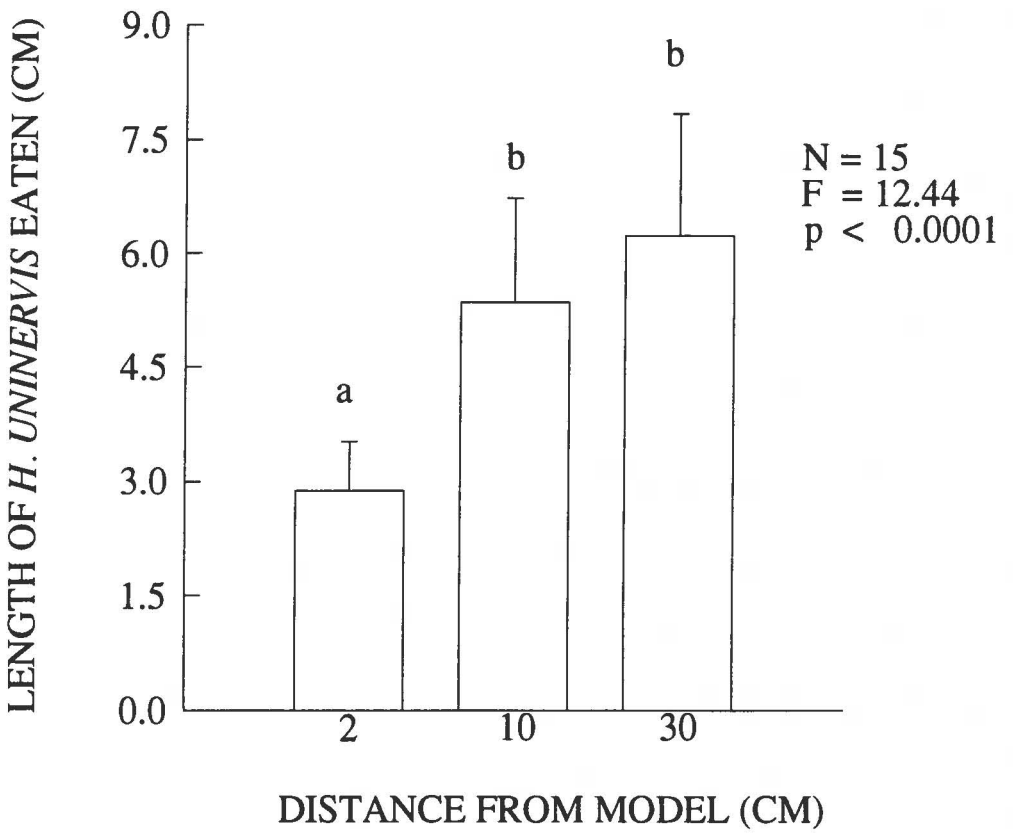

Figure 8. Mean length of seagrass grazed at distances of 2, 10, and 30 cm from 15 funnel-shaped models. Data were converted to a proportion of the total amount available for grazing and arcsine-square root transformed. A two-way ANOVA without replication was performed and p-value indicates that the amount of seagrass grazed was significantly different among the distances. Vertical bars are +1 SE. Identical letters above bars define means that are not significantly different (Tukey's HSD test, $p < 0.05$). Untransformed means are presented for clarity.

Removal Experiments and Algal Species Richness

Sinularia sp. removal experiments

There were no significant effects of treatment ($p = 0.716$) and treatment × zone ($p = 0.758$, $n = 7$, Table 9). This indicated that removing the soft corals did not encourage grazing of exposed macroalgae. However, there was a significant effect for zone ($p = 0.001$, Table 9) which suggested the same pattern of greater algal biomass next to soft corals than farther away. As in the previous biomass measurements, *Halimeda* spp. comprised the bulk of the total biomass in each zone around both removal and control sites. Upon examining the treatments separately, algal growth around intact colonies was significantly greater closer to the colonies than farther away, whereas removal sites had no pattern (Tukey's HSD, Figure 9).

For the longterm removal experiment, there was also no significant difference between treatments ($n = 11$, $p = 0.609$, Table 10). However, as above, there was a significant effect for zone ($p = 0.002$). I examined the treatments separately and found that algal growth decreased with distance around both intact colonies and removal sites (Tukey's HSD, Figure 10).

Algal species richness around soft corals and removal sites

No significant effects were observed for treatment ($p = 0.152$), treatment × distance ($p = 0.645$), and zone (0.501, Table 11). That is, species richness in zones

Table 9. Results of a two-way nested ANOVA of mean wet biomass of algae collected from zones (0-10, 10-20, and 20-30 cm) around 7 sites with intact *Sinularia* sp. and 7 sites from which colonies had been removed. Data were square root transformed before analysis. Treatments (*Sinularia* sp. colony intact or removed) and zones are fixed factors, and sites are random factors nested within treatments.

Source of variation	df	MS	F	p
Treatment (colony removed or intact)	1	0.479	0.140	0.716
Site	6			
Zone	2	3.500	10.440	0.001
Treatment × Site	12	3.460		
Treatment × Zone	2	0.094	0.280	0.758
Treatment × Site× Zone	24	0.335		

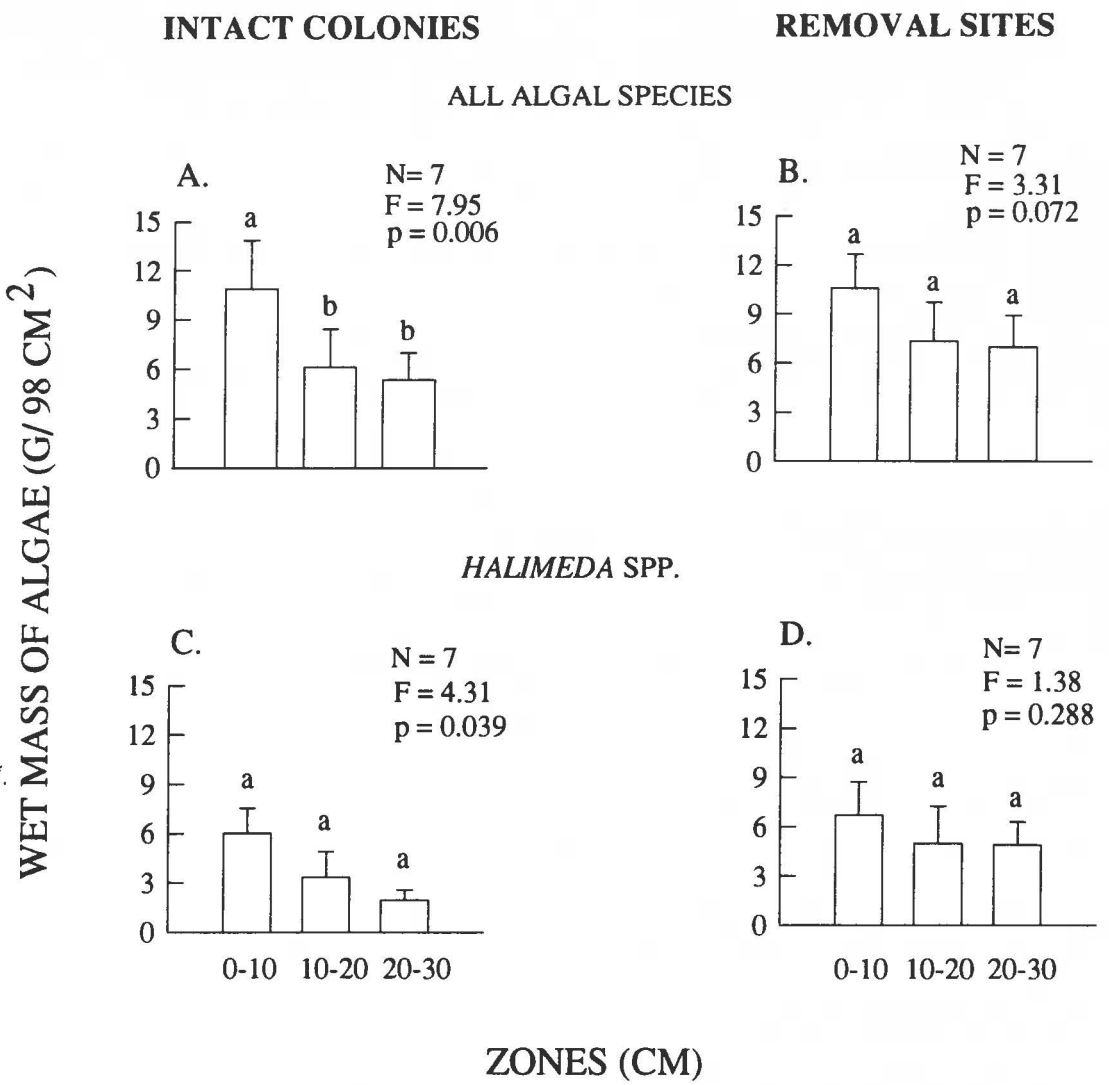

Figure 9. Mean biomass of all algal species (A,B), and *Halimeda* spp. (C,D) in zones around intact soft corals and sites from which colonies had been removed. Data were square root transformed before analysis by two-way ANOVA without replication. Untransformed means are presented for clarity. P-values < 0.05 indicate that mean biomass differs significantly among the zones. Vertical bars are +1 SE. Identical letters above bars define means that are not significantly different (Tukey's HSD test, $p < 0.05$).

Table 10. Results of a two-way nested ANOVA of mean wet algal biomass in zones 0-10, 10-20 and 20-30 cm around 11 sites with individual *Sinularia* sp., and 11 sites from which colonies had been removed. (Data were natural log transformed before analysis.) Treatments (colony intact or removed) and zones are fixed factors, and sites are random factors nested within treatments.

Source of variation	df	MS	F	p
Treatment (colony intact or removed)	1	1.858	1.870	0.186
Site	10			
Zone	2	2.094	7.290	0.002
Treatment × Site	20	0.992		
Treatment × Zone	2	0.144	0.500	0.609
Treatment × Site × Zone	40	0.288		

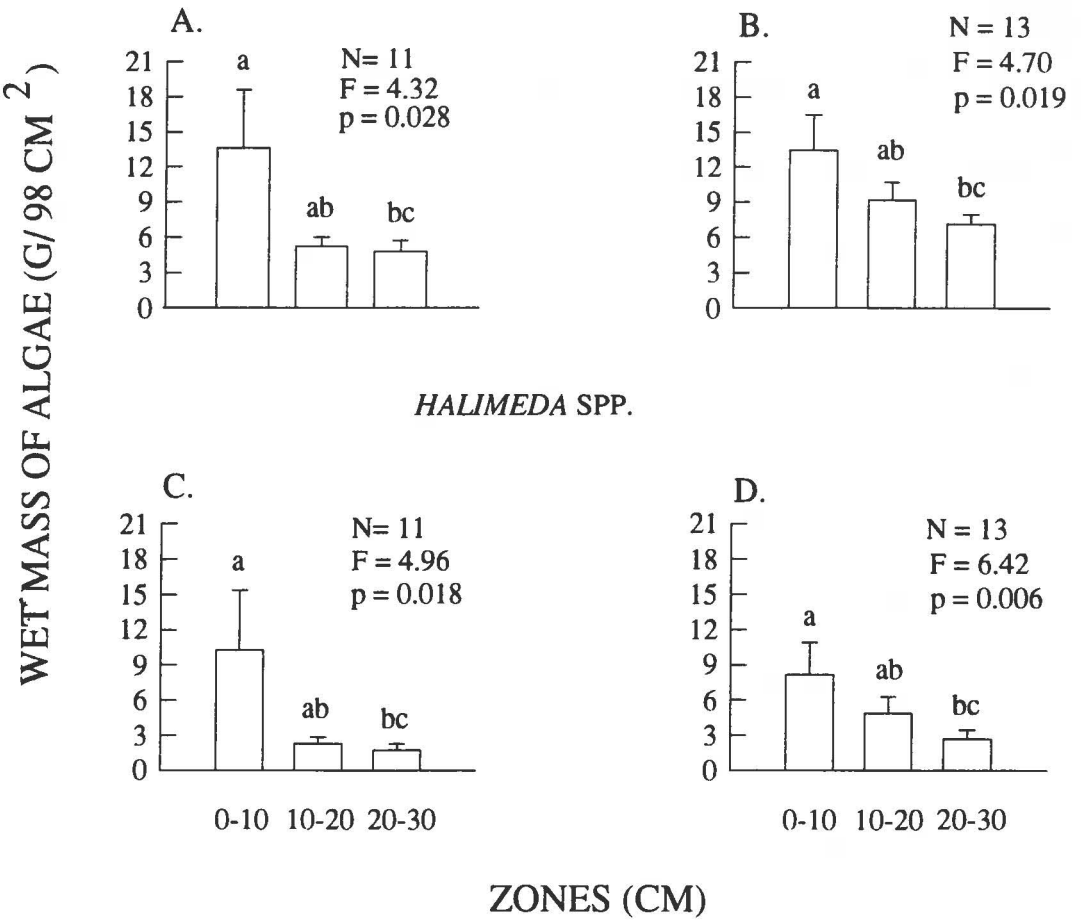

Figure 10. Mean biomass of all algal species (A,B) and *Halimeda* spp. (C,D) in zones around intact soft corals and sites from which colonies had been removed. Data were natural log transformed before analysis by two-way ANOVA without replication. Untransformed means are presented for clarity. P-values < 0.05 indicate that mean biomass differs significantly among zones. Vertical bars are +1 SE. Identical letters above bars define means that are not significantly different (Tukey's HSD test, $p < 0.05$).

Table 11. Results of a two-way nested ANOVA of the number of algal species found in zones, 0-10, 10-20, and 20-30 cm, around 11 sites with intact soft corals and 11 sites from which soft corals had been removed. The data did not require transformation before analysis. The number of species is the dependent variable, treatments and zones are fixed factors, and sites are random factors nested within treatments.

Source of variation	df	MS	F	p
Treatment (soft coral colony removed or intact)	1	62.061	2.22	0.152
Site	10			
Zone	2	3.197	0.70	0.501
Treatment × Zone	2	2.015	0.44	0.645
Treatment × Site	20	27.949		
Treatment × Site × Zone	40			

around intact colonies (n = 11) was similar to that found around removal sites (n = 11). Also, species richness was not correlated with distance from the colonies.

Longterm *Sinularia* sp. - algal removal experiment

For proportions of all algae, there were no significant effects of treatment ($p = 0.384$), zone ($p = 0.426$), and treatment × zone ($p = 0.486$, Table 12). The proportions of *Dictyota* spp., which were the dominant macroalgae, did not show any difference between treatments ($p = 0.890$, Table 12). Thus, algal recruitment was the same around intact colonies (n = 15) and removal sites (n = 15) at this stage of recruitment.

Experiments with Soft Coral Extracts

Beaker-shaped models with *Sinularia* sp. organic extracts

There were no significant effects of treatment ($p = 0.680$), treatment × distance ($p = 0.077$), and distance ($p = 0.455$, Table 13). That is, the fish grazed seagrass equally around models that contained extract and control models (n = 8). There was no interaction effect for location × model, thus pairing the extract and control models did not influence grazing around the sites ($p = 0.296$, Table 13).

Beaker-shaped models with *Sinularia* sp. aqueous extracts

As above, there were no significant effects of treatment ($p = 0.270$), treatment × distance ($p = 0.112$), and distance ($p = 0.051$, Table 13). The amount of

Table 12. Results of a two-way nested ANOVA of proportions of all algae (except cyanobacteria and crustose corallines) (TA) and *Dictyota* spp. (D) which had recruited to zones, 0-10, 10-20 and 20-30 cm, around 15 sites with intact *Sinularia* sp. and 15 sites from which soft corals had been removed. The proportions were arcsine-square root transformed before analysis. Treatments (soft coral colony intact or removed) and zones are fixed factors, and sites are random factors nested within treatments.

Source of variation	df	MS		F		p	
		TA	**D**	**TA**	**D**	**TA**	**D**
Treatment (colony removed or intact)	1	0.157	0.076	0.78	0.56	0.384	0.459
Site	14						
Zone	2	0.034	0.059	0.87	1.77	0.426	0.179
Treatment × Site	28	0.201	0.135				
Treatment × Zone	2	0.029	0.004	0.73	0.12	0.486	0.890
Treatment × Site × Zone	56	0.039	0.033				

Table 13. Results of two three-way ANOVAs of lengths of *H. uninervis* grazed at distances of 2, 10 and 30 cm around beaker-shaped carrageenan models containing *Sinularia* sp. organic extracts (O) paired with control models (n = 8), and models containing aqueous extracts (A) paired with control models (n = 8). The data were converted to a proportion of the total length available for grazing and then arcsine-square root transformed before analysis.

Source of variation	df	MS		F		p	
		O	**A**	**O**	**A**	**O**	**A**
Treatment (extract models or controls)	1	0.071	0.167	0.19	1.43	0.680	0.270
Location	7	0.223	0.241	0.58	2.07	0.755	0.180
Distance	2	0.076	0.254	0.83	3.71	0.455	0.051
Treatment × Location	7	0.384	0.117				
Treatment × Distance	2	0.284	0.176	3.10	2.57	0.077	0.112
Location × Distance	14	0.123	0.090	1.34	1.32	0.296	0.305
Treatment × Location × Distance	14	0.092	0.068				

seagrass grazed around the models containing aqueous extracts (n = 8) was not different from the amount grazed around the controls (n = 8). There was also no significant effect of location × distance, thus pairing extract and control models in the field had no influence on the amount of seagrass grazed among the distances (p = 0.305, Table 13).

Funnel-shaped models with *Sinularia* sp. organic extracts

There were no significant effects of treatment (p = 0.329) and treatment × distance (p = 0.561) (Table 14). Fishes grazed just as much seagrass around the extract models (n = 8) as around the controls (n = 8). Pairing the extract and control models also had no influence on grazing (location × distance, p = 0.835, Table 14). However, there was a significant effect for distance (p = 0.001, Table 14) which suggested that grazing occurred in the same pattern as observed for *Sinularia* sp. (Figure 4) and the first model experiment (Figure 8). I examined the treatments separately as described in the Statistical Analyses section. For extract models, significantly less seagrass was grazed next to the models than farther away (Tukey's HSD, Figure 11). For control models, the means were not significantly different (Tukey's HSD, Figure 11).

Funnel-shaped models with *Sinularia* sp. aqueous extracts

As above, there were no significant effects of treatment (p = 0.548) and treatment × distance (p = 0.464, Table 14). Pairing of the extract and control

Table 14. Results of 2 three-way ANOVAs of lengths of *H. uninervis* grazed at distances of 2, 10 and 30 cm around funnel-shaped carrageenan models containing *Sinularia* sp. organic extracts (O) paired with control models (n = 8), and models containing aqueous extracts (A) paired with control models (n = 8). The data were first converted to a proportion of the total length available for grazing. The data (proportions) for the organic extract models (and controls) were arcsine-square root transformed and the data for the aqueous extract models (and controls) required transformation by squaring before analysis.

Source of variation	df	MS		F		p	
		O	**A**	**O**	**A**	**O**	**A**
Treatment (extract models or controls)	1	0.150	0.020	1.10	0.40	0.329	0.548
Location	7	0.182	0.159	1.34	3.13	0.356	0.078
Distance	2	1.144	0.411	11.65	12.87	0.001	0.001
Treatment × Location	7	0.136	0.051				
Treatment × Distance	2	0.059	0.026	0.60	0.81	0.561	0.464
Location × Distance	14	0.058	0.031	0.59	0.96	0.835	0.533
Treatment × Location × Distance	14	0.098	0.032				

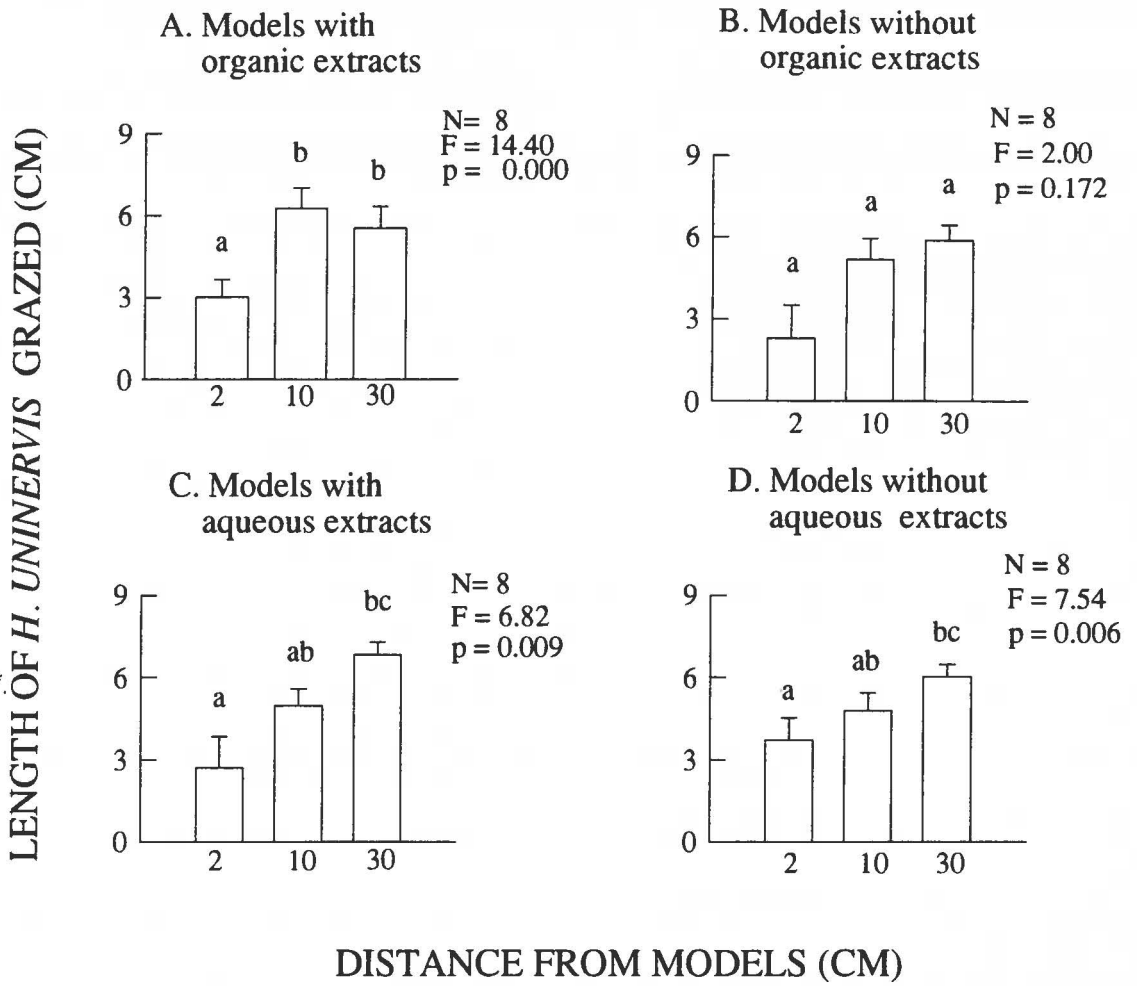

Figure 11. Mean lengths of seagrass grazed around funnel-shaped carrageenan models containing *Sinularia* sp. organic (A) and aqueous (C) extracts, and control models (B,D). Data were first converted to a proportion of the total amount available for grazing and then either arcsine-square root (A) or square transformed (B,C,D). Two-way ANOVAs without replication were performed separately for extract and control models. P-values < 0.05 indicate that lengths of seagrass grazed are significantly different among distances. Identical letters above bars define means that are not significantly different (Tukey's HSD test, $p < 0.05$). Untransformed means are presented for clarity.

models (n = 8) also had no effect on grazing (location × zone, p = 0.533, Table 14). However, there was a significant effect of distance (p = 0.001, Table 14). This suggested that differential grazing has also occurred around these models. I examined the treatments separately, as previously described , and found that for both extract and control models, significantly less seagrass had been grazed at 2 cm than 30 cm distant from the models (Tukey's HSD, Figure 10).

Rehydrated *Sinularia* sp.

There were no significant effects of treatment (p = 0.791) and treatment × distance (p = 0.066, Table 15). That is, the amount of seagrass grazed around colonies coated with extract (n = 15) was not different from that grazed around control colonies (n = 15). Pairing the extract and control colonies in the field also did not affect grazing around the colonies (location × distance, p = 0.535, Table 15). However, there was a significant effect of distance (p < 0.0001, Table 15) which suggested that the pattern of grazing remained around rehydrated colonies. I examined the treatments separately as previously described. For extract colonies, the means were not significantly different (Tukey's HSD, Figure 12). For control colonies, significantly less seagrass was grazed next to the colonies than farther away (Tukey's HSD, Figure 12).

Table 15. Results of a three-way ANOVA of lengths of *H. uninervis* grazed at distances of 2, 10 and 30 cm around 15 rehydrated *Sinularia* sp. colonies coated with organic extract, and 15 rehydrated colonies without extract. The data were converted to a proportion of the total length available for grazing and did not require transformation before analysis.

Source of variation	df	MS	F	p
Treatment (extract models or controls)	1	0.007	0.07	0.791
Location	7	0.215	2.34	0.061
Distance	2	0.491	12.61	< 0.0001
Treatment × Location	7	0.092		
Treatment × Distance	2	0.117	3.01	0.066
Location × Distance	14	0.038	0.97	0.535
Treatment × Location × Distance	14	0.039		

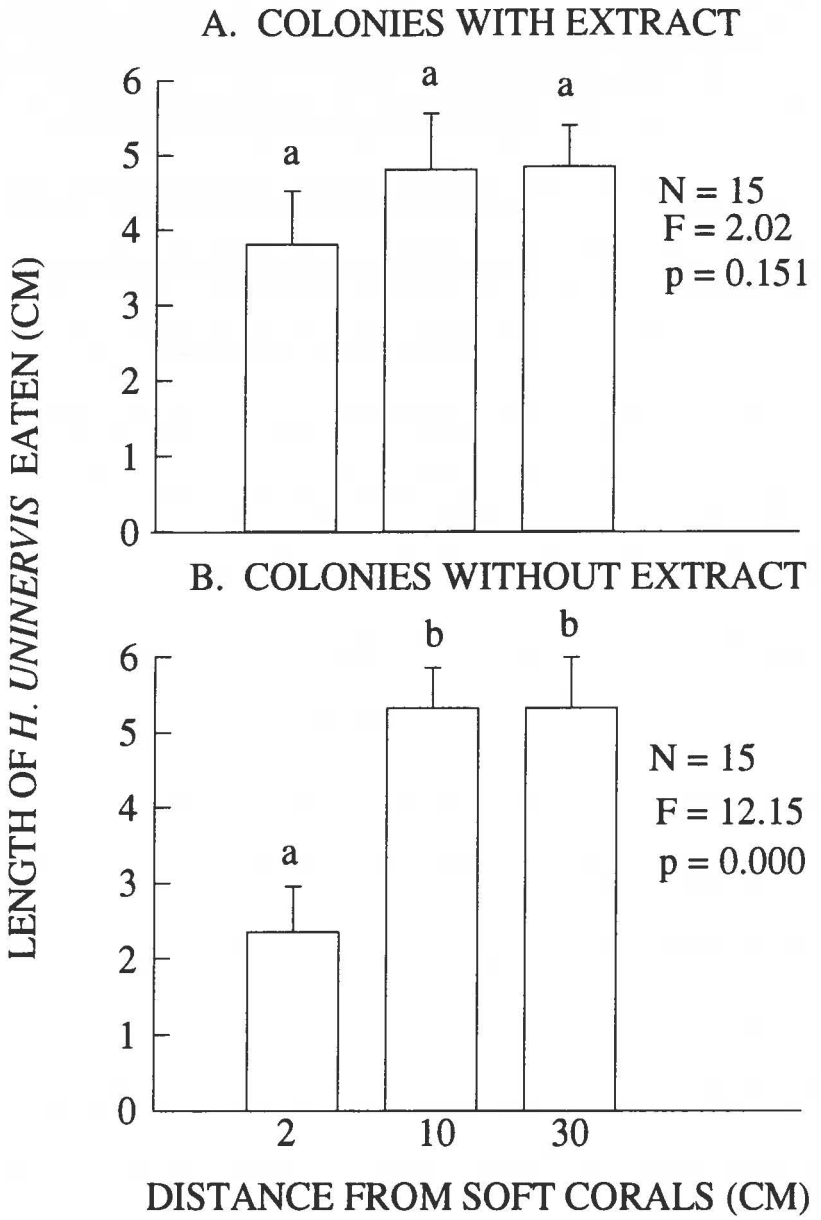

Figure 12. Mean lengths of seagrass grazed 2, 10, and 30 cm around 15 rehydrated *Sinularia* sp. colonies coated with organic extract (A), and 15 rehydrated colonies without extract (B). Data were converted to a proportion of the total length available for grazing and did not require transforming before analysis. Two-way ANOVAs without replication were performed separately for extract and control colonies and p-value < 0.05 indicates that the mean lengths of seagrass grazed are significantly different among the distances. Vertical bars are +1 SE. Identical letters above bars define means that are not significantly different (Tukey's HSD test, $p < 0.05$). Untransformed means are presented for clarity.

DISCUSSION

Sampling around individual soft coral colonies and clusters of colonies revealed that total algal biomass was greater closer to the colonies than farther away. *Halimeda* spp., which comprised the bulk of the biomass around the colonies, was the only genus that duplicated the pattern of decreasing biomass with distance from the soft corals. Many species of herbivorous fishes do not readily consume *Halimeda* spp. because they possess chemical (Paul and Hay 1986, Targett *et al.* 1986, Paul and Van Alstyne 1988 and 1992) and structural (Hay 1984, Lewis 1985, Schupp and Paul in press) defenses. However, parrotfish have been observed grazing young plants and newly formed tips of *Halimeda* plants, and rarely grazed mature, more calcified, segments (Hay *et al.*1988). In this study, fishes grazed significantly more young *Halimeda incrassata* strands that were placed in the open, than strands placed next to soft corals (Figure 3). Perhaps settling next to soft corals allows *Halimeda* to become established and mature to lengths that would not be possible farther away, since newly recruited algae growing away from soft corals would be grazed readily by parrotfish.

This hypothesis parallels the role of nurse plants in terrestrial habitats. For instance, the shrubs *Salvia leucophylla* and *Artemisia californica* form canopies which prevent herbivores, such as squirrels, pigs, and cattle, from locating and eating seedlings of the oak *Quercus douglasii* (Callaway 1992). Removal of the canopies resulted in shoot mortality similar to that observed in open grasslands (Callaway 1992). Similarly, perennial plants such as, *Ambrosia deltoidea* and *A. dumosa*, provide

dense cover that protects seedlings of the desert tree *Cercidium microphyllum* from grazing by rabbits (McAuliffe 1986).

Grazing of *Acanthophora spicifera* and *Halodule uninervis* revealed a pattern of differential grazing around the soft corals which was similar to the algal growth patterns. Established algae growing near the bases of soft corals did not prevent fish from locating and grazing seagrass strands placed close to the soft corals. When algae were removed from the bases of soft corals, the same grazing patterns were observed (Figure 5). Thus, the soft corals, and not associated macroalgae, appear to be responsible for producing the experimental grazing and algal growth patterns.

The presence of an object, other than a soft coral, did not present an obstacle to herbivory. As expected, the fish grazed indiscriminately around the rocks or outcrops of hard coral, whereas differential grazing occurred around the soft corals (less seagrass was eaten closer to the colonies than farther away) (Figure 6). Those results suggested that more than just the presence of an object prevented fish from grazing algae growing next to the soft coral. Subsequently, the results of the grazing experiment around funnel-shaped carrageenan models suggested that the shape of the soft corals may have an influence on the observed grazing patterns. These models, which were schematic representations of the arborescent structure of *Sinularia* sp. colonies, produced differential grazing patterns (Figure 8). However, the obstacles did not have an arborescent shape, which could explain the indiscriminate grazing that occurred around them.

Initially I thought that the inconclusive results of the first *Sinularia* sp. removal experiment could be explained by premature sampling of the sites. In the second soft coral removal experiment, the 4 mo waiting period should have allowed sufficient time for the fish to graze the algae around the removal scars so that a more obvious difference between the removal and control sites could be detected. Although a difference between treatments was not evident, total algal biomass around intact colonies and removal sites showed the same pattern of algal growth as observed in the earlier sampling survey. One possible explanation for these results is that the macroalgal species exposed by removing the soft corals were not particularly preferred by fish. *Halimeda* spp. that were exposed when soft corals were removed may have been too mature, i.e., highly calcified, for parrotfish to graze, and any grazing that occurred would most likely be restricted to newly formed tips (Hay *et al.* 1988). Also, juvenile rabbitfish, which recruited in great numbers to the waters around Guam in May 1994, appeared to have grazed only the tips of *Halimeda* plants located either next to soft corals, or in other areas of the study site (personal observation).

Although I did not test for the possibility that the areas around the bases of soft corals provide a substrate favorable for algal recruitment and settlement, it does not appear to be a likely explanation for the observed patterns of algal growth. At the study site, stands of *Halimeda* spp. and other macroalgae have been observed growing in other locations where grazing is less intense. For example, such algal stands are common around crevices in which territorial damselfish reside. Also, the structure of

the reef itself provides numerous holes and crevices which enable algae to become established and escape herbivory.

Algal species richness was not greater around the bases of soft corals than farther away. This was not consistent with results obtained in other studies of defense associations (Hay 1985 and 1986, McNaughton 1985, Littler *et al.*1986, Littler *et al.* 1987). For instance, several macroalgal species were significantly associated with *Stypopodium zonale* within a radius of 2 cm around the highly toxic alga, and twice as many taxa were found within a 10 cm radius of the alga as within 10 cm of points in which *Stypopodium* was absent (Littler *et al.* 1986). Also, algal species richness was positively correlated with percent cover of the unpalatable alga, *Sargassum filipendula*, (Hay 1986). Furthermore, in Serengeti grasslands, vegetation resilience to grazing ungulates was positively correlated with plant species diversity (McNaughton 1985). In this study, most of the species found around soft corals were rhodophycean turf or epiphytes. These species were associated with either *Dictyota* or *Halimeda*, which may provide protection by either cryptically concealing epiphytes or by virtue of being chemically well defended, or a combination of both. It is possible that species richness around the bases of soft corals is tempered by the surface area available for settlement on macroalgal species, as well as, the relative competitive abilities of the epiphytes.

The *Sinularia* sp. - algal removal experiment unfortunately did not yield results other than what might be expected for early successional algal species. That is, cyanobacteria and algal turf were the most frequent species encountered. After 4 mo,

Dictyota spp. were the most common macroalgae present in the experimental sites, however, no pattern of decreasing occurrence with distance from the soft corals was seen. Not much is known about temporal recruitment of the macroalgal species found at the study site, i.e., whether it is seasonal or episodic. Thus, it may take months before *Halimeda* spp. recruit to the sites. In any case, the final results of this experiment should provide more information on algal species richness and establishment around the bases of soft corals.

I saw no evidence that chemical defenses were involved in the observed differential grazing patterns around the soft corals. However, a significant effect of distance was shown for funnel-shaped models and rehydrated colonies. When I examined the treatments, grazing patterns around funnel-shaped models (Figure 11) and rehydrated *Sinularia* sp. colonies (Figure 12) usually resembled those previously observed around soft corals and the first funnel-shaped models (Figure 8). Moreover, the presence or absence of soft coral extracts did not mediate these patterns.

Many species of soft corals release chemicals which produce allelopathic effects on surrounding organisms, including hard corals (Sammarco *et al.* 1985), other soft coral species (LaBarre *et al.* 1986b), and algae (Coll *et al.* 1987). Such properties, although highly species-specific, enable soft corals to be hardy competitors for space. For instance, the soft coral *Sinularia flexibilis* which releases flexibilide and dihydroflexibilide to surrounding water at a concentration of 1 to 5 mg l^{-1} (Coll *et al.* 1982), induces necrosis and spacing behavior in other soft corals (LaBarre *et al.* 1986b). Thus, it is somewhat unusual that the chemistry of *Sinularia* sp. would not

prohibit algal species from settling in such close proximity. In this study, analysis of seawater extracts by TLC verified that soft coral models containing organic and aqueous extracts exuded secondary metabolites during the field experiments. However, since it is not possible to simulate the precise manner in which soft corals release secondary metabolites, the hypothesis that this relationship may be mediated by soft coral chemicals cannot be totally disregarded.

Herbivory has been discussed as a major influence on marine algal community structure (Hay 1981, Carpenter 1986, Lewis 1986, Hay and Fenical 1988). In this study, the shape of *Sinularia* sp. appears to influence herbivory in very small, well-defined areas and thus produces a distinct pattern of algal growth.

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