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Variability in the chemical defense of the sponge *Chondrilla nucula* against predatory reef fishes

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Abstract *Chondrilla nucula* is a common Caribbean demosponge that grows in a range of habitats, from coral reefs to mangrove swamps. On reefs, *C. nucula* grows as a thinly encrusting sheet, while in mangrove habitats it surrounds submerged mangrove roots as fleshy, lobate clumps. Previous feeding experiments using predatory reef fish revealed a high degree of variability in the chemical defenses of *C. nucula*. The present study was undertaken to determine whether a relationship exists between habitat, growth form, and chemical defense of *C. nucula*. Both laboratory and field feeding-assays of crude extracts confirmed that *C. nucula* possesses a chemical defense with high intercolony variability, but there was no significant variation in feeding deterrency between reef and mangrove habitats at either geographic location (Bahamas and Florida). Extracts of *C. nucula* collected during September and October 1994 from the Bahamas were significantly more deterrent than those collected during August 1993, May 1994, and May 1995 from Florida, and extracts of these spring and summer Florida collections were more deterrent than extracts of *C. nucula* collected in December 1994 and February 1995 in the same locations. There was no evidence that deterrent compounds were concentrated in the surface tissues of the sponge, or that chemical defense could be induced by simulated predation. Laboratory and field assays of the fractionated crude extract revealed that feeding deterrency was confined to the most polar metabolites in the extract. Field transplants were used to determine whether predation influenced the growth form of *C. nucula*. Uncaged sponges transplanted from the mangrove to the reef were readily consumed by spongivorous reef fishes. Lobate mangrove sponges became thinner after being caged on the reef for 3 mo, but encrusting reef sponges did not become thicker after being caged in the mangroves for the same period of time. Reef sponges that were caged for 3 to 15 mo thickened by only a small amount (< 1 mm) compared to uncaged and open-caged (i.e. in cages lacking tops) sponges. Simulated bite marks on both reef and mangrove sponges were repaired at a rapid rate (0.8 to 1.6 mm d^{-1}). Fish predation has an important impact on the distribution and abundance of *C. nucula*, but the thin growth form common to reef environments may be more the result of hydrodynamics than of grazing by spongivorous fishes.

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Introduction

Tropical marine sponges have yielded a wealth of novel secondary metabolites, which natural-products chemists have been isolating over the past three decades (Faulkner 1996, and previous reviews cited therein), but only recently have the ecological roles of these metabolites been addressed (Paul 1992; Pawlik 1993; Hay 1996; McClintock and Baker 1997). Although possible functions of secondary metabolites in sponges may include inhibition of fouling or overgrowth and protection from ultraviolet radiation, the most commonly advanced hypothesis is that these compounds act to deter potential predators (Paul 1992; Pawlik 1993). In tropical coral reef environments, the dominant predators are fishes (Hixon 1983).

We recently completed a survey of the chemical, structural and nutritional defenses of 71 species of Caribbean demosponges against predatory reef fishes (Chanas and Pawlik 1995, 1996; Pawlik et al. 1995). Although the majority of sponge species yielded crude organic extracts that were either unpalatable or palatable when incorporated into food pellets and presented to the reef fish *Thalassoma bifasciatum*, seven species exhibited considerable intraspecific variability, with

extracts ranging from highly deterrent to non-deterrent (Pawlik et al. 1995). One of these species was *Chondrilla nucula*, commonly known as the "chicken-liver sponge".

Chondrilla nucula is a common Caribbean demosponge that is unusual in that it grows in a wide range of habitats, from the reef crest to mangrove swamps. On coral reefs, *C. nucula* is especially common in shallow, well-lit areas (Wilkinson and Vacelet 1979), where it grows over substrata in thin (< 5 mm) recumbent sheets. *C. nucula* may overgrow 26 to 34% of the substratum on shallow Caribbean reefs (Suchanek et al. 1983), and was the dominant sponge species at 13% of shallow reef sites (depth < 7 m) sampled off Cuba (Alcolado 1994). In addition to the reef habitat where *C. nucula* grows as an encrusting sheet, *C. nucula* also grows in seagrass beds, where it forms thicker colonies (Thorhaug and Roessler 1977; Alcolado 1994), and in mangrove swamps where it surrounds mangrove prop-roots in thick (often 4 to 5 cm), lobate clumps. Despite these differences in growth form, the spicules and internal structure (Rützler 1986) of *C. nucula* from each of these habitats is identical, leaving no doubt that they are different growth forms of the same species.

Although generalist predatory reef fishes will not eat the sponges found on Caribbean coral reefs (Pawlik et al. 1995), a few species of fish (Randall and Hartman 1968) and turtles (Meylan 1988) feed predominantly on sponges, selecting mostly sponge species that are not chemically defended (Pawlik et al. 1995). *Chondrilla nucula* is one species preferred by both spongivorous fishes (Randall and Hartman 1968) and turtles (Meylan 1988; Bjorndal 1990). Although these sponge-eating predators are frequently observed in reef environments where *C. nucula* is present in encrusting sheets, these predators are generally absent from mangrove swamps, where *C. nucula* is found in its thick, lobate form.

The foregoing raises the following questions, which we have attempted to address in the present study: does the chemical defense of *Chondrilla nucula* vary with habitat, and therefore, growth form? Specifically, are encrusting, reef colonies of the sponge more chemically deterrent than lobate, mangrove colonies? Does the encrusting growth form result from the grazing activities of spongivorous predators on the reef? In order to answer these questions experimentally, we compared the deterrency of extracts of *C. nucula* from the two habitats at two different geographic locations (the Bahamas and Florida), and for one of these locations (Florida) during different times of the year. We compared the deterrency of extracts of the surface tissue of *C. nucula* with that of internal tissue to determine whether defensive metabolites were concentrated in the sponge surface. We compared the deterrency of extracts of sponges before and after simulated predation-events to determine whether defenses could be induced. Finally, we reciprocally transplanted reef and mangrove sponges, both in and out of cages, to assess the importance of predation on growth form.

Materials and methods

Description of sites

Sponges (*Chondrilla nucula*) for extraction were collected between 23 September and 12 October 1994 off the Bahama Islands (26°36′10″N; 77°54′22″W);. and during August 1993, May and December 1994, and February and May 1995 near the National Undersea Research Center in Key Largo, Florida (25°05′58″N; 80°26′29″W). The reef areas were shallow, generally < 6 m. The mangrove sites were in shallow tidal channels, with maximum collecting depth of ≃2 m. Sponge extractions and laboratory feeding-assays were conducted at the University of North Carolina at Wilmington and aboard the R.V. "Seward Johnson". Field feeding-assays and some wounding experiments were performed on reefs in the Bahamas. Sponge transplant and wounding experiments were conducted and monitored during May 1994 to August 1995 on patch reefs and a mangrove site near Key Largo, Florida: the reefs were Three Sisters (25°01′51″N; 80°23′61″W) and White Banks (25°07′35″N; 80°17′85″W), both shallow with a maximum depth of 7 m, while the mangrove site was Jewfish Creek (25°11′24″N; 80°23′27″W).

Tissue manipulation and extraction

Sponge volume was determined by displacement of water or solvent, and sponge tissue was extracted in a sequence of organic solvents. For small-volume extractions, 10 ml of sponge was extracted twice in 40 ml of 1:1 methanol/dichloromethane and once in 40 ml of methanol. Each step of the extraction process was allowed to proceed for ≃24 h. The solvents were removed by rotary evaporation or vacuum evaporation. Crude extracts were used in laboratory assays. For larger volumes of sponge (60 to 1000 ml), the tissue was extracted three times in methanol, twice in 1:1 methanol/dichloromethane, and once in dichloromethane. The organic extract was partitioned between solvents of increasing polarity: hexane, ethyl acetate, butanol, and water. Each partition was subjected to laboratory assay either separately or in combinations based on polarity. After extraction, the dry mass of extracts and extracted sponge tissue was recorded.

To determine whether deterrent chemistry was concentrated in the surface tissues of *Chondrilla nucula*, the pigmented, outer 2 mm of sponge tissue of 21 mangrove sponges was separated from the inner tissue and both were extracted and subjected to laboratory feeding assays. This thickness was chosen because the surface tissues harbor the pigmented microbial symbionts of the sponges, and because these tissues are the most susceptible to predation by fishes. To address the possibility of an induced chemical defense, ten mangrove sponges were tagged in the field, and approximately half the sponge volume was removed by cutting the sponge with a razor blade to simulate fish grazing. After 6 wk, additional tissue from each sponge was collected. The tissue collected at the onset of the experiment, and after 6 wk, were both extracted and subjected to laboratory feeding assays.

Laboratory feeding-assays

Crude extracts or partitioned fractions were mixed with 10 ml of alginate-based food (see Pawlik et al. 1995) until all organic and water-soluble components were distributed uniformly throughout the paste. Food coloring was added to both treated and control foods to make them the same color. The alginate food was then dispensed with a 10 ml syringe into a 0.25 *M* calcium chloride solution forming a strand that was allowed to harden for 2 min. The hardened strand was rinsed in filtered seawater and cut into 3 mm pellets. Control pellets were prepared identically but without the addition of crude extract. Because material is inevitably lost during any separation procedure (Cronin et al. 1995), partitioned fractions that were not deterrent at natural concentrations were

subsequently assayed at higher concentrations. Feeding assays were performed with groups of the bluehead wrasse *Thalassoma bifasciatum* using previously described methods regarding scoring and statistical analysis (Pawlik et al. 1995).

Field feeding-assays

For field assays, crude extracts or purified compounds from 60 ml vol sponge tissue were dissolved in a minimal volume of MeOH and combined with 60 ml of preheated carrageenan-based food (Chanas and Pawlik 1995). Food coloring was added to both treated and control foods to make them the same color. The mixture was then poured into molds crossed by lengths of cotton string and allowed to harden. After hardening, 20 string-embedded strips were cut from the molds. Control strips were prepared identically, but without the addition of crude extracts. Field assays were conducted on shallow water reefs off the Bahamas using previously described methods regarding deployment, retrieval, and statistical analyses (Chanas and Pawlik 1995).

Transplant and caging experiments

Predation on *Chondrilla nucula* was assessed by transplanting caged and uncaged lobate sponges from the mangrove habitat to the reef. Sponges were cut from mangrove prop-roots and transported in pairs to the laboratory, where they were weighed and tagged, and subsequently transplanted in pairs to a patch reef. Each pair consisted of one sponge that was fixed to a brick with a cable tie, 1 to 2 m distant from another sponge that was tied to a brick; this second sponge was also enclosed in a 40 cm^3 plastic mesh (Vexar) cage with 2 cm^2 openings. This mesh size was small enough to exclude potential fish predators, but large enough not to interfere with the filter-feeding activities of the sponges. Fifteen sponge pairs were placed haphazardly across the patch reef. After 3 d, sponges were collected and reweighed. A Wilcoxon paired-rank test was performed to determine the significance of differences in tissue loss between caged and uncaged sponges.

Reef sponges were also caged to determine if excluding large spongivores had an effect on growth. Cages were constructed as before, but with open bottoms and side-flanges for attachment to the substratum ("full cage"). In addition, cages without tops were used to test the effect of the cage on sponge growth without excluding spongivores ("open cage"). For each replicate, three sponges were located and tagged, and the thickness of each was recorded. One sponge was left uncaged, one was enclosed in a full cage, and one was enclosed in an open cage. Five replicates were deployed in May 1994, eight in February 1995, and nine in May 1995. The experiment was ended in August 1995, when the thickness of all sponges was again determined and the cages were removed. Data were pooled for all the replicates, and analysis of variance was used to determine if there were differences in sponge thickness between treatments.

Thickness was measured by piercing sponges with a needle until the tip reached the substratum. The shaft of the needle was marked at the tissue surface, the needle was removed, and the distance between point and shaft mark was recorded. The mean of eight random measurements of thickness was determined for each sponge.

Reciprocal transplants of *Chondrilla nucula* from reef and mangrove habitats were undertaken to assess the plasticity of growth form. Twenty sponges were collected from the reef by removing them along with the substratum on which they were growing. Sponge thickness was determined as before, holes were drilled in the adjacent coral rock for attachment of cable ties, and the rocks were attached to prop-roots in the mangroves adjacent to prop-roots supporting natural colonies of *C. nucula*. In the absence of any evidence of sponge predation in the mangrove habitat, cages were not used for transplants of reef sponge to the mangroves. Ten sponges were collected from the mangroves by removing the sponges along with the portions of the prop-roots on which they were growing. Sponge thickness was determined, the roots were attached to bricks with cable ties, and the bricks were placed in full cages on the reef. Reciprocal transplants were performed in May 1995, and retrieved in August 1995, at which time the thickness of the sponges was again determined.

Wounding experiment

To determine the speed at which *Chondrilla nucula* repairs tissue damage, and to compare the rate of repair to the rate of potential sponge growth in transplantation experiments, sponges were wounded by removing tissue with round cork borers (diameters of 9, 12 and 15 mm). Ten reef sponges were wounded in September 1994 on a patch reef off Sweetings Cay (Bahamas; 26°36′10″N; 77°54′22″W). Ten reef and 10 mangrove sponges were similarly wounded off Key Largo, Florida in May 1995. Sponges were left to heal for a period of 7 to 14 d, after which time the relative healing of the three different-sized holes was noted. The healing rate was determined by dividing the original diameter of the completely healed wounds (in mm) by the number of days elapsed.

Results

Variability in chemical defense

Crude organic extracts of samples of *Chondrilla nucula* collected from both habitats (reef and mangrove) from the Bahamas during September and October 1997, and from Florida during August 1993, May 1994 and May 1995, were deterrent to the wrasse *Thalassoma bifasciatum* in laboratory feeding-experiments (Fig. 1), although there was a high degree of variability between samples for collections at all sites. For Bahama collections, ≃80% of the samples assayed were deterrent; for the Florida collections, only half were deterrent. Samples of *C. nucula* collected in the Bahamas were significantly

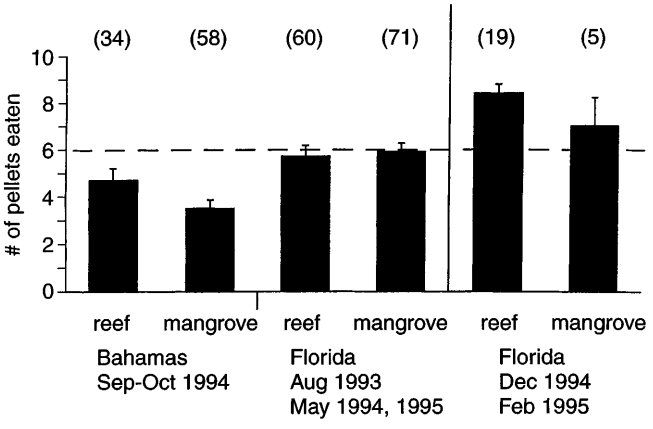

Fig. 1 *Chondrilla nucula* eaten by *Thalassoma bifasciatum*. Intraspecific variation in chemical defense of sponge as a function of location and time of year. Results of laboratory assays in which reef fish were fed foods containing crude extracts of sponge from reef and mangrove environments in the Bahamas and Florida. Data are mean (+ SE) number of treatment pellets eaten of ten offered. For any single assay, extract is considered deterrent if ≤6 treatment pellets were eaten out of 10 (*dashed line*; $p < 0.05$, Fisher exact-test, one-tailed). All control pellets were eaten in all assays (*Nos. in parentheses* number of colonies assayed for each mean)

more deterrent than those collected from Florida in August and May (two-way ANOVA on arcsine-transformed data, $p < 0.0001$). However, there was no difference in deterrency between reef and mangrove samples ($p = 0.25$). Additionally, there was no interaction effect between geographic location and growth form ($p = 0.19$). Student–Newman Keuls (SNK) comparisons indicated that there was a significant difference in deterrency between locations for sponges from mangrove habitats ($p < 0.05$).

Chondrilla nucula was also collected from Florida during the months of December 1994 and February 1995. These data were analyzed separately, because no winter collections were taken from the Bahamas for comparison. A significant temporal effect was evident (two-way ANOVA, $p < 0.05$); samples collected in the winter were less deterrent than those collected in Aug and May (Fig. 1). In fact, crude assays of extracts from sponges collected in the winter were still not deterrent even when performed at twice the natural concentration (mean number of pellets eaten = 7.1, $N = 10$). There was no significant difference in deterrency between reef and mangrove colonies ($p = 0.46$), and no significant interaction between habitat and season ($p = 0.42$).

Intracolony variation in defense and induced defense

Assays of crude extracts of the inner and outer tissues of mangrove sponges revealed no evidence of greater chemical defense in surface tissues of *Chondrilla nucula* (Table 1). Of 21 samples, only four yielded extracts in which outer tissues were more deterrent than inner tissues at either natural or twice-natural concentrations, but two samples exhibited the opposite pattern.

There was also no evidence of an induced defense when *Chondrilla nucula* from mangrove habitats were subjected to simulated predation (Table 2). Of the ten sponges used in this experiment, none yielded extracts that were more deterrent after simulated predation, and three became less deterrent.

Partial isolation of deterrent metabolites

Aquarium assays of partitioned fractions of the crude extract of *Chondrilla nucula* indicated that deterrency was confined to the most polar (water) fraction (Fig. 2). However, partitioned fractions had to be assayed at higher than natural concentrations for deterrency to be significant. For the less polar partitions, increasing the concentration of the partitioned fraction to 4 or 8 times the natural concentration did not result in inhibition of feeding. The active, polar fraction was suspended in methanol, and methanol-insoluble salts were filtered out; these salts also were not deterrent, neither when assayed alone nor in combination with the less-polar fractions.

Crude extracts of samples of *Chondrilla nucula* collected in the summer from the Bahamas deterred feeding of a natural suite of predatory reef fish in field assays (Fig. 3A). Field assays of fractions partitioned from the crude extract corroborated the aquarium assay results by revealing that deterrency was limited to the polar fractions (Fig. 3B, C). A crude extract of a mangrove sample of sponge from Florida derived from a mixed collection of sponge pieces was deterrent in the field at twice the natural concentration (Swearingen 1996), as

Table 1 *Chondrilla nucula*. Intracolony differences in chemical defenses. Results of laboratory feeding-assays in which reef fish *Thalassoma bifasciatum* were fed foods containing crude extracts of sponge from mangrove environments. Inner and outer tissue layers were assayed at natural (*1*×) and twice natural (*2*×) concentrations. Data are number of treated pellets eaten of ten offered; all control pellets were eaten (* Colonies in which outer tissues were significantly more deterrent; ** colonies in which inner tissues were significantly more deterrent)

Colony	Extract concentration			
	1×		2×	
	inner	outer	inner	outer
1	10	10		
2*	10	5		
3	10	8		
4	10	9		
5	10	9		
6	10	9		
7	10	9		
8	9	10		
9			9	10
10**			5	8
11			10	7
12			6	6
13**			6	10
14			9	7
15*			8	3
16*	10	6	5	2
17	7	8	7	7
18	8	7	9	7
19	10	9	3	2
20*	6	4	7	2
21	4	5	5	6

Table 2 *Chondrilla nucula*. Induced defenses. Results of laboratory feeding-assays in which reef fish *Thalassoma bifasciatum* were fed foods containing crude extracts of sponge from mangrove environments. Extracts were prepared from tissues of colonies (*Original assay*), the colonies were then wounded and extracts were prepared from their tissues 6 wk later (*Subsequent assay*). Data are number of treated pellets eaten of ten offered; all control pellets were eaten

Colony	Original assay	Subsequent assay
1	7	7
2	9	10
3	4	9
4	1	9
5	7	10
6	9	10
7	6	10
8	8	8
9	7	9
10	9	9

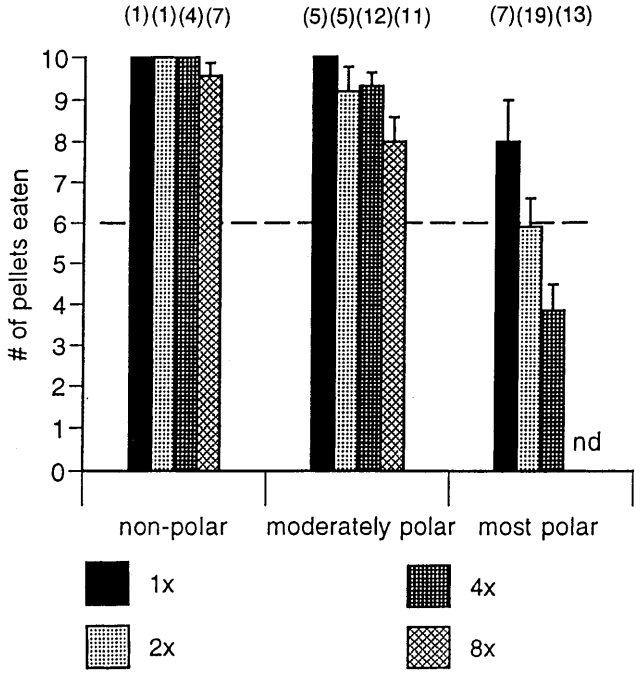

Fig. 2 *Chondrilla nucula.* Separation of defensive chemistry. Results of laboratory feeding-assays in which reef fish *Thalassoma bifasciatum* were fed foods containing solvent partitions of crude extract of sponge. Organic extracts of sponge tissues were partitioned between hexanes (*non-polar*), ethyl acetate or butanol (*moderately polar*) and water (*most polar*). Assays were performed at natural (*1*×) and higher (*2 to 8*×) concentrations (*nd* 8× concentration assay not done). Further details as in legend to Fig. 1

was the polar fraction of this extract, but the non-polar fraction at four times the natural concentration was preferred by predatory reef fishes.

Predation on transplanted sponges

There was heavy predation on uncaged *Chondrilla nucula* transplanted from the mangrove habitat to patch reefs (Fig. 4). The difference between caged and uncaged treatments was highly significant ($p < 0.0001$, Wilcoxon paired-sample test). Uncaged sponges lost an average of 65% of tissue weight during the 3 d experiment, while caged colonies gained weight during the same period. Nine of 15 uncaged sponges lost at least 50% of their original tissue weight. Several grey and french angelfishes (*Pomacanthus arcuatus* and *P. paru*, respectively) were observed consuming sponge tissue during the course of the experiment.

Growth of caged and transplanted sponges

Reef sponges were caged to determine whether protection from fish predation would result in a change in growth form. Although 22 replicates were deployed over a 12 mo span (each replicate consisting of a full cage, open cage, and no cage placed over a reef sponge), only 17 full cages and 15 open cages remained when the sites were revisited. Therefore, only the thickness of the 17 uncaged sponges adjacent to the remaining 17 full-caged sponges was measured, as well as the thickness of the 17 full-caged sponges and 15 open-caged sponges. Because there were no consistent differences in the data from treatments that had been deployed for 15 vs 3 mo, all the treatment data were pooled. There were no differences in the tissue thickness of the sponges that

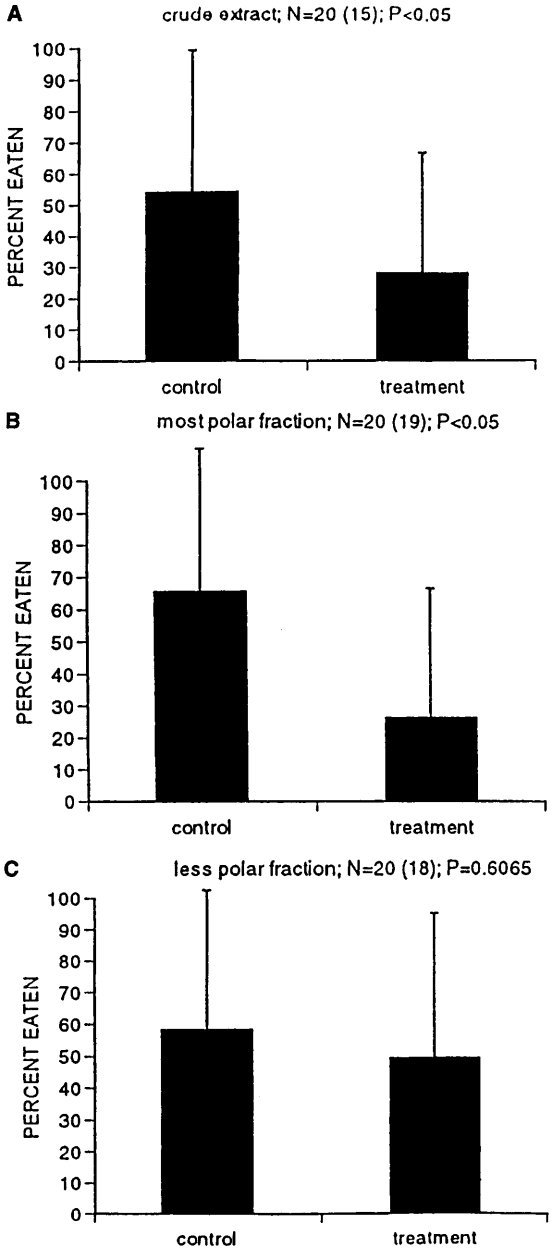

Fig. 3 *Chondrilla nucula.* Field assays of crude extract of sponge collected from reef in Bahamas in summer. Data are mean (+SE) percentage of food strips eaten by natural suite of predatory reef fishes in field. **A** Crude extract; **B** most polar partition of crude extract; **C** remaining partitions of crude extract. All treatments assayed at natural concentrations. Probabilities calculated using Wilcoxon paired-sample test [*N* number of paired control and treatment strips of 20 retrieved (= number of comparisons used in statistical analyses)]

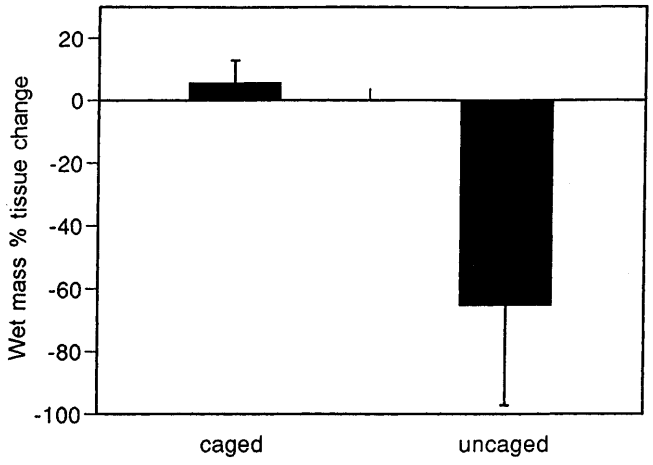

Fig. 4 *Chondrilla nucula*. Predation on mangrove sponges transplanted to reef. Percentage (mean + SD) change in wet mass of sponge transplanted from mangroves to shallow patch reefs in paired assay of caged and uncaged colonies ($N = 30$). Experiment ran for 3 d. Percentage change was significant ($p < 0.0001$; Wilcoxon paired-sample test)

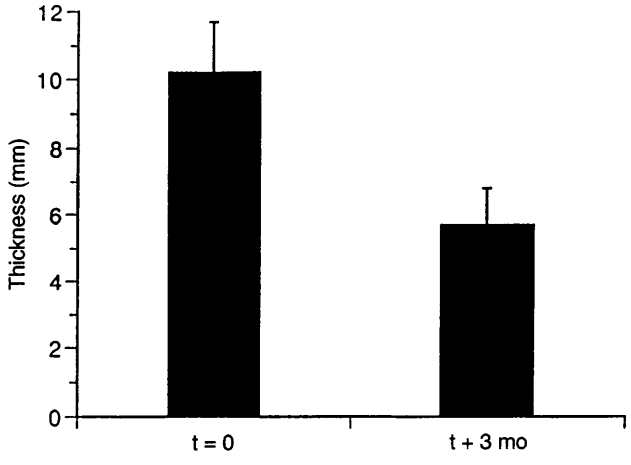

Fig. 6 *Chondrilla nucula*. Effect on growth form of mangrove sponges transplanted to reef. Mean (+SD; $N = 6$) thickness of mangrove colonies at time of collection ($t = 0$) and after 3 mo transplanted and caged on patch reefs ($t + 3$ *mo*)

were used for the three treatments at the onset of the experiment (ANOVA, $p = 0.27$) but measurable differences were evident at the conclusion of the experiment (Fig. 5). Caged sponges grew an average of 0.4 mm thicker, while both open-caged and uncaged sponges decreased in thickness (mean loss of 0.6 and 0.5 mm, respectively); the difference among treatments was significant (ANOVA, $p < 0.001$), with the thickness of caged sponges being significantly greater than that of open-caged or uncaged sponges (SNK, $p < 0.05$), but with no difference between open-caged and uncaged treatments.

Mangrove sponges that were transplanted to the reef changed both in thickness and in coloration over the 3 mo experiment. Only 6 of 10 sponges remained at the end of the experiment, but the morphology of these six more closely resembled those of reef sponges. Sponge coloration had changed from the olive drab and white of *Chondrilla nucula* from mangrove habitats to the uniform brownish color common to sponges from reefs. Sponge thickness had decreased by nearly two-fold (Fig. 6) Statistical analyses were not performed on these data because colonies were not also transplanted back into the mangroves for comparison.

Reef sponges that were transplanted to the mangrove habitat did not grow thicker after 3 mo, although the color of the recovered sponges had changed from brownish to white. Only 5 of 20 transplanted reef sponges remained when data were collected. There was no significant difference in sponge thickness between the beginning and end of the experiment, nor between reef sponges that were transplanted to the mangrove habitat and those that were transplanted back to the reef (Fig. 7; ANOVA).

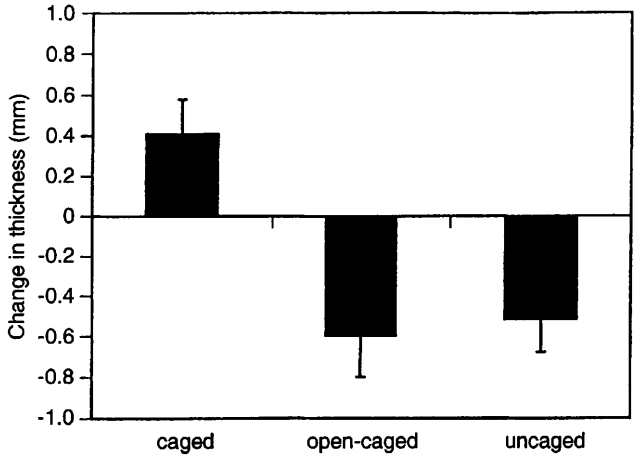

Fig. 5 *Chondrilla nucula*. Effect of caging on growth form of reef sponges. Mean (+SE) change in thickness of reef colonies that were caged or open-caged in Vexar mesh, or uncaged. Colony thickness was monitored for 3 to 15 mo. There was a significant difference in thickness between caged sponges and the other two treatments ($p < 0.001$, ANOVA)

Wound repair

Of ten sponges wounded on a Bahamas reef in September 1994, nine had completely healed 15 d later, and all three wounds (9, 12, and 15 mm) on the remaining sponge were only partially healed; therefore, the healing rate for 9 of 10 reef sponges was ≥ 1 mm d^{-1}. Of ten sponges wounded on a Florida reef in May 1995, four were completely healed, three had healed the 12 mm wound, and three had not completely healed in 9 d; therefore, the healing rate for 7 of 10 reef sponges was ≥ 1.3 mm d^{-1}. Despite being more fleshy, mangrove sponges healed more slowly. Of ten sponges wounded in a Florida mangrove habitat in May 1995, none had completely repaired the 15 mm wound after 11 d, three

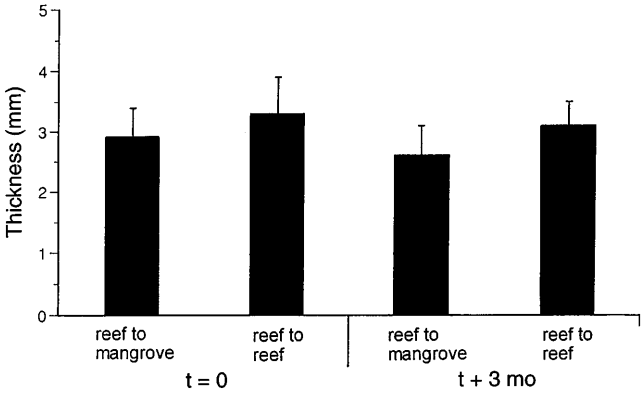

Fig. 7 *Chondrilla nucula*. Effect on growth form of reef sponges transplanted to mangroves. Mean (+SD, $N = 5$) thickness of reef colonies transplanted to mangroves or left on reef at time of transplantation ($t = 0$) and after 3 mo ($t + 3$ *mo*). There were no significant differences in thickness after 3 mo ($p = 0.22$; ANOVA)

had repaired both the 9 and 12 mm wound, and five had repaired only the 9 mm wound; therefore the healing rate was 0.8 to 1.1 mm d^{-1}.

Discussion

A previous survey by Pawlik et al. (1995) demonstrated that the crude extracts of most species of Caribbean demosponges are consistently deterrent, while others are consistently palatable. The present study focused on one of the few species that exhibited variability in chemical defense to determine whether this variability was associated with other differences in habitat, growth form, and intensity of predation. It was expected that *Chondrilla nucula* from predator-intensive reef habitats would exhibit higher levels of deterrent chemistry than samples from mangrove habitats, where spongivorous fishes are rare. Our data were not consistent with this expectation. In fact, mangrove samples of *C. nucula* from the Bahamas were, on average, more deterrent than reef samples (Fig. 1). These results are more surprising because samples of *C. nucula* from reef habitats were significantly more dense than those collected in mangrove habitats (data in Swearingen 1996), reflecting the thin encrusting vs thick lobate growth forms from the two habitats, respectively. As reef samples are more dense, and have less water per unit volume than mangrove samples, it might be expected that they would also have more defensive chemistry per unit volume, but the opposite was true. Alternatively, because the deterrent metabolites are water-soluble compounds (Fig. 2), sponge tissue with a higher water content per unit volume may contain greater concentrations of these metabolites. In point of fact, samples of *C. nucula* collected in Florida were significantly more dense than those collected in the Bahamas (data in Swearingen 1996), a result that parallels the significantly greater deterrency of samples from the Bahamas (Fig. 1).

The significant difference in deterrency between spring and summer (May and August) and winter (December and February) collections of *Chondrilla nucula* from Florida should be interpreted with caution, because sponges were sampled only during one winter season in this study. Nevertheless, the difference was fairly dramatic (Fig. 1), and if confirmed through additional experimentation, it may have some basis in the polarity of the deterrent metabolites. If the chemical defense is polar enough to be slowly released into seawater over time, the concentration of metabolites in sponge tissues may decrease during the winter months when light levels and temperatures are lower, growth and metabolism are presumed to be slower, and synthesis of deterrent metabolites is likely to decrease. This may particularly be true if deterrent metabolites are synthesized by photosynthetic endosymbionts in the sponge. However, extracts of specimens of *C. nucula* collected from dark locations (e.g. caves, or deep in mangroves) were completely white in appearance, presumably lacked photosynthetic endosymbionts, and nevertheless exhibited the same variable levels of deterrency as pigmented specimens collected from well-lit locations (Swearingen 1996).

A prominent evolutionary model for predicting intraspecific patterns of chemical defenses in terrestrial plants has been the optimal defense theory (Rhoades 1979; Coley and Aide 1990), which predicts that defenses are differentially allocated to those parts of a plant that are more susceptible to herbivory, or that defenses may be induced in response to herbivory. Recent evidence suggests that marine algae may exhibit the latter phenomenon (Cronin and Hay 1996). The results of the present study suggest that *Chondrilla nucula* is not optimally defended by either mechanism: deterrent chemistry was not concentrated in the surface tissue of mangrove samples of *C. nucula*, nor was deterrency of crude extracts enhanced by simulated predation over a 6 wk period (Tables 1 and 2). Sponge-eating angelfishes and tilefishes generally take large bites out of the sponges on which they feed, including *C. nucula* (Dunlap and Pawlik 1996); therefore, there may be no adaptive advantage to concentrating a chemical defense in a sponge that has a maximum thickness of 4 to 5 cm.

Why does the chemical defense of *Chondrilla nucula* exhibit such a high degree of intraspecific variability? Differences between location and season have already been discussed, but the underlying question of why this sponge has a variable defensive strategy is unclear. Specifically, two questions remain: (1) Why are some specimens heavily defended and some not at all? and (2) Why do so many specimens exhibit a comparatively weak chemical defense (5 or 6 pellets eaten)? Many other sponge species are extremely deterrent on a consistent basis; others are not chemically defended at all (Pawlik et al. 1995). Food value does not seem to be the driving force behind such differential adaptations; *C. nucula* is known to have a fairly high food value, with a protein content ranked eighth out of the 71 species tested

(Chanas and Pawlik 1995). Sponges that do not rely on a chemical defense may have more metabolic energy to invest in growth and/or reproduction. Coley et al. (1985) have suggested that the potential growth rates of terrestrial plants are inversely related to the level of defensive investment. Thus, more palatable sponges may maintain their presence by simply growing or reproducing fast enough to overcome the effects of predation (Pawlik 1997). Although growth and reproductive patterns were not studied, *C. nucula* may use a moderate chemical defense as a supplement to a strategy involving growth or reproductive rates. Another possibility is that as *C. nucula* colonizes a habitat, undefended colonies are consumed to a higher degree until the population consists of primarily chemically-deterrent specimens. Potential predators then would learn not to attempt to eat this sponge. Therefore, it would then not be necessary for all colonies in the population to be chemically defended. Non-defended specimens would obtain an associated defense from those specimens in the population that were chemically defended. This type of automimicry may be important, in that a specimen could receive the benefits of feeding deterrence without having to allocate resources to the production of defensive metabolites.

The wounding experiments demonstrated that *Chondrilla nucula* is able to regenerate tissue very quickly, although the process appears to be slightly slower in mangrove habitats. There may be greater competition for space in the mangroves; bare patches created in mangrove specimens often rapidly filled with hydroids and algae (Swearingen personal observation). The healing process is evidently faster than normal rates of growth, because specimens monitored in fish-exclusion experiments for periods exceeding one year did not show obvious increases in surface area.

What is responsible for the morphological difference in specimens of *Chondrilla nucula* from the two habitats? Spongivorous reef fish readily consume the lobate, mangrove morph (Fig. 4); could grazing by fishes result in the encrusting morphology found on reefs? Caging experiments suggest otherwise. The relative difference in thickness of the caged reef colonies vs uncaged colonies averaged < 1.0 mm; caged reef colonies came nowhere near to attaining the thickness of colonies found in the mangroves. Some of the colonies were caged for well over a year, and none changed in appearance (color or texture) nor exhibited any morphological change except for the slight increased thickness of caged colonies. The small loss of thickness of uncaged colonies (< 0.6 mm) does not appear to have been a caging artifact, because a similar loss occurred in partially caged colonies also (Fig. 5). However, the difference in the flow regime experienced by sponges in full cages vs open cages or no cages may have been sufficient to produce the small (but significant) differences in growth seen in this experiment. In any case, the caging data suggest that predation on *Chondrilla nucula* is not the principal factor determining its growth form on the reef, otherwise colonies shielded from predation would probably have grown into the thicker morph common to the mangroves.

Hydrodynamics, rather than predation, may be more responsible for growth form differences in *Chondrilla nucula* between reef and mangrove sites. Shallow reefs experience much more wave action and surge than do mangrove channels. The movement of water through mangrove channels is unidirectional, based on the tide, with very little sudden fluctuation. On shallow reefs however, water flow is not only strong but constantly changing direction. Given the texture of *C. nucula*, this sudden acceleration of the surge could be an important factor that necessitates a flat growth form on the reef. During an initial trial in which mangrove sponges were transplanted to cages on the reef, the transplanted specimens were ripped from their cable-tie attachments after only one day. Only when sponge specimens were taken along with their mangrove prop-root substata, and the roots firmly cable-tied to bricks, were the sponges able to withstand the surge over the short-term; nevertheless, four of ten replicates were lost during the 3 mo experiment. After this period, the remaining specimens were, on average, half as thick as at the commencement of the experiment (Fig. 6). The skeletal morphology of *C. nucula* may prevent it from maintaining a lobate morphology in all but the calmest habitats. In mangrove channels that experience relatively greater tidal currents, lobes of *C. nucula* are often drawn out into long projections by the prevailing flow. Detached projections are frequently found on the bottom of mangrove channels, and this process may represent a mechanism for asexual reproduction and colonization.

If hydrodynamics were the only factor responsible for affecting the morphology of *Chondrilla nucula*, then it would be expected that reef specimens transplanted to the mangrove environment would increase in thickness, but this had not happened after a period of 3 mo time (Fig. 7). Most of the specimens transplanted from the reef to the mangrove habitat did not survive, and those that did had lost their original coloration and become white. According to Arillo et al. (1993), colonies of *C. nucula* that were transplanted into caves (and thus deprived of light to support cyanobacterial symbionts) underwent "metabolic collapse." Healthy, white specimens of *C. nucula* can be found in caves and in the mangroves under dark overhangs. It may be that the 3 mo period of the experiment was too short to allow reef sponges transplanted to the mangroves to recover their endosymbionts and to respond (with an increase in growth) to milder hydrodynamic conditions.

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