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Does the skeleton of a sponge provide a defense against predatory reef fish?

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Abstract Sponge tissue often contains two structural components in high concentrations: spicules of silica, and refractory fibers of protein (spongin). Some terrestrial plants contain analogous structures, siliceous inclusions and refractory lignins, that have been demonstrated to deter herbivory. We performed feeding experiments with predatory reef fish to assess the deterrent properties of the structural components of three common Caribbean demosponges, *Agelas clathrodes*, *Ectyoplasia ferox*, and *Xestospongia muta*. The concentrations of spicules and spongin in the tissues varied widely between the three species, but when assayed at their natural volumetric concentrations, neither spicules (all three species assayed) nor the intact spiculated spongin skeleton (*A. clathrodes* and *X. muta* assayed) deterred feeding by reef fish in aquarium or field assays using prepared foods of a nutritional quality similar to, or higher than, that of sponge tissue. Spicules deterred feeding in aquarium assays when incorporated into prepared foods of a nutritional quality lower than that of sponge tissue (15–19 times less protein), but spiculated spongin skeleton was still palatable, even in prepared foods devoid of measurable protein, and even though spicules embedded in spongin were oriented in their natural conformation. Based on comparisons of the nutritional qualities of the tissues of the three sponge species and of the prepared foods, sponge tissue would have to be much lower in food value (5 times less protein or lower) for spicules to provide an effective defense, and spicules in combination with the spongin skeleton would be unlikely to provide an effective defense regardless of the nutritional quality of the tissue. Unlike terrestrial plants, marine sponges may use silica and refractory fibers solely for structural purposes.

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Introduction

Sponges are important members of the coral reef ecosystem, where they rival hard corals in both biomass and diversity (Rützler 1978; Suchanek et al. 1983; Van Soest 1994). Because sponges are sessile and soft-bodied (lacking a mineralized shell or hard exoskeleton), they appear to be physically vulnerable to predation. Grazing by fish is intense on coral reefs and acts to control the distribution and abundance of many benthic invertebrates (Bakus 1964; Hixon 1983; Huston 1985; Jones et al. 1991), but spongivory is largely restricted to a few species of sea turtles (Meylan 1988; Bjorndal 1990) and fish (Randall and Hartman 1968; Wulff 1994). The lack of predation on sponges is thought to result from the elaboration of physical (e.g., Hartman 1981) and chemical defenses (e.g., Bakus and Green 1974). Purified secondary metabolites from some sponges have been found to deter feeding by potential predators (Pawlik et al. 1988; Paul 1992; Pawlik 1993; Albrizio et al. 1995), and in a recent survey of Caribbean sponges, crude extracts from 44 of 71 species exhibited antifeedant properties in fish feeding assays (Pawlik et al. 1995).

Demosponges have two types of skeletal components: inorganic siliceous (glass) spicules and organic proteinaceous fibers (Bergquist 1978). Spicule concentrations range from 3.8 to 67.1% of total tissue dry mass for some Caribbean species (Rützler and Macintyre 1978) and spicules serve an important role in colony support by increasing tissue rigidity (Bergquist 1978; Koehl 1982). It has also been hypothesized that sponges derive a physical defense from spicules, ostensibly through the irritation of the mouthparts and guts of potential predators (Randall and Hartman 1968; Hartman 1981; Wainwright et al. 1982), although the few species of fish that prey largely on sponges are not deterred by spicules (Randall and Hartman 1968; Wulff 1994). Gorgonians and soft

corals have calcareous sclerites in their tissues that are similar in shape and size to sponge spicules. Octocoral sclerites have been reported to serve an important structural role (Lewis and Von Wallis 1991) and have also been demonstrated to deter feeding by some generalist predators (e.g., Harvell et al. 1988).

In addition to spicules, most demosponges are supported by an internal matrix of proteinaceous fibers called spongin (Bergquist 1978). In some sponges, spicules or sand grains may be embedded in the spongin fibers (Bergquist 1978; Wainwright et al. 1982). The rigidity and toughness of sponge tissue is not only dependent on the density and thickness of spongin fibers (Storr 1964) but also on spicule morphology and orientation (Koehl 1982; Wainwright et al. 1982). Spongin can constitute a significant portion of total colony biomass; for example, Reiswig (1973) reported that spongin accounted for ~30% of the total dry mass of *Mycale* sp. (probably *M. laxissima*). In addition to its structural role, spongin is also believed to be difficult to digest (Bjorndal 1990; Meylan 1990). It stands to reason, therefore, that the combination of poorly digestible spongin and indigestible silica may result in tissue of sufficiently low nutritional quality that predators are deterred from eating sponges. Moreover, low nutritional quality has been demonstrated to increase the effectiveness of sponge chemical defenses (Duffy and Paul 1992; Pennings et al. 1994).

Predation on sponges is much like herbivory on plants. Reef sponges are abundant, apparent, clonal, lack behavioral defenses, are often autotrophic (due to the presence of symbiotic algae and cyanobacteria; Arillo et al. 1993), and are non-fatally grazed by reef fish (Wulff 1994). Plants employ various structural components as defenses against herbivory: terrestrial plants contain siliceous phytoliths (Kaufman et al. 1981) and lignified fiber (Coley 1983), while some marine algae have calcified tissues (Hay et al. 1994). We therefore pose the following question: do sponges, like some plants, use silica and refractory fibers as defenses against predation?

To examine the role that physical components of Caribbean demosponges play in deterring predation, we studied three conspicuous reef species that differ in spongin content as well as spicule concentration and morphology. *Agelas clathrodes* (Schmidt) forms large (up to 1.5 m) orange flabiform colonies that possess a strong, highly elastic spongin network embedded with acanthostyle spicules (Zea 1987). *Ectyoplasia ferox* (Duchassaing and Michelotti) grows as moderately-sized (~0.25 m) orange-brown colonies that are compressible but fragile because of thin spongin fibers and short styles (Zea 1987). *Xestospongia muta* (Schmidt) forms large (up to 2 m) brown barrel-shaped colonies; the tissue has a brittle consistency because of a low spongin content and a high concentration of long oxeas (up to 500 μm; Zea 1987). All three species are abundant throughout the Caribbean Sea (Alvarez et al. 1985; Zea 1993). To elucidate the deterrent properties of their structural components, we manipulated the nutritional quality of prepared foods containing the spicules and spiculated skeleton of these three sponge species and employed the resulting foods in feeding experiments with predatory reef fish.

Methods

Isolation of spicules and spiculated skeleton

Spicules were obtained by completely oxidizing 10 cm^3 of sponge tissue in two to five changes of a 5% solution of sodium hypochlorite. Spicules were then isolated by filtration, washed with 0.1 M sodium thiosulfate to neutralize any residual sodium hypochlorite, and rinsed thoroughly with distilled water. Spicules from three different colonies of each of the three sponge species were tested in aquarium assays.

Whole skeleton (spiculated spongin) was isolated by lyophilizing sponge tissue of a known volume (10 cm^3 for aquarium assays, 60 cm^3 for field assays). The lyophilized tissue was then extracted sequentially in 1:1 dichloromethane:methanol for 6 h and methanol for 2 h to remove all lipid-soluble compounds. The skeleton was dried, submerged in 0.5% sodium hypochlorite for 3 min to remove any remaining cellular material, washed with 0.1 M sodium thiosulfate, and rinsed thoroughly with distilled water. Microscopic examination of cleaned skeletons showed no obvious damage due to isolation methods. The spongin network of *E. ferox* was too fragile for skeleton isolation and consequently was not used. Spiculated skeleton from three different colonies of both *A. clathrodes* and *X. muta* was tested in aquarium assays, and spiculated skeleton from one colony of *A. clathrodes* and *X. muta* was tested in field assays.

Aquarium assays

Aquarium assay techniques were modified from Pawlik and Fenical (1992). For aquarium assays of spicules, isolated spicules were mixed with 10 ml of an alginic-acid based food, hereafter referred to as "alginate food", consisting of 0.5 g lyophilized, powdered squid mantle, 0.3 g alginic acid, and 10 ml distilled water mixed to form a homogeneous paste. The amount of lyophilized squid mantle used in this recipe was chosen to approximate the mean protein content of the tissues of Caribbean demosponges (Chanas and Pawlik 1995). The mixture of spicules and alginate food was dispensed with a 10-ml syringe into 0.25 M calcium chloride and allowed to harden for 2 min to form spaghetti-like strands; the strands were then rinsed with filtered seawater and cut or pulled apart into 4 mm pellets. Control pellets were prepared identically but without addition of spicules. Microscopic examination of spicules subsequently isolated from treated pellets showed no obvious spicule breakage due to mixing.

For aquarium assays of sponge skeleton, isolated whole skeleton was sliced into 3 mm×1 mm×1 mm pieces and embedded by dipping the pieces into alginate food mixtures of sequentially increasing concentrations of alginic acid (0.05 g, 0.1 g, 0.2 g, 0.3 g per 10 ml of distilled water) to insure that the mixture would enter the interstices of the skeleton. The surface of the embedded skeleton was wiped of excess mixture, then the pieces were hardened in 0.5 M calcium chloride and rinsed with filtered seawater. Control pellets were cut of hardened alginate-squid mixture without addition of skeletal material.

The bluehead wrasse, *Thalassoma bifasciatum*, was employed as an aquarium assay fish because it is an abundant generalist predator on Caribbean reefs (see further discussion in Pawlik and Fenical 1992; Pawlik et al. 1995). A control and a treated pellet were offered to each of ten independent groups of three fish which were kept apart in divided, opaque-walled aquaria located at a marine research facility at Wrightsville Beach, North Carolina. Feeding by each group was scored as to whether the treated pellet was eaten or rejected: a rejection was scored if the treated pellet was spit out three or more times by one or more fish in each group. A

third control pellet was offered to confirm that fish had not ceased feeding. Assays were scored only if a control pellet was eaten before and after the treated pellet was offered. The Fisher exact test (one-tailed) was used to determine whether feeding on treated pellets was significantly reduced relative to feeding on control pellets for each assay (Zar 1984). A treatment was considered deterrent if the number of treated pellets eaten was less than or equal to 6 ($P \leq 0.043$).

Field assays

Field assay methods were modified from Pawlik and Fenical (1992). For field assays of sponge skeleton, isolated spiculated skeleton was cut into 20 strips (5 cm×1 cm×1 cm) and threaded with monofilament line. The skeleton strips were placed in molds and embedded by pouring a preheated mixture of carrageenan-based food (hereafter referred to as "carrageenan food"), consisting of 3 g of lyophilized, powdered squid mantle, 1.5 g of type I carrageenan (Sigma Chemical Co.), and 60 ml of distilled water, into the molds. Special care was taken to make certain that the food mixture permeated the interstices of the skeleton strips. The mixture was allowed to harden and the 20 embedded strips were cut apart. Control strips were prepared from carrageenan food alone. Field assays were conducted on shallow reefs off Key Largo, Florida. One treatment and one control strip each were tied to a 50-cm length of three-strand polypropylene rope at a distance of ~4 and 12 cm from one end of the rope (the order was haphazard). Twenty polypropylene ropes (each with a treated and a control strip attached) were deployed along the reef and monitored for 30–60 min. A rope was removed from the reef when approximately 50% of the total food offered per rope was consumed. Strips were scored by measuring the percentage length that was eaten (to the nearest 5%). The Wilcoxon paired-sample test (two-tailed) was used to determine if consumption differed significantly between control and treated pairs of strips (Zar 1984).

Manipulation of nutritional quality

The nutritional quality of prepared foods was controlled by changing the amount (percentage by volume) of lyophilized, powdered squid mantle used in the alginate food recipe. The standard alginate food recipe contained 0.5 g (5%) of lyophilized squid mantle which was similar in protein content to the mean protein content of 71 species of Caribbean sponges (Chanas and Pawlik 1995). Lower nutritional quality recipes, containing 0.05 g (0.5%) and 0 g (0%) of lyophilized squid mantle, were used to determine if the level of nutritional quality altered the effectiveness of the structural defenses. Spicules and spiculated skeleton were assayed as before but with mixtures of lower food value. Spicules of three colonies of the three sponge species were tested in aquarium assays using 0.5% and 0% squid mixtures. Spiculated skeleton of three colonies of *A. clathrodes* and *X. muta* was tested in aquarium assays using 0.5% and 0% squid mixtures.

Nutritional quality analysis

Nutritional quality data (protein, carbohydrate, lipid, energy, and ash content) for *A. clathrodes*, *E. ferox*, and *X. muta*, as well as for the 5% squid-alginate and carrageenan assay foods, were presented in Chanas and Pawlik (1995); the 0% and 0.5% squid-alginate foods used in the present study were analyzed using the same nutritional quality methods described therein. It should be noted that the method for protein analysis (Bradford 1976) measured only NaOH-soluble (cellular) protein, and not the refractory protein bound in spongin fibers; therefore, any nutritional value of spongin is above and beyond the concentration of protein measured in this study. Spicule content of the three sponge species was determined by dissolution of a known volume of tissue in 5% sodium hypochlorite and isolation of spicules by vacuum filtration. Salt content of the sponge samples was estimated by assuming a salinity of 35‰ for all water of the tissue wet mass and calculating the mass of salt contained in that volume of water. All measures of nutritional quality, ash, and spicule mass for each sponge species or prepared food analyzed were expressed on a dry mass-to-volume basis (calculated as in Chanas and Pawlik 1995).

Results

Nutritional quality analysis

Nutritional quality values are expressed as dry mass per tissue volume, because sponges are consumed on a volumetric basis, not as dry mass (see Chanas and Pawlik 1995). Tissues of *A. clathrodes* contained the highest protein content of the three sponge species, while tissues of *E. ferox* had the highest carbohydrate content and lipid content (Table 1). The highest total ash and spicule content was measured in tissues of *Xestospongia muta* (Table 1). Spongin content could be estimated for each species using the following equation (derived from McClintock 1986): Σ(ash mass + ash-free mass) - Σ(soluble protein mass + carbohydrate mass + lipid mass + spicule mass + salt mass) = spongin mass. Estimated spongin content varied from 114 and 86 mg ml^{-1} in tissues of *E. ferox* and *A. clathrodes*, respectively, to 24 mg ml^{-1} in tissues of *X. muta*. Using previously reported mean values of tissue dry mass per ml (Chanas and Pawlik 1995), spongin accounts for approximately 60, 56 and 14% of the dry mass of *E. ferox*, *A. clathrodes* and *X. muta*, respectively. The differences in spongin mass were reflected by the differences in energy content: tissues of *A. clathrodes* and *E. ferox* were high in energy (2.2 and 2.7 kJ ml^{-1}, respectively), while tissues of *X. muta* were lower

Table 1 Nutritional quality analysis of *Agelas clathrodes*, *Ectyoplasia ferox*, *Xestospongia muta*, and prepared foods (% squid in alginate- or carrageenan-based mixture). Values expressed as mean ± SE (number of replicates). Some data previously reported (see Methods)

	Protein mg ml^{-1}	*Carbohydrate* mg ml^{-1}	*Lipid* mg ml^{-1}	*Total ash* mg ml^{-1}	*Spicule mass* mg ml^{-1}	*Energy content* kJ ml^{-1}
Agelas clathrodes	21.3±0.9 (4)	2.0±0.4 (3)	9.9±0.3 (4)	38.0±7.5 (4)	6.8±6.3 (4)	2.2±0.1 (4)
Ectyoplasia ferox	19.0±3.8 (5)	11.4±1.8 (3)	11.5±2.0 (5)	59.4±8.2 (5)	16.7±10.5 (4)	2.7±0.3 (3)
Xestospongia muta	16.4±4.5 (7)	2.8±0.2 (3)	8.6±2.3 (6)	99.5±8.0 (6)	91.6±20.5 (4)	1.4±0.2 (4)
5% squid in alginate	13.2±0.2 (3)	0.4±0.2 (3)	3.6±0.7 (3)	14.7±0.4 (3)	0.0	1.1±0.1 (3)
0.5% squid in alginate	1.1±0.7 (3)	0.5±0.2 (3)	0.4±0.1 (3)	11.0±0.3 (3)	0.0	0.4±0.1 (3)
0% squid in alginate	0.0±0.0 (3)	0.8±0.3 (3)	0.3±0.2 (3)	11.2±0.5 (3)	0.0	0.3±0.1 (3)
5% squid in carrageenan	8.9±0.1 (3)	10.6±1.0 (3)	3.1±0.1 (3)	10.0±0.9 (3)	0.0	1.1±0.8 (3)

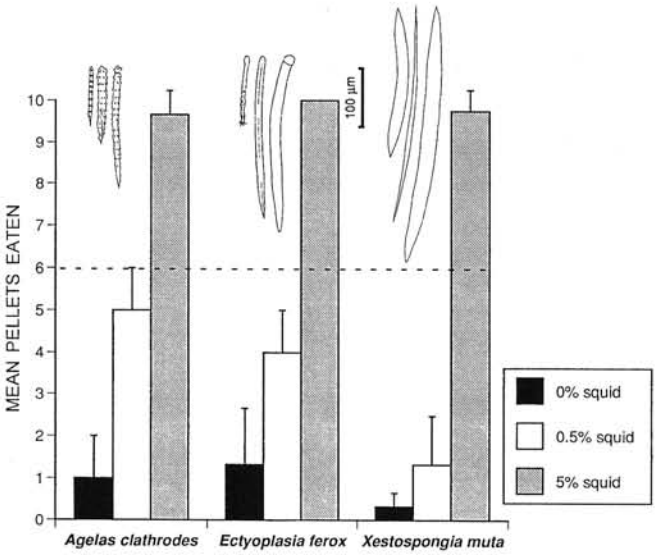

Fig. 1 Aquarium assays of spicules from *Agelas clathrodes*, *Ectyoplasia ferox*, and *Xestospongia muta* in prepared foods of variable nutritional quality (0, 0.5 or 5% by volume of dry powdered squid mantle). 1 SD above the mean number of treated pellets eaten is indicated. All control pellets were eaten in all assays. Three replicate assays were performed for each treatment. For any individual assay, a treatment was considered deterrent if the number of treated pellets was less than or equal to 6 ($P \leq 0.043$, Fisher exact test, one-tailed), as indicated by the dotted line. Drawings of representative spicule types are indicated for each species (adapted from Zea 1987)

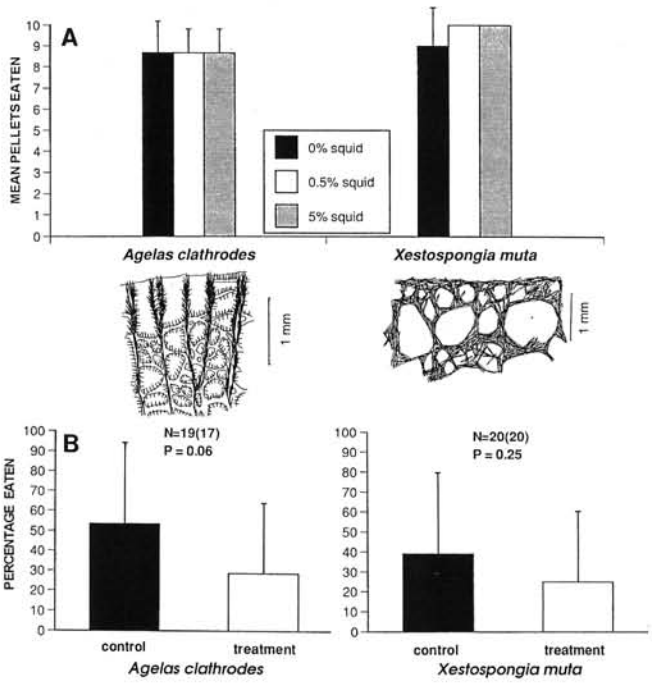

Fig. 2A, B Aquarium and field assays of spiculated skeleton from *Agelas clathrodes* and *Xestospongia muta*. **A** Aquarium assays of spiculated skeleton in prepared foods of variable nutritional quality (0, 0.5 or 5% by volume of dry powdered squid mantle). 1 SD above the mean number of treated pellets eaten is indicated. Three replicate assays were performed for each treatment. All control pellets were eaten in all assays. Statistics are the same as in Fig. 1. **B** Field assays of spiculated skeleton in carrageenan containing 5% by volume of dry powdered squid mantle. 1 SD above the mean percentage of food strips is indicated. P-values computed using Wilcoxon paired-sample test. *N* number of ropes retrieved out of 20 ropes deployed (number of ropes used in statistical analysis). Drawings of spiculated skeletons are indicated for both species (adapted from Zea 1987)

(1.4 kJ ml^{-1}; Table 1). Both alginate- and carrageenan-based foods with 5% squid were similar in protein content to tissues of the sponges measured, but their energy content was lower.

Assays of spicules alone

For all three species, spicules did not significantly deter feeding by *Thalassoma bifasciatum* in aquarium assays using alginate food with 5% squid (Fig. 1). Fish that consumed spicule-treated pellets suffered no noticeable immediate or long-term effects (fish were maintained in aquaria for 6 months after assay). Spicules of *A. clathrodes*, *E. ferox*, and *X. muta* were also found to deter feeding in field assays in a previous study (Chanas and Pawlik 1995). However, spicules of all three species did deter feeding by *T. bifasciatum* when assays were conducted using alginate foods with lower nutritional qualities of 0.5% and 0% squid ($P \leq 0.04$; Fig. 1).

Assays of spongin with spicules

In aquarium assays using alginate food with 5% squid, spiculated skeleton from *A. clathrodes* and *X. muta* did not deter feeding by *T. bifasciatum* ($P \geq 0.24$; Fig. 2A). Spiculated skeleton from *A. clathrodes* and *X. muta* also did not deter feeding by predatory fish in field assays using carrageenan food with 5% squid ($P \geq 0.06$; Fig. 2B). Fish that sampled the food strips during field assays included wrasses (*Thalassoma* and *Halichoeres* spp.), snappers (*Ocyurus chrysurus*), parrotfish (*Scarus* and *Sparisoma* spp.), filefish (*Cantherhines* spp.), and sergeant majors (*Abudefduf* spp.). Contrary to the assay results using spicules alone (Fig. 1), whole sponge skeleton was palatable to *T. bifasciatum* in aquarium assays using alginate foods with 0.5% and 0% squid ($P \geq 0.11$; Fig. 2A).

Discussion

Spicules of *A. clathrodes*, *E. ferox*, and *X. muta* did not deter feeding by generalist predatory reef fish when assayed in prepared foods of approximately the same nutritional quality as sponge tissue. This was true for aquarium assays conducted with the wrasse *T. bifasciatum* (Fig. 1), and for assays conducted in the field with a natural suite of reef fish (Chanas and Pawlik 1995). Although spicule concentration and morphology varied among all three sponge species (see Table 1, Fig. 2), they

were all similarly non-deterrent. Spicules only deterred fish feeding when assayed in prepared foods of very low quality, suggesting that sponge tissue would have to be much lower in nutritional quality for spicules to be an effective predatory defense. Assuming a conservative maximum concentration to elicit rejection of 3 mg ml^{-1} protein in a spicule-laden food, the sponge tissue used in this study was 5–7 times higher than this threshold maximum of soluble protein. The mean protein content of 71 species of Caribbean sponges was 20.7 mg ml^{-1}, with the lowest measured protein concentrations still over 3 times greater than threshold (Chanas and Pawlik 1995). Moreover, the assay for protein used in this study only indicated the presence of soluble (cellular) protein; the protein bound in the spongin skeleton would also potentially be available to fish predators if it could be digested. In point of fact, despite the questionable nutritional value of the spongin skeleton, its inclusion with spicules in prepared foods containing no additional protein negated any deterrent effect of the spicules (Fig. 2).

Sponge spicules and octocoral sclerites are similar in some physical characteristics (e.g., morphology, concentration found in tissues), but they differ in composition and in their ability to deter predation. Sclerites of *Gorgonia ventalina*, *Leptogorgia virgulata*, *Pseudopterogorgia acerosa*, and *Sinularia* spp. deterred feeding at their natural concentrations, which ranged from 31 to 82% of total tissue dry mass (Gerhart et al. 1988; Harvell et al. 1988; Van Alstyne and Paul 1992; Van Alstyne et al. 1992). In a subsequent study of *Sinularia* spp. to determine the relative importance of chemical and physical defenses, sclerites assayed at concentrations found in tips (31–47% by dry mass) did not deter feeding when compared to controls (Fig. 9 in Van Alstyne et al. 1994). Similarly, sclerites of the sea whip *Junceela* sp. did not deter feeding at natural concentration (~45% of dry mass; reported in Van Alstyne et al. 1992). The calcitic spicules of the ascidian *Tridemnum solidum* also did not deter feeding at natural concentration (~83% of dry mass) (Lindquist et al. 1992). In the present study, spicule concentrations, expressed as percentage dry mass, in *Agelas clathrodes* (3%) and *Ectyoplasia ferox* (9.7%) were well below the range reported for deterrent octocoral sclerites (31–82%). However, the concentration of spicules in *Xestospongia muta* (48.6%) fell within the deterrent range for octocoral sclerites, but were also palatable at this concentration. Rather than a concentration effect, it may be that the differences in palatability of sponge spicules and octocoral sclerites are due to differences in composition. The defensive function of sclerites may be dependent on a chemical mechanism, the alteration of gut pH by the dissolution of calcite, rather than a physical mechanism; such a hypothesis has been proposed recently for the defense of some calcified algae (Hay et al. 1994).

In contrast to sponge spicules, biomineralized silica in terrestrial plants is thought to play both a defensive and a structural role (Abrahamson 1989). While silica deposition occurs in dicotyledons, the highest concentrations of siliceous phytoliths are found in monocotyledons, namely the Poaceae (grasses), and Cyperaceae (sedges) (Kaufman et al. 1981). Grasses lack the elaborate chemical defenses (e.g. phenols) found in dicotyledonous species (McNaughton 1979). High concentrations of opaline silica found in Serengeti grasses are thought to be the consequence of intense coevolution between ungulates and grasses, wherein grasses respond to grazing pressure by increasing silica deposition in future seasonal growth (Brizuela et al. 1986; McNaughton and Tarrants 1983). In feeding trials using smaller mammalian herbivores, high silica and low nitrogen content in grass tissues has been demonstrated to significantly lower consumption (Gali-Muhtasib et al. 1992). Among grasses, silica is concentrated in plant parts vulnerable to herbivory, namely leaf blades and inflorescences (McNaughton et al. 1985). Silica phytoliths affect herbivores by accelerating tooth wear (Baker et al. 1959) and by causing esophageal cancer (O'Neill et al. 1980) and silica urolithiasis (Bailey 1981). Surprisingly, marine spongivores do not appear to suffer ill effects from the consumption of sharp siliceous spicules (Randall and Hartman 1968; Meylan 1988; Birenheide et al. 1993).

The results reported herein suggest that spicules in their natural configurations are an ineffective defense against predatory reef fish. The two species for which the spiculated skeletons were assayed provide an interesting contrast in spicule morphology and architecture: barbed acanthostyles in *A. clathrodes* are oriented perpendicular to the spongin fibers and the sponge surface, while smooth oxeas in *X. muta* lie in tracts parallel to choanosome chambers and the sponge surface (see Fig. 2; Zea 1987). The spiculated skeletons of *A. clathrodes* and *X. muta* did not deter feeding when foods of low nutritional quality were used, indicating that spongin alone may represent a potential food source.

Estimated spongin content constituted the largest portion of organic mass in all three sponge species measured. Although *A. clathrodes* had roughly 3.5 times more spongin than *X. muta*, there were no differences in the palatability of pieces of the spiculated skeleton of either species. Spongin fibers can be high in energy (Reiswig 1973) but they are resistant to enzymatic hydrolysis (Garrone 1978) and thus difficult to digest fully. The skeletons of fibrous sponges eaten by hawksbill turtles were unaffected by digestive processes (Meylan 1990). Similarly, the Antarctic asteroid *Perknaster fuscus antarcticus* digests only the cellular material of its prey sponge, *Mycale acerata*, leaving the spongin skeleton intact (Dayton et al. 1974). In contrast to the aforementioned spongivores, examination of the guts of Caribbean angelfish (Pomacanthidae) revealed intact skeletal networks of consumed sponges in the foregut, but the skeletons were completely digested in the hindgut and feces (Randall and Hartman 1968; B. Chanas personal observation). Aquarium and field assay results indicate that spongin, while difficult to digest, is palatable to generalist predators and, even in the absence of cellular material, counteracts the deterrent effect of spicules.

Protein is the preferred source of dietary energy for fish (Tacon and Cowey 1985) and demosponges could represent a rich source of protein and energy for browsing reef predators (Chanas and Pawlik 1995). The protein content of prepared foods used to test chemical defenses is known to alter palatability significantly (Duffy and Paul 1992; Pennings et al. 1994). Duffy and Paul (1992) reported that secondary metabolites deterred feeding when incorporated in a low-quality (low protein) food, but did not deter feeding in a high-quality (high protein) food. In the present study, high-quality prepared foods (5% squid in alginate- and carrageenan-based mixtures) were similar in protein content to the tissues of the three sponge species measured (Table 1).

This study has been limited in its scope to predation by reef fish because the feeding activities of fish are generally believed to structure benthic invertebrate communities on coral reefs (Bakus 1964; Hixon 1983). Sponges are noted for harboring a diversity of small invertebrates, many of which feed on sponge tissue (e.g., Pawlik 1983), but their activities are less likely to affect sponge distributions. Some larger invertebrates eat sponge tissue (Dayton et al. 1974; Pawlik et al. 1988; Bierenheide et al. 1993), but these are uncommon on Caribbean reefs. Nevertheless, the impact of nocturnal arthropod predators, such as crabs and lobsters, cannot be ruled out, as these invertebrates may have very different responses to the structural elements of sponges. Experiments with invertebrate predators are currently ongoing, and will be the subject of a subsequent report.

It is surprising, given the parallels between herbivory on terrestrial plants and grazing on marine sponges, and considering that siliceous inclusions and refractory fibers are present in the tissues of both, that there should be good evidence of structural defenses for plants but not for sponges. Clearly, spicules and spongin play an important role in the support of the sponge colony (Koehl 1982). Do these materials play any defensive role? Available evidence suggests that most sponge predators either avoid spicules and spongin (starfish: Dayton et al. 1974, worms: Pawlik 1983) or ingest these materials without apparent ill effects (urchins: Birenheide et al. 1993, fish: Randall and Hartman 1968, turtles: Meylan 1988). In addition, the results of the present study suggest that generalist predatory reef fish are not deterred by spicules, with or without spongin. While terrestrial plants have pursued both structural and chemical defense tactics to reduce herbivory, it appears that, as far as grazing by reef fish is concerned, marine sponges have followed only the latter course.

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