Co-occurrence of chemical and structural defenses in the gorgonian corals of Guam

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ABSTRACT: Chemical and structural defense mechanisms have been reported to co-occur in algae and some soft corals. This study addressed the co-occurrence of chemical and structural defense mechanisms in 7 of the common gorgonian species found around Guam, specifically focusing on patterns of within-colony variation of these 2 modes of defense. Lipophilic crude extract and sclerite concentrations were compared among the base, mid-axis and tip of multiple colonies of each species. Crude extract concentrations differed significantly among parts of *Annella mollis*, *A. reticulata*, *Subergorgia suberosa*, and *Viminella* sp. colonies. Sclerite concentrations differed significantly within colonies of *Astrogorgia* sp. and *Villogorgia* sp. Crude extracts and sclerites from the mid-axes and tips of the colonies were assayed in the field against natural assemblages of reef fishes. Extracts from the tips of 6 of the 7 species were unpalatable to fishes. Of these 6 species, extracts from the mid-axes of *A. mollis*, *A. reticulata* and *S. suberosa*, all of the family Subergorgiidae, deterred fish feeding. Chemical and structural defenses co-occurred in representatives of the families Plexauriidae (*Astrogorgia* sp. and *Villogorgia* sp.) and Subergorgiidae (*A. mollis* and *S. suberosa*). Only 1 gorgonian coral, *Viminella* sp., appeared to rely primarily upon structural defenses rather than chemical defenses. To determine if size and shape were factors associated with sclerite structural defenses, a second set of feeding assays was conducted with powdered and whole sclerites from the unpalatable species. Fishes avoided the powdered sclerites from 4 of the 5 species when offered at natural concentrations, suggesting that other properties remaining after the structural integrity of the sclerites was destroyed optimize the calcite skeletal elements for a defensive role.

KEY WORDS: Extracts · Sclerites · Chemical Ecology · *Annella* spp. · *Subergorgia suberosa* · *Rumphella* sp. · *Villogorgia* sp. · *Viminella* sp. · *Astrogorgia* sp. · Guam

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INTRODUCTION

Chemical (i.e. secondary metabolites) and structural defense mechanisms (i.e. $CaCO_3$) have been reported to co-occur in algae (Paul & Hay 1986, Pennings & Paul 1992, Hay et al. 1994, Schupp & Paul 1994, Pennings et al. 1996) and octocorals (Gerhart et al. 1988, Harvell et al. 1988, Harvell & Fenical 1989, van Alstyne & Paul 1992, van Alstyne et al. 1994, Slattery 1999, Koh et al. 2000, Puglisi et al. 2000). Production of multiple defense mechanisms by sessile marine organisms may be to their advantage, allowing them to overcome predation pressures from the high diversity of tropical consumers (Paul & Hay 1986, Hay et al. 1994, Schupp & Paul 1994, Pennings et al. 1996). Schupp & Paul (1994) observed differences in the effects of diterpenoid secondary metabolites from the green alga *Halimeda macroloba* and aragonite (the $CaCO_3$ form found in *H. macroloba*) when offered at natural concentrations to different herbivorous fishes in laboratory feeding assays.

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Scarus sordidus and *Ctenochaetus striatus* avoided the diterpenes but not the aragonite, while *Naso lituratus* and *Siganus spinus* avoided the aragonite but not the diterpenes (Schupp & Paul 1994). A limited number of comparative studies have been conducted among different species in the same phylum other than calcified algae (Paul & Hay 1986). Studies such as these are important to determine whether general patterns of defense exist among related groups of organisms.

Gorgonians, like other sessile marine organisms, produce a variety of secondary metabolites (Faulkner 2000a,b and references therein). These metabolites are concentrated in the coenenchyme (polyp bearing connective tissue surrounding the gorgonian skeleton) which also contains small calcite skeletal elements called sclerites (Bayer 1955). Feeding studies with crude extracts and secondary metabolites from sea fans and whips have demonstrated that many species of gorgonians are chemically defended (Pawlik et al. 1987, Harvell et al. 1988, 1993, 1996, Fenical & Pawlik 1991, Pawlik & Fenical 1992, Maia et al. 1999, Slattery 1999, Koh et al. 2000, Puglisi et al. 2000). However, broad surveys of the common species of gorgonians in the Caribbean and Guam show that the production of chemical defenses is not ubiquitous in this group of animals (Pawlik et al. 1987, Puglisi 2001).

Sclerites are the microscopic components of a meso-skeleton that prevent damage to the tissues caused by heavy wave action and currents (Esford & Lewis 1990, Lewis & von Wallis 1991). Sclerites differ considerably in size, shape and color among species of octocorals and are used as a taxonomic delineation (Bayer 1955). The few feeding studies conducted with gorgonian sclerites have demonstrated that the Caribbean gorgonians *Briareum asbestinum* (Harvell et al. 1993, West 1997, 1998), *Gorgonia ventalina* (van Alstyne & Paul 1992, Cronin et al. 1995, Slattery 1999), *Leptogorgia virgulata* (Gerhart et al. 1988), *Pseudopterogorgia acerosa* (Harvell et al. 1988) and the Singapore gorgonians *Cteno-cella pectinata*, *Ctenocella* sp. and *Echinogorgia* sp. (Koh et al. 2000) are structurally as well as a chemically defended. In a broad survey of Caribbean species, the extracts from species with small sclerites were usually found to be unpalatable to fishes (Pawlik et al. 1987, Harvell & Fenical 1989). The importance of the size, shape and density of sclerites in the coenenchyme to palatability has only been addressed for *B. asbestinum* (West et al. 1993, West 1998). Short, densely packed sclerites in colonies at shallow depths deterred fishes, while longer, larger, more loosely associated sclerites in colonies at deeper depths did not (West et al. 1993, West 1998). In addition, simulated predator damage to colonies resulted in the induction of the smaller sclerites that deter predators, suggesting that defense can be a major role for sclerites (West 1997).

Qualitative and quantitative variation of chemical and structural components within colonies occurs in some species of soft corals (Harvell & Fenical 1989, van Alstyne & Paul 1992, Kim et al. 2000, Puglisi et al. 2000). In an early study of the chemical and structural components of *Pseudopterogorgia* spp., metabolites were reported to be concentrated at the tips of colonies whereas sclerites were more concentrated in the mid-axes and bases, suggesting a potential tradeoff in resource allocation between chemical and structural components (Harvell & Fenical 1989). Van Alstyne & Paul (1992) observed similar patterns for the soft corals *Sinularia polydactyla* and *Sinularia* sp. where higher concentrations of metabolites where found in the terminal branches of colonies. Kim et al. (2000) recently reported the crude extracts from the tips of healthy *Gorgonia* spp. colonies to be more resistant to the fungal pathogen *Aspergillus sydowii* than extracts from other parts of the colony. This activity was correlated with higher crude extract concentrations in the tips of the colonies. However, 2 independent studies of *G. ventalina* by van Alstyne & Paul (1992) and Slattery (1999), found no within-colony variation of crude extract and sclerite concentrations in the upper portions of the colonies. In a recent study, feeding assays with crude extracts and sclerites from the Pacific gorgonians *Annella mollis* and *A. reticulata* showed that crude extracts from the tips of colonies deterred fish feeding, while similar concentrations of extracts from the mid-axes were not always effective deterrents (Puglisi et al. 2000). Crude extract concentrations did not differ between mid-axes and tips for either species, thus suggesting that quantitative differences in the concentration of the crude extract does not reflect differences in the active components.

In addition to providing an advantage to the organism by affording multiple defenses against a multitude of predators, the components of a multiple defense system may act additively or synergistically (Gerhart et al. 1988, Hay et al. 1994, Hay 1996). The components are additive if the combined effect is equal to the sum of the expected activities predicted by the dose response curve of the individual components. They are synergistic if the combined effect of the components is greater than the sum predicted by the dose response curves of the individual components (Berenbaum 1989). One advantage of synergism to the host organism is that multiple components of the defense system can be maintained within the tissues in a non-toxic state. Evidence for synergism between chemical defenses and $CaCO_3$ in feeding studies has been reported for the green alga *Halimeda goreauii* (Hay et al. 1994) and the gorgonian *Leptogorgia virgulata* (Gerhart et al. 1988, but see Hay 1996).

Studies of the co-occurrence of chemical and structural defenses of gorgonian corals have been limited to

a few species common to Caribbean and Singapore reefs. In this study we addressed within-colony variation of lipophilic crude extract and sclerite concentrations of 7 common species of gorgonian corals on Guam representing 4 families, *Annella mollis*, *A. reticulata*, *Astrogorgia* sp., *Subergorgia suberosa*, *Rumphella* sp., *Villogorgia* sp. and *Viminella* sp. The concurrent analysis of 7 species allowed us to ask the following questions: (1) Is within-colony variation in the concentrations of the chemical and structural components characteristic of the common gorgonian species of Guam? (2) Do chemical and structural components exhibit reciprocal gradients within the colony? (3) Is the co-occurrence of chemical and structural defense mechanisms characteristic of the common gorgonian species of Guam? (4) Are shape and size of sclerites important aspects of structural defense?

MATERIALS AND METHODS

Study organisms. The chemical and structural defense mechanisms of 7 of the most common gorgonians encountered on Guam's reefs were surveyed. *Viminella* sp. (Gray 1870), in the family Ellisellidae, is a red sea whip characterized by red double head and short spindle sclerites (Grassoff 1999). This gorgonian has a smooth surface and grows as a single unbranched whip up to 1 m. *Rumphella* sp. cf. *aggregata* (hereafter referred to as *Rumphella* sp.) (Nutting), in the family Gorgoniidae, is a zooxanthellate, uniform gray-brown weeping fan standing at 35 to 40 cm with transparent club-like sclerites (Chen & Chang 1991). *Astrogorgia* sp. (Verrill 1868), in the family Plexauriidae, is a dark reddish violet fan with free branches standing at 35 cm. The large violet sclerites (some larger than 2.0 mm) are mainly spindles with irregularly arranged tubercles (Grassoff 1999). *Villogorgia* sp. (Duchassing & Michelotti 1860), also in the family Plexauriidae, is a delicate violet fan with violet thorn-scales that grows in tall bushes up to 1 m (Williams 1992). *Annella mollis* and *A. reticulata* (Grassoff 1999), in the family Subergorgiidae, are large sea fans (<2 to 3 m) with branches closely anastomosed as a network. The sclerites are small and colorless (Chen & Chang 1991). *A. mollis* is typically brown or orange with oblong cells. This species is characterized by the 'double-wheel' sclerites densely packed in the cortex (Grassoff 1999). *A. reticulata* is pink to orange with small cells and is characterized by the 'double-head' sclerites in the cortex. *Subergorgia suberosa* (Pallas 1766), also in the family Subergorgiidae, is a yellow-brown fan with free branches growing to 1 m. Deep longitudinal furrows on either side of the branches characterize this species; the sclerites are mainly yellow spindles (Chen & Chang 1991).

Collection sites and sampling methods. Sea fans and whips were collected by SCUBA from sites around Guam between March and May 1998. *Annella mollis* and *Rumphella* sp. were collected from Cocos Wall (13° 30.332′ N, 144° 46.301′ E) at 20 to 35 m. *A. reticulata* and *Villogorgia* sp. were collected from Hospital Point (13° 30.462′ N, 144° 46.595′ E) at 30 to 50 m. *Astrogorgia* sp. was collected from the Blue Hole (13° 30.457′ N, 144° 46.565′ E) at 30 to 40 m. *Subergorgia suberosa* was collected from Anae Island (13° 30.389′ N, 144° 46.605′ E) at 15 to 20 m. *Viminella* sp. was collected from Uruno Point (13° 36.324′ N, 144° 49.551′ E) at 30 m.

Five or 7 individual colonies of each species were removed from the reef by cutting the base of the animal with underwater shears. Colonies were separated into the base (lowest 10 cm of colony), mid-axis (the center of the colony between the base and tip) and tip (top 5 cm of colony around the outer edges) sections (Harvell & Fenical 1989, van Alstyne & Paul 1992, Puglisi et al. 2000). Colonies were extracted immediately at the University of Guam Marine Laboratory (UOGML). Voucher specimens were preserved in 70% ethanol or 10% formalin and air-dried. A representative of each species was deposited in the collections at the UOGML and/or the Museum and Art Gallery of the Northern Territory, Darwin.

Crude extract and sclerite preparation. Two 3 cm samples of fresh animal tissue were removed from the base, mid-axis and tip of each colony. One sample from each section was extracted for analytical evaluation of crude extract and sclerite concentrations in the coenenchyme. Protein concentrations were determined with the duplicate set of samples. The remainder of each colony was separated into mid-axis and tips for bulk extraction to obtain crude extracts and sclerites for feeding assays. To determine the crude extract and sclerite concentrations, each sample was weighed and exhaustively extracted in 1:1 dichloromethane:methanol over 72 h. The extracted tissue was then dried in an oven for 24 h at 64°C. After determining the dry mass, the samples were dissolved in 5.25% sodium hypochlorite (bleach) solution to free the sclerites and the gorgonin skeleton. These were rinsed with fresh water and then dried in an oven for 24 h at 64°C. Crude extracts were evaporated under reduced pressure and weighed. Total dry mass equaled the dry mass of the extracted tissue plus the dry mass of the crude extract. Dry extracts were stored at 0°C. Natural concentrations of the crude extracts and sclerites were calculated as a portion of the coenenchyme as follows:

$$\text{Yield (\%)} = \frac{\text{Crude extract (or sclerite) mass}}{\text{(Total dry mass} - \text{gorgonin skeleton mass)}} \times 100$$

Protein concentration. Protein concentrations were determined by a modified Bradford protein assay (Karentz et al. 1997, Puglisi et al. 2000). Approximately 50 mg of tissue was removed from the gorgonin skeleton and digested for 12 h in 5 ml of 1N NaOH. Five ml of 1N HCl was added to neutralize the solution. 200 µl of each sample was diluted with 5 ml of Bio-Rad protein solution and the absorbance (595 nm) was recorded on a DU-65 Beckman spectrophotometer. Protein concentrations were calculated with a calibration curve generated from standards with bovine serum albumin that were prepared following the same digestion procedure described above. Mean protein concentrations did not differ significantly between parts (p = 0.34) or among species (p = 0.12). The artificial diets were prepared to approximate the mean protein concentration of 25 % per g dry mass.

Multiple comparison feeding assays with extracts and sclerites. Previous studies addressing chemical and structural defenses in gorgonian corals have offered fishes a choice between one treatment (chemical and/or structural components) and a control in paired assays (Pawlik et al. 1987, Gerhart et al. 1988, Harvell et al. 1988, 1993, 1996, Fenical & Pawlik 1991, Pawlik & Fenical 1992, van Alstyne & Paul 1992, Maia et al. 1999, Slattery 1999, Koh et al. 2000, Puglisi et al. 2000). The feeding assay design for this study is similar to van Alstyne et al. (1994). Natural assemblages of reef fishes were offered 3 treatment ropes (crude extract, sclerites, a combination of the crude extract and sclerites) side-by-side and 1 control rope each carrying 4 food cubes. The 4 ropes were set out on the reef adjacent to one another. Feeding experiments were conducted with the crude extracts and sclerites from the mid-axes and tips of the gorgonian corals at Western Shoals, Guam from April to June 1998 and in February 2001. Crude extracts and sclerites were incorporated into the artificial fish food at the mean (n = 5 or 7) concentrations determined for each species or, in the case of mid-axis sclerites, at the lower end of the range of concentrations to determine if sclerites deterred fish feeding at the lowest concentrations (Table 1). The artificial diet was prepared with 5.0 g Kruses Brand catfish food, 2.5 g carrageenan (Type 1) and 80 ml water. Carrageenan was stirred into a 250 ml beaker containing the water and microwaved on high until the mixture boiled, approximately 2 min. The fish food and extract (dissolved in ethyl acetate) were stirred into the beaker after the carrageenan cooled for approximately 30 s. Control and sclerite cubes were prepared by adding 1 ml of ethyl acetate, the carrier

solvent for the crude extracts. Twenty-five 1 × 1 cm food cubes were prepared by pouring the mixture into a partitioned tray. Four cubes were mounted on each rope with safety pins. The assays were scored by counting the number of food cubes eaten from each rope. Only the food cubes completely removed from the O-rings on the SCUBA tanks were scored as eaten. The ropes were removed from the reef when fishes removed all of the food cubes from 1 to 3 of the ropes. In all cases, fishes would avoid eating more than one cube from a rope if the food was unpalatable. Feeding assays lasted approximately 1 h each.

Schools of damselfishes *Abudefduf sexfasciatus* and *Amblyglyphidodon curacao*, juvenile parrotfishes *Scarus schlegeli* and *S. sordidus*, the occasional wrasses *Cheilinus fasciatus* and *Thalossoma hardwickii*, surgeonfishes *Naso vlamengii*, *N. lituratus* and *Acanthurus triostegus*, and the butterflyfish *Chaetodon auriga* represent the fishes most frequently encountered during the feeding assays.

Powdered sclerite feeding assays. In the first series of feeding experiments, sclerites from some species of gorgonian corals deterred fish feeding while others did not. While these assays did suggest that some of the observed unpalatability might be a result of the concentration of sclerites in the artificial food, it was unclear what other differences in the sclerites accounted for the variability. A second series of feeding assays was conducted with powdered sclerites from the unpalatable species *Annella mollis*, *Astrogorgia* sp., *Subergorgia suberosa*, *Villogorgia* sp. and *Viminella* sp., to determine if the shape and size of the sclerites was an important property for deterring fish feeding. Sclerites were crushed into a fine powder in a mortar and pestle and incorporated into the artificial diet at the same concentration as that used for the whole sclerites in the previous assays. Fishes were offered a choice among the powdered sclerites, whole sclerites and plain food without sclerites. Feeding assays were conducted at Western Shoals with ropes in sets of 3.

Table 1. Concentration of crude extracts and sclerites in the feeding assays from the mid-axes and tips of the colonies

Species	Mid-axis		Tips	
	Crude extract Conc. (%)	Sclerite Conc. (%)	Crude extract Conc. (%)	Sclerite Conc. (%)
Annella mollis	9.71	41.83	7.78	56.40
Annella reticulata	3.98	61.34	4.92	62.69
Astrogorgia sp.	4.99	43.74	11.62	83.99
Rumphella sp.	9.00	56.71	15.00	80.47
Subergorgia suberosa	6.96	43.46	12.53	40.24
Villogorgia sp.	5.51	43.74	11.38	83.99
Viminella sp.	7.30	83.32	12.33	71.65

Statistical analyses. Data for the crude extracts and sclerite concentrations did not meet the requirements for parametric analysis. Therefore, differences in base, mid-axis and tip yields among parts and within individuals were calculated using Friedman's test (Sokal & Rohlf 1981). The factors used were location within the colony and individual. A simple regression was used to compare extract and sclerite concentrations for each of the 7 species to investigate if patterns of within-colony distribution could be found with respect to chemical and structural components. Data collected in the protein analysis did not meet the requirement for parametric analysis either. Therefore, differences in the protein concentrations of mid-axes and tips within colonies of the different species were calculated using a Kruskal-Wallis test. The data generated from the 4-way and 3-way feeding assays were analyzed with Friedman's test (Sokal & Rohlf 1981). Comparisons between treatments were analyzed by pair-wise Wilcoxon signed rank comparisons with α adjusted for the number of analyses. All statistical analyses were generated with Statview 5.01 for Macintosh (Abacus Concepts).

RESULTS

Within colony variation of crude extract and sclerite concentrations

Four of the 7 gorgonians surveyed, *Annella mollis*, *A. reticulata*, *Subergorgia suberosa*, and *Viminella* sp., exhibited significant differences in the crude extract concentration among parts of the colonies (Fig. 1a,b, e,g). Although small sample sizes did not allow for statistical comparisons among parts, crude extract concentrations appeared to increase from the base to the
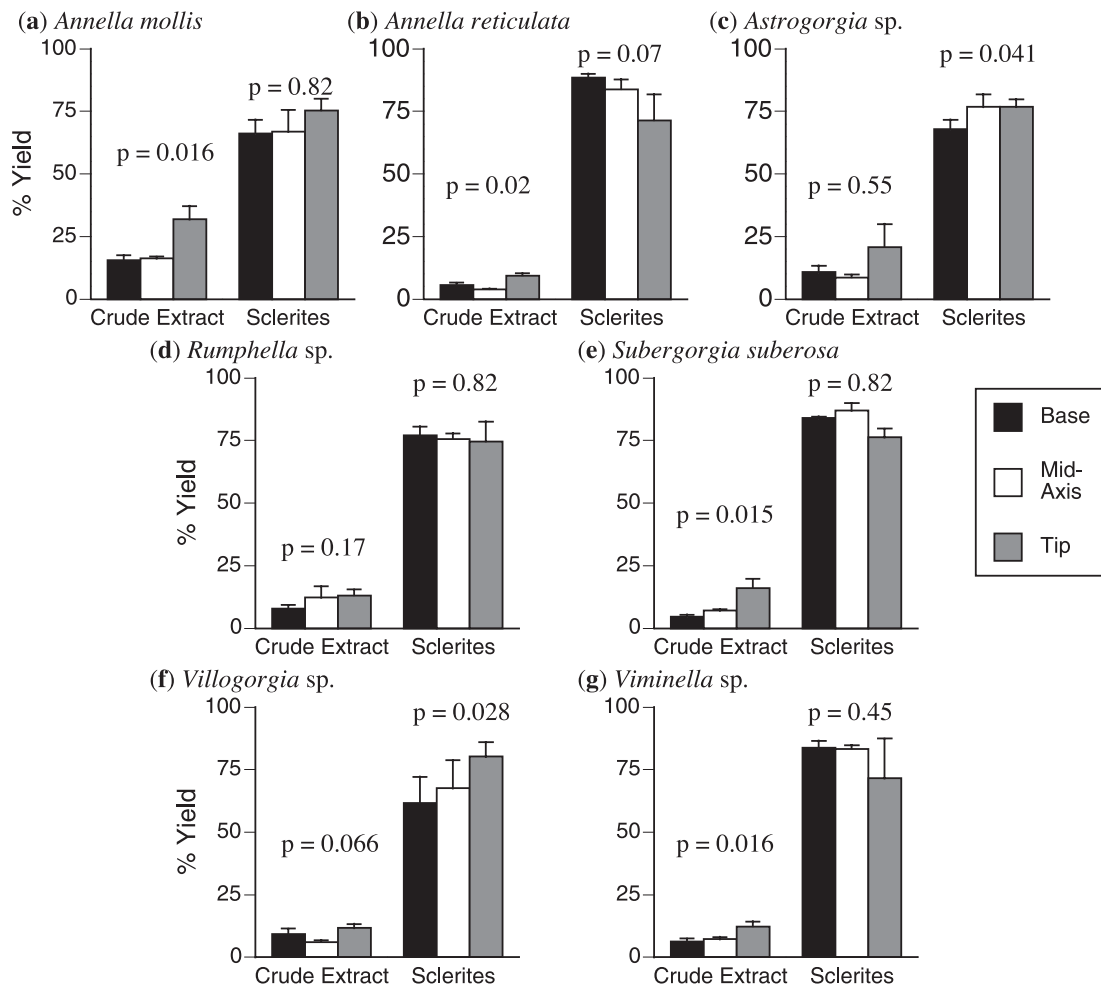

Fig. 1. Crude extract and sclerite concentrations in the coenenchyme of colonies of (a) *Annella mollis* (n = 5), (b) *A. reticulata* (n = 5), (c) *Astrogorgia* sp. (n = 5), (d) *Rumphella* sp. (n = 5), (e) *Subergorgia suberosa* (n = 5), (f) *Villogorgia* sp. (n = 7) and (g) *Viminella* sp. (n = 5). Differences in base, mid-axis and tip yields among sites and within individuals were calculated using Friedman's test

tips of the colonies. *Villogorgia* sp. also exhibited this trend (Fig. 1f). *Astrogorgia* sp. and *Rumphella* sp. had uniform crude extract concentrations throughout the colony (Fig. 1c,d; p = 0.55 and p = 0.17, respectively). Crude extract concentrations did not vary among individual colonies for any of the 7 species surveyed (*A. mollis*: p = 0.32; *A. reticulata*: p = 0.57; *Astrogorgia* sp.: p = 0.75; *Rumphella* sp.: p = 0.066; *S. suberosa*: p = 0.20; *Villogorgia* sp.: p = 0.72; *Viminella* sp.: p = 0.25).

Sclerite concentrations differed significantly among parts of *Astrogorgia* sp. and *Villogorgia* sp. colonies (Fig. 1c,f), while sclerite concentrations were fairly uniform throughout the colonies of *Annella mollis*, *Rumphella* sp., and *Viminella* sp. (Fig. 1a,d,g). *A. reticulata* exhibited a trend toward a significant difference in sclerite concentration among parts of the colonies (Fig. 1b). Although small sample sizes did not allow for statistical comparisons among parts, sclerite concentrations appeared to increase from the bases to the tips

of colonies, similar to crude extract concentrations. Sclerite concentrations did differ significantly among individual colonies of *Villogorgia* sp. (p = 0.008) but did not vary among individual colonies for any other species (*A. mollis*: p = 0.97; *A. reticulata*: p = 0.10; *Astrogorgia* sp.: p = 0.90; *Rumphella* sp.: p = 0.28; *S. suberosa*: p = 0.39; *Viminella* sp.: p = 0.41). No overall correlations could be found between crude extract and sclerite concentrations within colonies when crude extract and sclerites were compared by a simple regression for any of the species surveyed.

Multiple comparison feeding assays with extracts and sclerites

Crude extracts from the tips of 6 of the 7 species in this study deterred feeding by local assemblages of fishes at natural concentrations (Fig. 2a–f; p < 0.001).
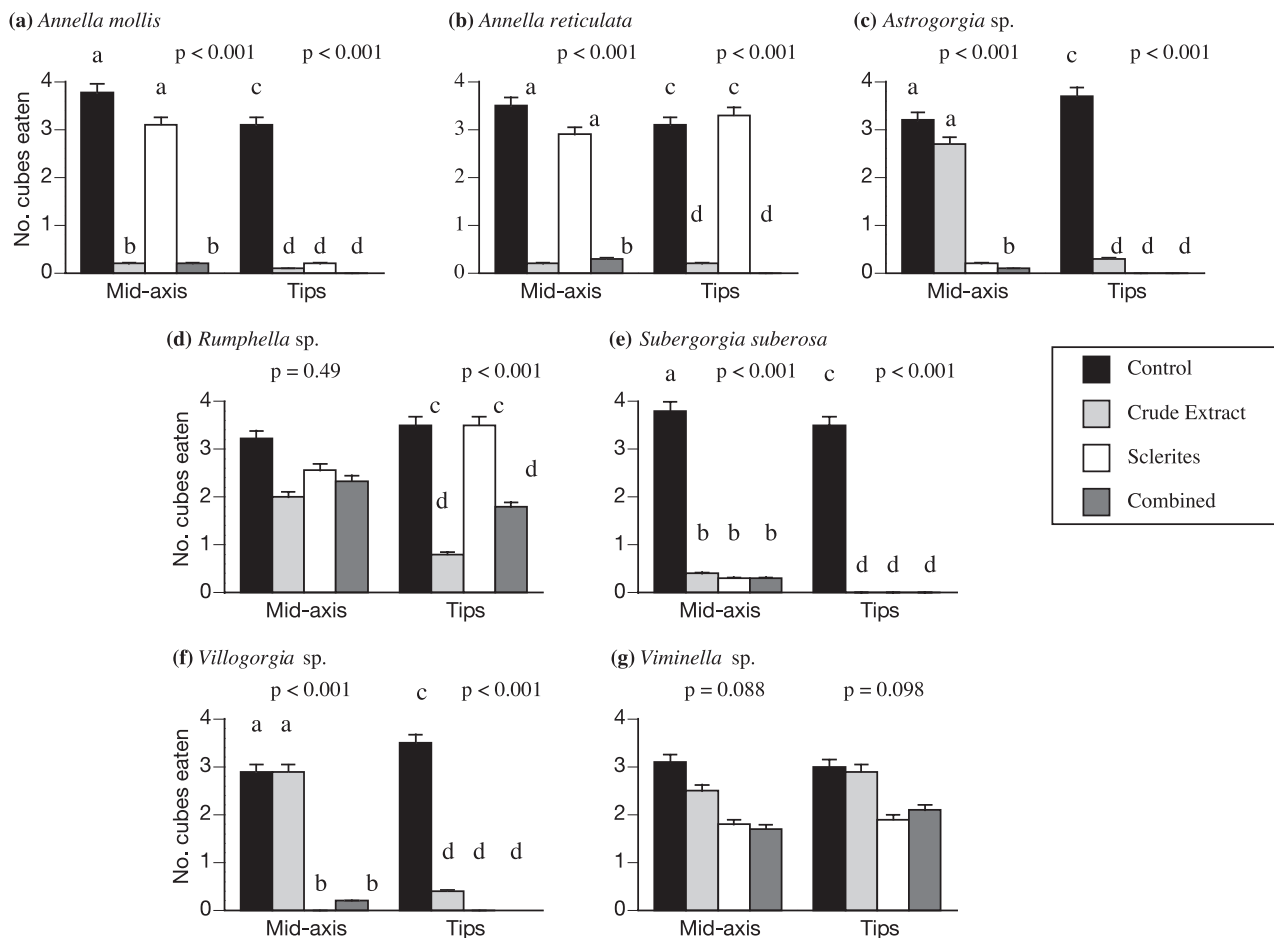

Fig. 2. Feeding assay results for crude extracts and sclerites from the mid-axis and tips of (a) *Annella mollis* (n = 10), (b) *A. reticulata* (n = 10), (c) *Astrogorgia* sp. (n = 10), (d) *Rumphella* sp. (crude extract: n = 9, sclerites: n = 10), (e) *Subergorgia suberosa* (n = 10), (f) *Villogorgia* sp. (n = 10) and (g) *Viminella* sp. (n = 10). Similar means among samples are indicated with same letters above the histogram bars

The crude extracts from the mid-axis of the 3 species of gorgonians in the family Subergorgiidae, *Annella mollis*, *A. reticulata* and *S. suberosa*, also deterred feeding by generalist fishes (Fig. 2a,b,e; p < 0.001). The mid-axis and tip extracts from *Viminella* sp. did not significantly deter fish feeding, however, the results show a trend (Fig. 2g; p = 0.088 and p = 0.098, respectively) suggesting that the sclerites from the mid-axes and tips may play a role in defense.

Sclerites from *Astrogorgia* sp., *Subergorgia suberosa* and *Villogorgia* sp. were unpalatable at the mid-axis and tip concentrations (Fig. 2c,e,f; p < 0.001). In addition, the high concentration of sclerites from the tips of *A. mollis* deterred fish feeding (Fig. 2a; p < 0.001). In this series of assays, sclerites from *A. reticulata* and *Rumphella* sp. did not deter fish feeding (Fig. 2b,d).

Powdered sclerite feeding assays

The powdered sclerites from 4 of the 5 species, *Annella mollis*, *Astrogorgia* sp., *Subergorgia suberosa* and *Viminella* sp., deterred fish feeding when offered at the same concentration as the whole sclerites (Fig. 3). Except for *A. mollis* and *Astrogorgia* sp., less of the treated food containing the whole sclerites was eaten compared to food containing the powdered sclerites.

DISCUSSION

Chemical and structural defenses co-occurred in 4 of the 7 species surveyed, *Annella mollis* and *Subergorgia suberosa* in the family Subergorgiidae and suborder Scleraxonia, and *Astrogorgia* sp. and *Villogorgia* sp. in the family Plexauriidae and suborder Holaxonia. In previous studies, the co-occurrence of chemical and structural defenses was reported for *Briareum asbestinum* in the family Briaridae, suborder Scleraxonia (Harvell et al. 1993, West 1997, 1998), *Gorgonia ventalina* (van Alstyne & Paul 1992, Cronin et al. 1995, Slattery 1999), *Pseudopterogorgia acerosa* (Harvell et al. 1988) and *Leptogorgia virgulata* (Gerhart et al. 1988) in the family Gorgoniidae, suborder Holaxonia, *Echinogorgia* sp. (Koh et al. 2000) in the family Plexauridae, suborder Holaxonia and *Ctenocella pectinata* and *Ctenocella* sp. (Koh et al. 2000) in the family Ellisellidae, suborder Calcaxonia.

In general, chemical and structural defense mechanisms were more frequently encountered at the outer edges of the gorgonian colonies. The members in the family Subergorgiidae, *Annella mollis*, *A. reticulata*, and *Subergorgia suberosa*, were the only species whose mid-axis and tip extracts were both unpalatable to fishes. Variation in crude extract concentrations in the coenenchyme was characteristic of only 4 of the 7 species surveyed. The limited sample size of n = 5 did not allow for post-hoc Wilcoxon tests for paired comparisons to determine the significant differences among specific parts of the gorgonian colonies.

During vegetative reproduction new polyps are added to the outer colony edges (Szmant-Froelich 1974). Therefore, it was not surprising to find the tips of the gorgonian colonies better defended against generalist predators. Assuming a cost associated with the production of chemical and structural defenses, the feeding assay results presented here may be consistent with the predictions of the optimal defense hypothesis that defenses will be concentrated in the more susceptible parts of the plant or animal if damage to the tips by predation causes reduced growth in the colony (McKey 1974, 1979, Rhoades 1979).
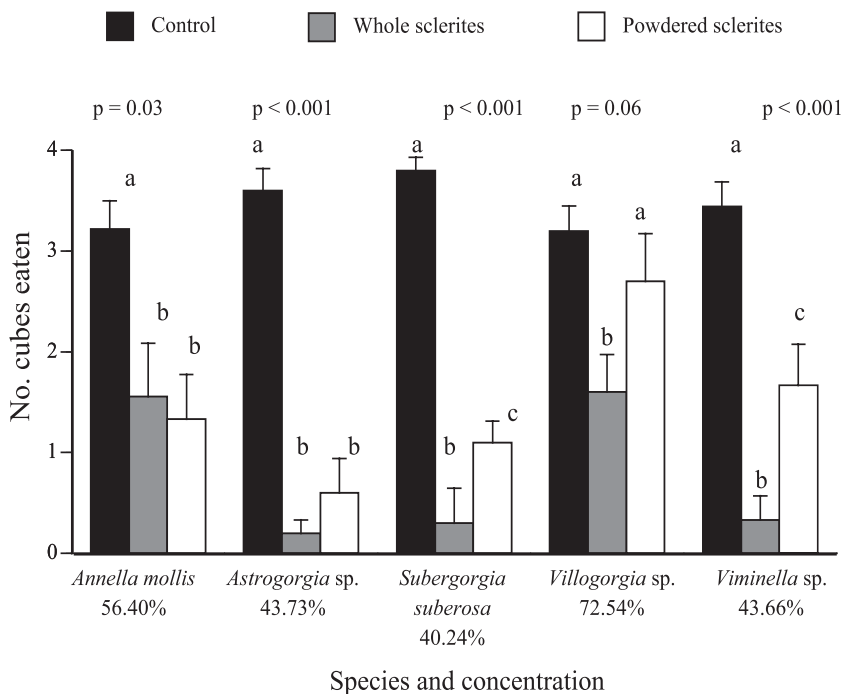

Fig. 3. Feeding assay results for powdered and whole sclerites from *Annella mollis* (n = 9), *Astrogorgia* sp. (n = 10), *Subergorgia suberosa* (n = 10), *Villogorgia* sp. (n = 10) and *Viminella* sp. (n = 9). Similar means among samples are indicated with same letters above the histogram bars

Fewer studies have addressed structural defenses of gorgonians (Gerhart et al. 1988, Harvell et al. 1988, 1993, van Alstyne & Paul 1992, Cronin et al. 1995, West 1997, 1998, Slattery 1999, Koh et al. 2000, Puglisi et al. 2000). Sclerites in cnidarians and spicules in other phyla, when densely packed, have been shown to increase rigidity and resist excessive deformation and tearing that could result from environmental stresses such as heavy currents (Koehl 1982, Lewis & von Wallis 1991, West et al. 1993). The major role for sclerites in gorgonian corals is purported to be the support of new tissues at the tips before an internal gorgonin skeleton can be deposited (Szmant-Froelich 1974), whereas defense is thought to be a secondary or, perhaps, an incidental role. However, West et al. (1993) and West (1998) provided direct evidence that sclerites of the Caribbean gorgonian *Briareum asbestinum* serve as structural defenses by showing that short, densely packed sclerites in the coenenchyme of colonies at shallow depths deterred fishes while longer, more loosely associated sclerites in colonies at deeper depths did not. In addition, simulated predator damage to colonies of *B. asbestinum* resulted in the induction of the smaller predator-deterrent sclerites (West 1997). In this survey, sclerites from 5 of the 7 species surveyed were unpalatable to fishes. The sea whip *Viminella* sp. was unusual because extracts from the colonies did not deter predators, even at the outer edges. This is the first instance where a gorgonian coral has been experimentally shown to rely solely upon sclerites to deter feeding by generalist predators, providing further evidence that sclerites can play a major role as structural defenses as well as structural support.

In the second set of feeding experiments conducted with sclerites powdered in a mortar and pestle versus whole sclerites, fishes avoided the powdered sclerites and the whole sclerites from 4 of 5 species. With the exception of *Annella mollis* and *Astrogorgia* sp., fewer food cubes containing the whole sclerites were consumed compared to the food cubes containing powdered sclerites, suggesting that size and shape do account for some of the observed feeding deterrence activity; however, other chemical and physical properties of the sclerites may also be important aspects that contribute to the ability of sclerites to deter fishes. Feeding studies by Pennings & Svedberg (1993) and Schupp & Paul (1994) showed that ground $CaCO_3$ was unpalatable to a variety of herbivores. Powdered sclerites are essentially finely ground $CaCO_3$, which has been proposed to act on predators by a number of mechanisms including (1) the inhibition of digestion by neutralizing acid in the gut of the consumer, (2) altering the effectiveness of enzymes by shifting the gut pH or (3) increasing the effect of co-occurring metabolites if their activity is pH dependent (Feeny 1970, Pennings & Svedberg 1993, Schupp & Paul 1994).

There was no evidence in the feeding experiments conducted here to suggest that the combination of the chemical and structural components showed an additive or synergistic effect, contrary to the results reported for feeding study with crude extracts and sclerites from colonies of *Leptogorgia virgulata* by Gerhart and his colleagues (1988). In this earlier study, the chemical and physical components of the gorgonian colony did not deter feeding when offered separately to fishes but appeared to act synergistically to deter fishes when offered in combination (Gerhart et al. 1988). Gerhart and his colleagues proposed 2 explanations for this phenomenon: (1) the simultaneous response to 2 negative stimuli and (2) that sclerites pierce the mouth of the fish allowing the crude extract to pass into the internal tissues. Our results suggest that synergism between chemical and structural components does not occur widely in gorgonian corals. Rather, it is more likely that the co-occurrence of chemical and structural defenses in this group of organisms is an adaptive advantage against a variety of potential consumers found on coral reefs (Paul & Hay 1986, Hay et al. 1994, Schupp & Paul 1994, Pennings et al. 1996).

Although some previous studies have shown that soft corals and gorgonians deploy inverse gradients of chemical and structural defense mechanisms suggesting a trade-off between chemical and structural defenses within colonies (Harvell & Fenical 1989, van Alstyne et al. 1994), our results mostly contradict that pattern suggesting that these few examples do not represent general patterns for gorgonians. While 4 of the 7 gorgonians surveyed, *Annella mollis*, *A. reticulata*, *Subergorgia suberosa*, and *Viminella* sp., did exhibit significant differences in the crude extract concentration among parts of the colonies, sclerite concentrations did not differ significantly within the colonies. Alternatively, sclerite concentrations differed significantly among parts of *Astrogorgia* sp. and *Villogorgia* sp. colonies, whereas the crude extract concentrations did not. These results do not suggest any relationship between crude extract and sclerite production in the colonies. Further, we propose that concentration is not necessarily a determining factor for gorgonian structural defense mechanisms, which is implied if we consider a 'trade-off hypothesis'. For example, in the feeding experiments conducted in this study, sclerites from the mid-axes and tips of *A. reticulata* and *Rumphella* sp. did not deter feeding when offered to fishes at similar concentrations to those from *A. mollis*, *Astrogorgia* sp., *Subergorgia suberosa*, *Villogorgia* sp. and *Viminella* sp. These results provide further evidence that other chemical and physical properties of sclerites are important for structural defense mechanisms (van Alstyne et al. 1992).

In summary, the multiple-choice feeding experiments conducted in this study demonstrated that chemical and structural defenses produced by gorgonian corals of Guam are more frequently encountered at the outer edges of colonies. The co-occurrence of chemical and structural defense mechanisms occurred in colonies of *Annella mollis*, *Subergorgia suberosa*, *Astrogorgia* sp. and *Villogorgia* sp. We suggest that further studies of chemical and structural defense mechanisms in gorgonians will show trends in phenotypic expression at the family level and may provide insight into the evolution of defenses produced by gorgonians. Moreover, this is the first instance where a gorgonian, *Viminella* sp., is reported to rely primarily upon structural defense. This result supports the hypothesis that defense is a major role for sclerites in some species of gorgonian corals in addition to providing structural support of the colony. Feeding experiments with the powdered sclerites also demonstrated that size and shape do explain some of the feeding deterrence activity of sclerites, however, other physical and chemical properties are also likely to be important factors in structural defense of gorgonian corals.

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