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A reappraisal of the chemical and physical defenses of Caribbean gorgonian corals against predatory fishes

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ABSTRACT: Anti-predatory properties of crude organic extracts and calcitic sclerites from the tissues of 32 species of Caribbean gorgonians were examined at natural volumetric concentrations in laboratory feeding assays using the bluehead wrasse *Thalassoma bifasciatum*. The nutritional qualities of the gorgonian samples were analyzed by determination of protein content, total energy content, and ash mass. All of the species tested (100%) yielded predation-deterrent crude organic extracts, but the sclerites of only 2 species (6.3%), *Pterogorgia citrina* and the encrusting form of *Briareum asbestinum*, were deterrent. The mean NaOH-soluble protein content, total energy content and ash mass of the gorgonian species were 17 ± 8 mg ml^{-1}, 4 ± 2 kJ ml^{-1}, and 400 ± 100 mg ml^{-1}, respectively. There was no apparent relationship between the nutritional quality of a species and its chemical or physical defense. Contrary to previous studies, results indicated that sclerites do not generally afford gorgonians protection against generalist fish predators, and there was no evidence that the presence of sclerites in gorgonian tissue decreases the nutritional value sufficiently to deter predation. As in sponges, secondary metabolites are the primary means of defense against fish predators for Caribbean gorgonians. Results confirm that feeding assays conducted on the basis of tissue volume yield different results than those conducted on the basis of tissue mass.

KEY WORDS: Gorgonian · Chemical defense · Physical defense · Predation · Nutritional quality · Sclerites · Spicules

INTRODUCTION

Gorgonian corals are rarely consumed on Caribbean reefs, despite their abundance and the prevailing conditions of high predation in these ecosystems (Grigg et al. 1984). With the exception of relatively small-scale or localized predation by certain specialists such as the snail *Cyphoma gibbosum* (Harvell & Suchanek 1987), the butterflyfish *Chaetodon capistratus* (Lasker 1985), and the bristleworm *Hermodice carunculata*, gorgonians are generally unexploited as a food source (Preston & Preston 1975). Randall (1967) reported that the gut contents from only 11 of 212 species of reef fishes contained gorgonian tissue, and only 1 of these (*Alutera scripta*) had consumed gorgonians in excess of 5% of its diet. Such a low level of predation is surprising considering gorgonian abundance, with typical values ranging from 5.43 colonies m^{-2} (Preston & Preston 1975) to 25.1 colonies m^{-2} (Goldberg 1973), making gorgonians the most conspicuous sessile organisms on Caribbean reefs (Goldberg 1973, Lasker & Coffroth 1983).

Generalists may not prey on gorgonians for several reasons. Like many sponges and algae, gorgonians may be chemically defended, or the presence of calcitic sclerites in their coenenchyme may deter predators. An alternative possibility, however, is that gor-

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gonians lack sufficient nutritional quality to sustain most generalist reef predators.

Evidence of chemical defense among gorgonians is abundant. While the chemistry of only ~20% of Caribbean gorgonian species has been studied in detail, a large number of novel metabolites have been discovered in the tissue of these organisms (reviewed in Rodriguez 1995). Many natural products isolated from gorgonians are active in pharmacological assays, often exhibiting anti-cancer and anti-inflammatory properties (Fenical 1987, Rodriguez 1995). Hypotheses regarding the benefits of these metabolites to gorgonians have also been advanced. There is experimental evidence that the compounds may inhibit larval settlement, increase resistance to fungal pathogens, or prevent or inhibit overgrowth by other sessile organisms (Standing et al. 1984, Rodriguez 1995, Kim et al. 2000). Organic extracts of gorgonians do not appear to have strong antimicrobial activity against marine bacteria (Jensen et al. 1996). The most common hypothesis is that secondary metabolites are anti-predatory agents, and studies have consistently demonstrated anti-predatory properties of gorgonian crude extracts and purified compounds (Pawlik et al. 1987, Fenical & Pawlik 1991, Pawlik & Fenical 1992, Van Alstyne & Paul 1992, Harvell et al. 1993, Epifanio et al. 1999, Maia et al. 1999, Koh et al. 2000).

Another hypothesis that has been proposed is that calcitic sclerites defend some gorgonians from potential predators (Harvell et al. 1988, Harvell & Fenical 1989, Van Alstyne & Paul 1992, Van Alstyne et al. 1992, West 1993). Sclerites are embedded in the soft tissue and may act as physical deterrents by irritating the mouth or digestive lining of predators; alternatively, they could provide an inorganic chemical defense by reacting with stomach acid after tissue is consumed, as proposed for calcified algae by Hay et al. (1994). Such a reaction could potentially increase stomach pH, thereby decreasing the efficiency of digestive enzymes.

Not all of the available evidence corroborates the hypothesis that sclerites serve as predator deterrents, however. While Van Alstyne et al. (1992) demonstrated significant deterrence of reef fishes by sclerites from 3 species of the soft coral *Sinularia*, they also found that sclerite concentrations decreased from the base of colonies to the tips. This is incongruent with the notion that sclerites function to deter generalist predators, because attacks by fish predators would be most frequent at colony tips. However, this does not exclude the possibility that sclerites act as deterrents against specialist predators. As Harvell & Fenical (1989) proposed, a greater density of sclerites at the base of colonies may be a strategy to deter specialist invertebrate predators that are unaffected by chemical defenses. In a later study intended to quantify the relative importance of structural and chemical defenses in 3 species of *Sinularia*, Van Alstyne et al. (1994) proposed that the primary function of sclerites is structural colony support, adding that defense could be efficiently achieved by secondary metabolites alone.

The experimental method used in conducting feeding experiments may greatly affect the conclusions drawn about sclerite deterrency. Lindquist et al. (1992) concluded from field assays of ascidian sclerites at approximately natural volumetric concentrations that most ascidians do not rely on sclerites as a primary means of antipredator defense. Similarly, Chanas & Pawlik (1995) found that natural volumetric concentrations of siliceous spicules from sponges were palatable to fish in laboratory and field assays. It is unlikely that results from similar assays with gorgonian sclerites would be much different unless calcitic sclerites act specifically via the buffering mechanism proposed by Hay et al. (1994) or occur in much greater concentrations than sponge spicules. Most previous studies supporting the defensive role of gorgonian sclerites have been based on dry weight (gravimetric) concentrations, despite the fact that fish predators consume hydrated gorgonian tissue. As Harvell et al. (1988) noted, tissues of low dry mass may occupy large volumes when hydrated. Thus, sclerite (or extract) concentrations encountered by fishes depend on the volume, not the mass, of the tissue consumed. Similarly, because the amount of water present in gorgonian tissue is unaccounted for in nutritional analyses based on mass, such values do not accurately reflect the nutrition available to a predator consuming a certain volume of tissue. Therefore, feeding experiments performed on a volumetric basis better reflect the natural condition in which generalist predators encounter prey, and may give different results than similar assays performed on a gravimetric basis.

A few studies have investigated whether organisms may be able to avoid consumption by having tissues of low food value (Duffy & Paul 1992, Pennings et al. 1994, Chanas & Pawlik 1996). Nutritionally deficient tissues may deter predators by (1) supplying too little energy per unit effort to sustain continued predation, (2) decreasing tissue palatability, or (3) increasing the effectiveness of physical and/or chemical defenses. Duffy & Paul (1992) reported that reef fishes fed preferentially on high-quality food in field assays with paired sets of high- (22% protein by dry mass) and low-quality food (4% protein by dry mass). Additionally, paired assays with high- and low-quality foods containing 1 of 3 purified secondary metabolites from either a brown alga or a sponge showed that these compounds were less effective at deterring predators

when incorporated into high-quality food. Perhaps more striking, results of multiple-choice assays indicated that the importance of nutritional value in determining palatability was equal to or greater than that of secondary chemistry. The subsequent study by Chanas & Pawlik (1996), in which assay foods containing natural concentrations of sponge spicules were palatable to *Thalassoma bifasciatum* when nutritional value was similar to sponge tissue, but became unpalatable when nutritional quality was decreased to ≤10% of that found in most sponge tissues, also supports this notion and underscores the importance of accurately simulating the nutritional quality of a volume of tissue that would be consumed by a predator.

In a preliminary survey of gorgonian chemical defenses by Pawlik et al. (1987), organic extracts were applied to freeze-dried pieces of krill, which were offered to *Thalassoma bifasciatum* in ship-board feeding assays. Concentration analyses were gravimetric, and therefore, the results are subject to the above criticisms. The concentration of extract in the krill and the nutritional quality of the assay food relative to the extract concentration and nutritional quality of a volume of gorgonian tissue were unknown. Additionally, the more recent work by Lindquist et al. (1992) and Chanas & Pawlik (1995) demonstrating the palatability of ascidian sclerites and sponge spicules at natural volumetric concentrations suggests that gorgonian sclerites may not serve as predator deterrents, as studies based on gravimetric data suggest. Advances in the field of chemical ecology since the publication of Pawlik et al. (1987), improvements in the design of ecologically relevant assays, and the realized importance of nutritional quality as a factor in palatability studies warranted a re-evaluation of the chemical and physical antipredatory defenses of Caribbean gorgonians. Therefore, the objectives of this study were to survey extract and sclerite palatability and nutritional quality of the most common Caribbean gorgonians. Palatability was tested in laboratory feeding assays using the bluehead wrasse *Thalassoma bifasciatum*, and nutritional quality was quantified through protein assays, bomb calorimetry, and determination of ash mass. Specifically, we addressed the following questions: (1) Do crude organic extracts of gorgonian tissue deter feeding? (2) Do sclerites deter feeding? and (3) Is there a correlation between the nutritional quality of gorgonian tissue and the presence of chemical or physical defenses?

MATERIALS AND METHODS

Gorgonian collection and identification. This study was conducted over the course of 5 research expeditions: 3 in the Bahamas on board RV 'Seward Johnson' during September 1998, August 1999, and March 2001 and 2 in Key Largo at the National Undersea Research Center during May and October of 1999. Bahamian collections were taken from the reefs surrounding the islands of South Bimini (25°33.126′N, 79°18.151′W), Cat Island (24°08.364′N, 75°22.501′W), Egg Island (25°28.807′N, 76°53.840′W), Little San Salvador (24°35.167′N, 75°58.476′W), Long Island (23°37.841′N, 75°21.690′W), San Salvador (24°03.095′N, 74°32.372′W), Santo Domingo (21°43.162′N, 75°45.714′W), and Sweetings Cay (26°33.664′N, 77°52.538′W). Collection sites in Key Largo were North Dry Rocks (25°07.850′N, 80°17.521′W), Canon Patch (25°06.72′N, 80°20.43′W), Pickles Reef (24°59.272′N, 80°24.660′W), and Three Sisters Reef (25°01.51′N, 80°23.61′W). Nonfatal collections were made by cutting branches or portions of colonies, leaving the base and a substantial amount of the upper colony intact. Replicate samples of a given species were taken from geographically distant sites to avoid the collection of clones. Replicates were either immediately frozen and stored until use, or small portions were removed for immediate bioassays and the remainder subsequently frozen and stored for nutritional analysis. Gorgonians were identified using morphological characters of the colony and sclerite analyses according to Bayer (1961).

Extraction and sclerite isolation. Gorgonian pieces cut from the branch tips of each replicate were added to a graduated centrifuge tube containing 45 ml of a 1:1 dichloromethane:methanol mixture to a final volume of 50 ml. The centrifuge tubes were capped, agitated, and stored at 4°C for 24 h. The resulting extract was vacuum-filtered through celite, the solvent removed by rotary evaporation, and the flask containing the dry extract stored at 4°C. A second extraction of the tissue was performed in methanol under the same conditions for a period of 24 h, filtered into the same flask used in the first extraction, and dried by rotary evaporation. The combined extract was then transferred into a 20 ml scintillation vial using a minimum volume of solvent. Extracts were then completely dried using a vacuum concentrator.

After extraction was completed, sclerites were isolated from the same 5 ml of tissue. Commercial chlorine bleach (5.25% sodium hypochlorite) was used to oxidize the soft tissue, leaving behind the gorgonian skeleton and calcitic sclerites. Enough bleach was added to the centrifuge tube to cover the tissue, and the solution was allowed to sit until either the tissue stopped bubbling or was completely dissolved (30 to 45 min). If tissue remained, the supernatant was slowly decanted, fresh bleach added, and the process repeated until all of the tissue was oxidized. After no tissue remained in the tube, the bleach was carefully

decanted, the gorgonin skeleton removed with forceps, and the sclerite pellet washed with distilled water until no bleach odor remained. After the water was decanted, the tube was filled with acetone and its contents poured into a 150 ml Buchner funnel. The sclerites were dried under suction for 10 to 15 min, weighed, and transferred to a scintillation vial.

Laboratory assays. Aquarium feeding assays were performed as described in Pawlik et al. (1995), on board RV 'Seward Johnson' and in the wet laboratory of the Center for Marine Science in Wilmington, North Carolina. The bluehead wrasse *Thalassoma bifasciatum* was chosen as the assay organism, because it is one of the most abundant reef fishes throughout the Caribbean, does relatively well in captivity, and is commonly used as a model organism for generalist predator studies (Pawlik et al. 1995). The assay food used was a squid-alginate paste containing 5.0 g of freeze-dried powdered squid mantle, 3.0 g of alginic acid, and 100 ml of distilled water. Extracts and sclerites were incorporated into assay food at natural volumetric concentrations by mixing the entire 5 ml organic or sclerite extract into 5 ml of squidalginate paste. The treated assay food was then extruded through a 10 ml syringe into a 0.25 M calcium chloride solution where it hardened into a spaghetti-like strand. The strand was removed from the calcium chloride and cut into 3 to 5 mm long pellets. Control pellets lacking extracts and sclerites were prepared in a similar fashion, with the addition of liquid food coloring to match the color of treatment pellets when necessary. A control pellet was offered to a set of 2 to 3 fish and if consumed, a treatment pellet followed. The treatment pellet was considered rejected if it was (1) taken into the mouth cavity and spit out at least 3 times, (2) completely ignored, or (3) mouthed and then completely ignored. Only assays in which fish immediately consumed a control pellet after 1 of the above behaviors (indicating that the fish were still feeding) were scored. Ten assays were performed for each replicate, and at least 3 replicates of each gorgonian species were assayed to determine the mean number of pellets eaten of that species. Using the Fisher exact test (Zar 1996), a significant difference in the number of treated and control pellets eaten was determined for an individual 10 replicate assay if 4 or more pellets were rejected ($p \leq 0.043$, 1-tailed test). Therefore, extracts or sclerites of a species were considered deterrent if the mean number of pellets eaten was ≤6.

Nutritional quality analysis. Frozen tissue samples were measured by displacement in distilled water, weighed, and freeze-dried. Dry weights were taken immediately after removal of the sample from the freeze-dryer. These data were used to calculate wet weight to volume and dry weight to volume ratios. The freeze-dried colonies were subsequently ground to a fine powder using a Wylie mill, and the ground samples were used in all nutritional quality analyses.

Protocols for measuring protein and total energy content followed those used for sponges by Chanas & Pawlik (1995). Briefly, protein assays were conducted as follows: approximately 0.20 g of ground gorgonian and 20 ml of 1 M NaOH were added to a scintillation vial, agitated, and chilled for 24 h. After the extraction period, the vial was centrifuged to pellet the tissue and the supernatant decanted. NaOH-soluble protein content of the supernatant was measured with a microtiter plate reader using Bio-Rad total protein reagent and bovine serum albumin as a standard (Bradford 1976). The protein content of each replicate was the mean of duplicate subsamples of the protein extract.

Total energy content was determined by combustion of 0.8 to 1.4 g of sample in a Parr oxygen bomb calori-

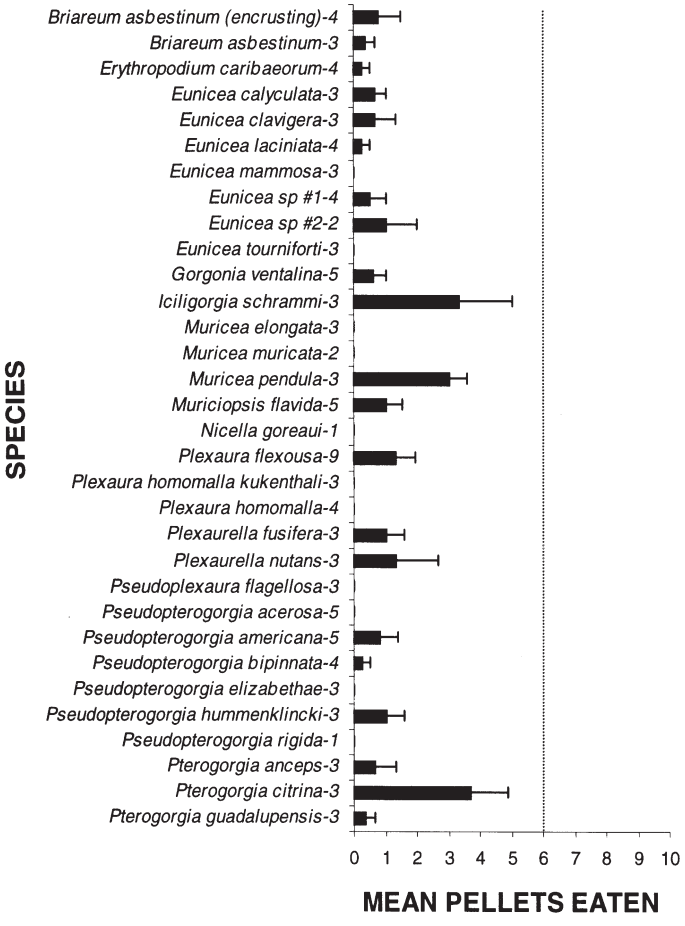

Fig. 1. *Thalassoma bifasciatum.* Consumption of food pellets (mean ± SE) containing crude organic extracts of gorgonians (10 assays replicate^{-1}; no. of replicates given after species name). Extracts were considered deterrent if the mean number of pellets eaten was ≤ 6

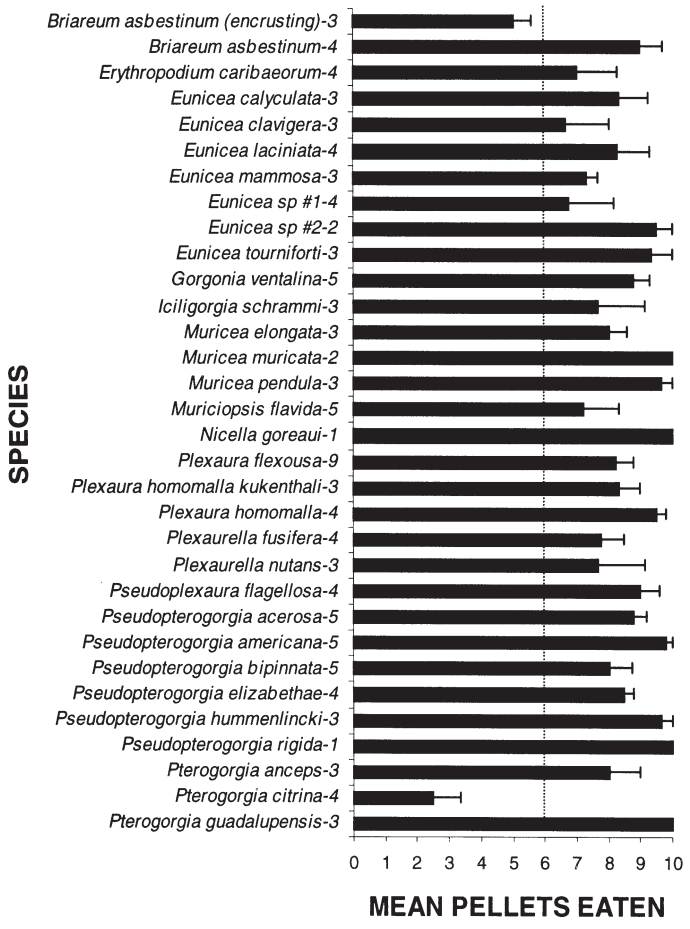

Fig. 2. *Thalassoma bifasciatum.* Consumption of food pellets (mean ± SE) containing spicules of gorgonians (assays and replicates as in Fig. 1). Spicules were considered deterrent if the mean number of pellets eaten was ≤ 6

meter at 20 to 30 atm of O_2. The energy content each replicate sample was the mean of 3 subsamples.

Methods of ash mass determination were adapted from Harvell & Fenical (1989). For each species, 1.0 g of ground gorgonian from 3 replicates was ashed in a muffle furnace at 450°C for 4 h, a temperature and interval which effectively ashes organic material while retaining waters of hydration in mineralized elements (Paine 1964, Harvell & Fenical 1989). Samples were immediately re-weighed after removal from the muffle furnace.

Dry weight to volume ratios were used to convert values of protein and total energy content and ash mass to volumetric measurements. The reported values for each species are the means of 3 or more replicates, except in circumstances where fewer than 3 individuals of a species could be found in different locations and accurately identified. Nutritional quality analyses were also performed on the squid-alginate paste used in the palatability assays.

RESULTS

Extract and sclerite deterrency

Natural volumetric concentrations of crude organic extracts from all 32 species of Caribbean gorgonians (100%) deterred feeding of *Thalassoma bifasciatum* in laboratory assays (Fig. 1). Thus, no interspecific trend in the production of deterrent secondary metabolites was observed. Conversely, food pellets containing sclerites of all but 2 species (6.3%) were readily consumed (Fig. 2). The sclerites of *Pterogorgia citrina* and the encrusting form of *Briareum asbestinum* deterred feeding in 4 out of 4 and 2 out of 3 replicate assays, respectively.

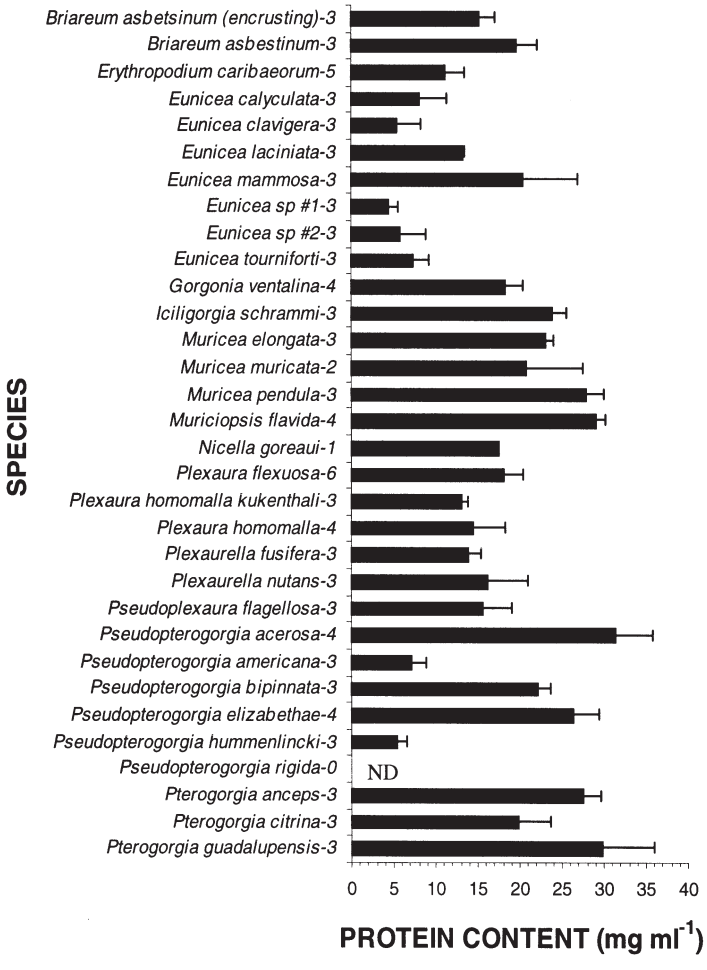

Fig. 3. Protein content of gorgonians. No. of replicates given after species name. ND: no data

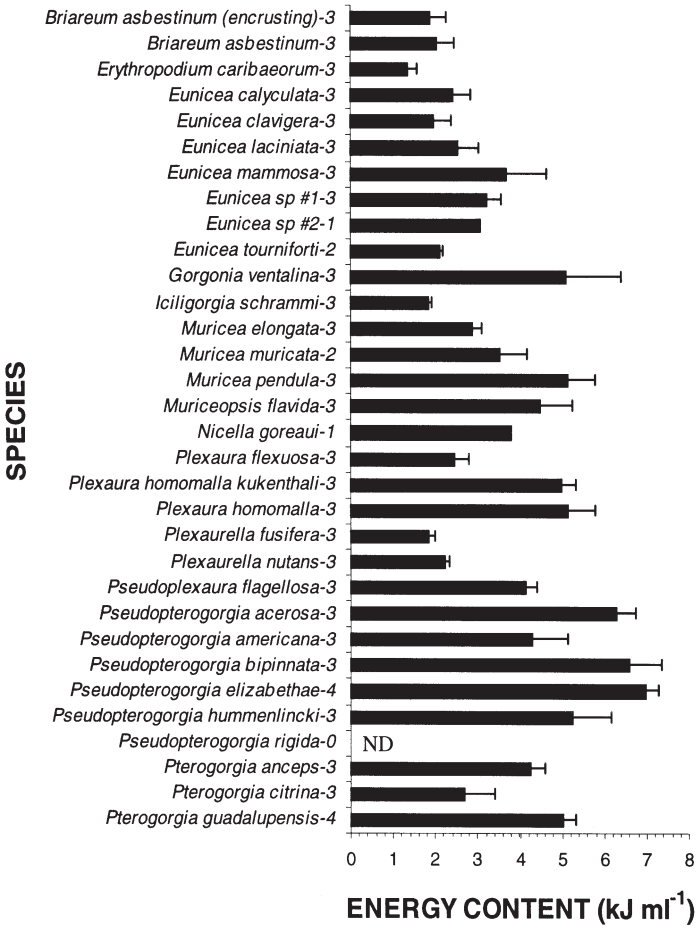

Fig. 4. Total energy content of gorgonians. No. of replicates given after species name. ND: no data

Nutritional quality

The protein content, total energy content and ash content of each species are shown in Figs. 3, 4 & 5, respectively. The mean soluble protein content of the species tested was 17 ± 8 mg ml^{-1}. The interspecific range of soluble protein concentration was 4 to 31 mg ml^{-1}. The range of total energy content was 1.3 to 6.9 kJ ml^{-1}, and the mean total energy content was 4 ± 2 kJ ml^{-1}. The mean ash content was 400 ± 100 mg ml^{-1} (range 130 to 873 mg ml^{-1}).

Analyses of the squid alginate assay food indicated that it closely approximates gorgonians in food value. The mean total protein and energy contents of 3 samples of assay food were 25 ± 4 mg ml^{-1} and 1.35 ± 0.01 kJ ml^{-1}, respectively. The ash content of the squid matrix (9 ± 3 mg ml^{-1}) was lower than the mean value for Caribbean gorgonians, reflecting the lack of inorganic structural elements in squid mantle.

DISCUSSION

Do the organic extracts or sclerites of Caribbean gorgonians deter generalist predators?

The results of this study indicate that secondary chemistry is the primary means of defense for Caribbean gorgonians against fish predators. Crude organic extracts from each of the 32 species tested (100%) were unpalatable to *Thalassoma bifasciatum* in laboratory assays, but the sclerites of only 2 species (6.3%) deterred consumption of assay food. The species of wrasse used in this study has often been considered a model generalist predator, and the results of assays with *T. bifasciatum* in previous studies have consistently agreed with the results of field studies in which the deterrent effects of metabolites or spicules were tested on a natural assemblage of reef fishes (Fenical & Pawlik 1991, Chanas & Pawlik 1995, Swearingen & Pawlik 1998, Wilson et al. 1999, Kubanek et al.

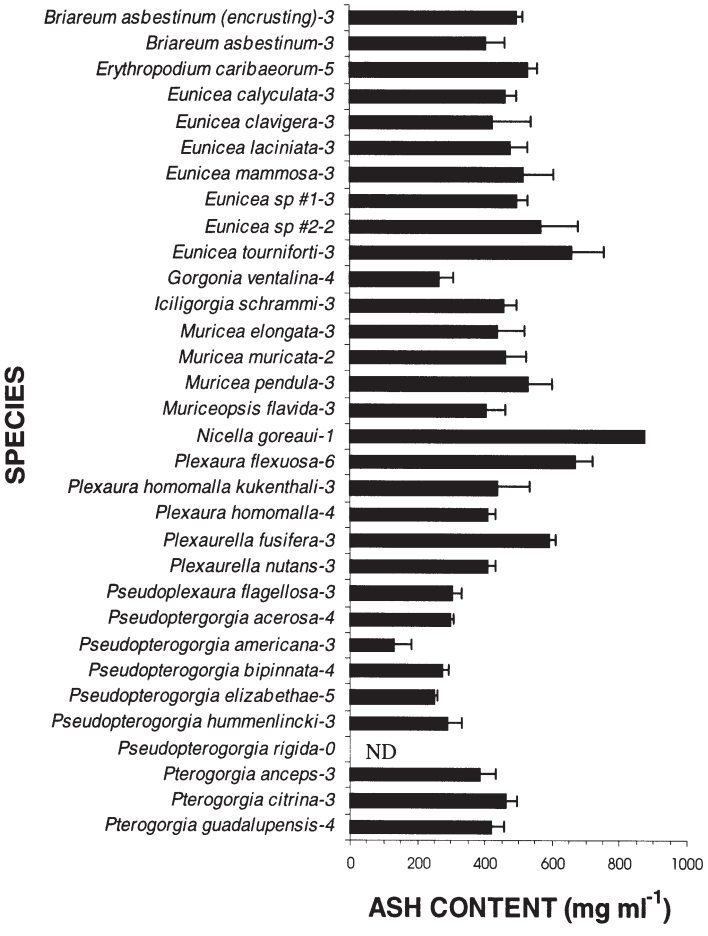

Fig. 5. Ash content of gorgonians. No. of replicates given after species name. ND: no data

2000). Thus, our results suggest that in most gorgonian species, it is a chemical component of the tissues and not the calcitic sclerites that deters consumption by generalist predatory fishes.

The results of the present survey did not match those of the only previous survey of the chemical defenses of Caribbean gorgonians (Pawlik et al. 1987). In that study, only 51% of the 37 surveyed gorgonian 'types' yielded crude organic extracts that were deterrent to *Thalassoma bifasciatum*; 11% were classified as moderately unpalatable and the remaining 38% were palatable. The disparity in the results can be attributed to differences in sample handling and in assay technique. In the Pawlik et al. (1987) study, gorgonian samples were dried in the sun prior to analysis. It is possible that this process altered the chemical composition of the gorgonian tissues and therefore, the defensive properties of the organic extracts. In the present study, gorgonians were either immediately extracted after collection or immediately frozen for subsequent extraction.

With respect to assay technique, Harvell et al. (1988) reported that volumetric assays enable exact dosage control, while gravimetric assays do not. Because gorgonian tissue is hydrated, the volume occupied by any particular mass of tissue may vary considerably, depending on the specimen. For example, the dry weights and volumes of 2 *Gorgonia ventalina* samples, A and B, used in the present study were 19.72 g and 27 ml and 22.86 g and 57 ml, respectively (i.e. the mass of B was 16% greater than that of A, but occupied 111% more volume). In a gravimetric assay, the extracts of these 2 samples would be applied to very similar masses of food of unknown volume. In a volumetric assay, however, the extract of B would be applied to more than twice the volume of food as A. The relative concentrations of the 2 extracts would be very different in the gravimetric and volumetric assays, as likely would be the responses by the assay organism. Because marine predators consume hydrated tissue, the concentration of secondary metabolites or sclerites they encounter depends on the volume, not the mass, of the tissue consumed. Volumetric assays, such as those used in the present study, effectively control for differences in tissue volume, whereas gravimetric assays fail to do this. Additionally, gravimetric feeding assays fail to control for the nutritional quality of the target organisms. Gorgonian tissue contains nutritionally valuable (e.g. protein) and non-valuable components (e.g. sclerites). In the gravimetric preparation of assay food, the mass of the nutritionally poor portion is replaced almost entirely by an equivalent mass of nutritionally valuable matrix. As a result, a gravimetrically prepared assay food cannot replicate the nutritional quality of tissue. In the volumetric preparation of assay food, however, analyte from a volume of tissue is added to a volume of assay food with a similar nutritional quality. The proportion of nutritional components is controlled for in a volumetric assay. Thus, volumetric assays are more ecologically relevant than their gravimetric counterparts, because the assay food more closely models hydrated tissue in both nutritional quality and extract or sclerite concentration.

In the Pawlik et al. (1987) study, the assay protocol and interpretation of the results were based on gravimetric analyses. Additionally, the nutritional quality of the krill used as the assay food relative to gorgonian food value was unknown. As a result, there is little basis for comparing the feeding assay results of that study with the ability of the extracts to defend gorgonians. Furthermore, the volume-based concentration of a gorgonian extract should be less than the respective gravimetric concentration, because volumetric analyses account for the water present in gorgonian tissues. Thus, assays based on volumetric techniques are more conservative measures of deterrency, and the results of the present study more convincingly affirm the role of secondary chemistry in the defense of these gorgonian species from generalist fish predators.

Volumetric feeding assays of Indo-Pacific gorgonian extracts also support the results of the present study. In a recent survey, 100% of the organic extracts from 65 Indo-Pacific gorgonians deterred predation by a generalist predator (Eve 2001). Additionally, Koh et al. (2000) found that crude organic extracts from 8 species of Singapore gorgonians were deterrent in laboratory assays.

The finding that the presence of sclerites does not deter predation by generalists is in agreement with results of similar studies on gorgonians and other sessile invertebrates. Koh et al. (2000) found that natural volumetric concentrations of sclerites from 6 of 8 Singapore gorgonian species were palatable to fish in field assays. Similarly, Lindquist et al. (1992) and Chanas & Pawlik (1995) found that natural volumetric concentrations of sclerites and spicules from ascidians and sponges, respectively, were palatable to generalist predators. The physical orientation of sclerites in the assay food was not controlled in the current study, but it is unlikely that this factor affected the results. Chanas & Pawlik (1996) explored the effect of sponge spicule orientation in deterring fish predation, and determined that feeding results using naturally spiculated sponge skeleton were no different than those using the pellet assay employed herein.

Many studies on the palatability of sclerites from gorgonians and other soft corals have suggested that these structures are unpalatable (Harvell et al. 1988, Van Alstyne & Paul 1992, Van Alstyne et al. 1992,

1994). The discrepancy between our results and those of previous researchers is probably due to assay technique. Despite the problems associated with gravimetric assays, most investigators have continued to use gravimetric sclerite concentrations. Increased sclerite concentrations in gravimetrically prepared assay foods probably result in falsely deterrent results, as high spicule concentrations lead to rejection (Chanas & Pawlik 1996). Additionally, in none of the previous studies were the nutritional qualities of the assay foods known relative to the organisms. Thus, nutritional effects may also have confounded the results of those studies.

There were 2 species for which the sclerite assay indicated that these structures are effective defenses. No more than 4 pellets containing sclerites of *Pterogorgia citrina* and 6 pellets containing those of the encrusting form of *Briareum asbestinum* were consumed in a single assay. These sclerites are not conspicuously different from those of the other 30 species (Bayer 1961). While the tissue of these 2 species is generally not as thick as that of most other gorgonians, the sclerites of *Erythropodium caribaeorum*, which almost always has a thinner tissue layer than either *P. citrina* or *B. asbestinum* (encrusting), were not found to be deterrent (mean pellets eaten = 7); however, 2 of the 4 replicate assays of sclerites from *E. caribaeorum* did yield deterrent results (4, 6, 8, and 10 pellets eaten in replicate assays). Thus, there may be some relationship between the deterrency of sclerites and their concentration relative to digestible material. However, this supposition is not supported by nutritional quality analyses as *P. citrina* and *B. asbestinum* (encrusting) do not have lower total energy, protein, or ash content values than most other gorgonians. Thus, there are no simple distinguishing characteristics of these 2 species that predict the deterrency of their sclerites.

This study shows that the primary function of gorgonian sclerites is not to defend these organisms from generalist predators, but these structures may conceivably deter predation by specialist fishes or invertebrates. Several studies have reported that sclerites deter the feeding of the specialist predator *Cyphoma gibbosum* (Van Alstyne & Paul 1992, West et al. 1993, West 1998); however, these studies were not based on natural volumetric concentrations. As the present investigation demonstrates, natural volumetric analyses often provide results that are significantly different from those obtained by gravimetric methods. There is significant evidence that the major functions of spicules and sclerites are to support colony structure and enhance rigidity (Koehl 1982, Lewis & Von Wallis 1991, Van Alstyne et al. 1994, West 1998), and the results of the present study also suggest that this is the case.

Relationship between nutritional quality, and physical and chemical defenses

There was no relationship between extract or sclerite deterrency and the nutritional quality of the gorgonians in this study. Protein content, total energy content, and ash mass were highly variable between species, whereas extracts were consistently unpalatable and sclerites were generally palatable. As discussed above, the 2 species with contrary sclerite assay results did not appear to deviate from the mean value of any measure of food value. Protein content, total energy content, and ash mass were 20 mg ml^{-1}, 3 kJ ml^{-1}, 460 mg ml^{-1} and 15 mg ml^{-1}, 1.9 kJ ml^{-1}, 490 mg ml^{-1} for *Pterogorgia citrina* and *Briareum asbestinum* (encrusting), respectively. All of these values are within 1 SD of the mean values for the 32 species tested, except for the energy content of *B. asbestinum* (encrusting), which falls just outside of 1 SD. Protein content alone is likely the single most important factor when considering the value of reef organisms as prey and has been correlated with the predatory behavior of an assortment of reef fishes (Millikan 1982, Duffy & Paul 1992). The variability in both protein and total energy content indicate that the value of Caribbean gorgonian species as prey may also be highly variable. Any differences in the values of species as prey, however, do not appear to translate into differences in the effectiveness of chemical or sclerite defenses.

If sclerites defended gorgonians from predation by decreasing nutritional quality, gorgonians should have low protein content, low total energy content, and high ash mass. Chanas & Pawlik (1996) found that squid-alginate assay food containing sponge spicules became deterrent when protein and total energy contents were lowered to 4.4% (1.1 mg ml^{-1}) and 30% (0.4 kJ ml^{-1}), respectively, of the values of the assay food used in this study. If gorgonian sclerites were present in concentrations high enough to lower gorgonian nutritional quality to these values, they may consistently offer a mechanism of predatory defense. The data presented in the current study, however, indicate that natural volumetric concentrations of sclerites are not high enough to deter generalist predators by decreasing the nutritional quality of gorgonian tissues.

Gorgonians appear to be quite similar to sponges in nutritional quality, the other dominant sessile invertebrate on Caribbean reefs. Chanas & Pawlik (1995) reported that the mean protein and energy contents of 71 species of demosponges common on Caribbean reefs were 20.7 ± 11.6 mg ml^{-1} and 2.0 ± 0.9 kJ ml^{-1}, respectively. Gorgonian protein and total energy contents (17 ± 8 mg ml^{-1} and 4 ± 2 kJ ml^{-1}, respectively) fall within 1 SD of the sponge values. Like gorgonian sclerites, natural volumetric concentrations of sponge

spicules do not appear to deter generalist fish predators (Chanas & Pawlik 1995), nor do they deter hermit crabs (Waddell & Pawlik 2000a) or sea stars (Waddell & Pawllik 2000b). Interestingly, however, there is a much greater variability in chemical defense among Caribbean sponges than among gorgonians. Crude organic extracts of 31% of the 71 sponge species did not deter *Thalassoma bifasciatum* in the same assay used in the present study (Pawlik et al. 1995). Although the reasons for this difference are unknown, there are some intriguing possibilities. Some of the most abundant sponges on Caribbean reefs were chemically undefended (Pawlik et al. 1995). One hypothesis regarding the prevalence of chemically undefended sponges on coral reefs is that these species compensate for the lack of defense by increased rates of growth or reproduction. Similarly, it may be that gorgonians, which appear to have slower growth rates than the most common sponges, may have developed chemical defenses to compensate for the inability to regenerate quickly. Alternatively, the greater frequency of chemical defenses among gorgonian species may reflect their conspicuous placement on coral reefs, where predators have greater access to them. The majority of chemically undefended sponges frequently occur in cryptic habitats that have low light regimes, such as in caves or under ledges. The most common Caribbean gorgonians do not survive or grow in cryptic habitats, because their photosynthetic zooxanthellae must be positioned in direct sunlight. It is also possible that the zooxanthellae or other symbiotic microorganisms harbored by gorgonians are partially or wholly responsible for the production of deterrent secondary metabolites.

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