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## Patterns of chemical defense among Caribbean gorgonian corals: a preliminary survey

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**Abstract:** Ship-board assays employing the common Caribbean wrasse *Thalassoma bifasciatum* (Bloch) were undertaken to determine the palatability of food pellets coated with freshly-extracted, lipid-soluble metabolites of 37 types of Caribbean gorgonian corals representing at least 19 species from 11 genera. Extracts of 19 types (51%) were highly unpalatable (zero or one of five pellets eaten), four types (11%) were moderately unpalatable (two or three of five pellets eaten) and 14 (38%) were palatable (four or five of five pellets eaten) to fish in feeding assays. Gorgonians of the genera *Pterogorgia* (three types) and *Eunicea* (nine types) were consistently highly unpalatable, those of the genus *Plexaurella* (four types) were palatable and those of the genus *Plexaura* were most frequently palatable (six of eight types). Further assays of serial dilutions of extracts from seven representative, unpalatable types revealed that extracts inhibited fish feeding at pellet concentrations near or below the concentrations that metabolites occur in the gorgonian soft tissue. Extracts of *Erythropodium caribaeorum* (Duchassaing & Michelotti) and *Pseudopterogorgia rigida* (Bielschowsky) deterred fish feeding at pellet concentrations less than an order of magnitude lower than those found in the soft tissues of the corresponding gorgonians. Thin layer chromatographic analyses of extracts revealed the presence of lipid-soluble, secondary metabolites in a majority of the highly unpalatable extracts, although secondary metabolites were also present in a smaller percentage of palatable extracts. These data support the hypothesis that the soft tissues of many gorgonian corals contain lipid-soluble feeding deterrents which act as a defense against predation.

**Key words:** Gorgonacea; Gorgonian; Chemical defense; Predation; Palatability

### INTRODUCTION

The octocorals comprising the order Gorgonacea (see whips and fans) occur at their greatest diversity and abundance in the tropical northwestern Atlantic, where they frequently dwarf the scleractinian corals in both numbers and size (Bayer, 1961; Lasker, 1985). Despite their relative abundance, however, gorgonian corals appear to have few predators. The polychaete *Hermodice carunculata* is known to consume gorgonian tissue (Preston & Preston, 1975), and in some localities butterflyfishes prey on extended polyps (Lasker, 1985), but gorgonians otherwise appear unmolested in an environment noted for nutrient scarcity and intense predation (Grigg *et al.*, 1984). A definitive explanation for the low predation rates on gorgonians has not been provided, but may

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include both physical defenses (calcite spicules in the soft tissues, proteinaceous axial skeleton; Kinzie, 1974) and chemical methods of predator deterrence.

Studies of the natural products chemistry of gorgonian corals have yielded a wealth of novel metabolites (for recent reviews, see Fenical, 1982; Faulkner, 1984, 1986), which occur in numerous Caribbean species at astonishingly high concentrations. *Plexaura homomalla*, for example, contains esterified prostaglandins at concentrations frequently as high as 5% of dry tissue wt (Schneider *et al.*, 1977) and was the subject of considerable attention among pharmaceutical manufacturers in the early 1970's (Bayer & Weinheimer, 1974). Although many gorgonian-derived metabolites have been recognized to possess potent pharmacological activities (Weinheimer & Matson, 1975; Weinheimer *et al.*, 1977; McEnroe & Fenical, 1978; Fenical *et al.*, 1981; Bandurraga *et al.*, 1982; Izac *et al.*, 1982; Grode *et al.*, 1983; Cimino *et al.*, 1984; Fusetani *et al.*, 1985; Groweiss *et al.*, 1985; Look *et al.*, 1985), their importance to the producing organisms has remained a mystery. Some recent evidence indicates that the secondary metabolites of some gorgonians may play a role in reducing surface fouling by autotrophic microorganisms (Targett *et al.*, 1983; Bandurraga & Fenical, 1985).

Most experimental assessments of the anti-predatory role of the secondary metabolites of marine invertebrates have involved immersing assay fish (most frequently freshwater species) in aqueous extracts of the test organism and observing toxic effects (Bakus & Thun, 1979; Coll *et al.*, 1982). Only recently have more ecologically meaningful studies been undertaken (cf. Pawlik *et al.*, 1986). Gerhart (1984) investigated the defensive role of unesterified prostaglandins derived from the gorgonian *P. homomalla* by performing in situ feeding experiments with the reef fish *Halichoeres garnoti*. Food pellets that had been treated with prostaglandins at concentrations approximating those of the esters found in the gorgonian soft tissue caused assay fishes to vomit repeatedly. However, no results were reported from assays of the naturally-occurring prostaglandin esters.

In this study, we performed ship-board assays employing a common predatory reef fish to survey the palatability of lipid-soluble extracts of 37 types of Caribbean gorgonian corals. Assays undertaken in this fashion carried two important experimental advantages: they were performed on fresh extracts in which potentially unstable metabolites would have undergone minimal decomposition or air oxidation, and they were performed with predatory fish collected from the same reefs as the gorgonians under investigation. The primary disadvantage of a ship-board survey of this type was the difficulty in accurately weighing tissue extracts, a problem circumvented by retaining extracts for subsequent accurate analysis in the laboratory.

## MATERIALS AND METHODS

### GORGONIAN COLLECTION, EXTRACTION AND EXTRACT QUANTIFICATION

Gorgonians were collected by SCUBA from shallow depths (6–12 m) on reefs off the Caribbean islands of Nevis, St. Kitts, Antigua, Guadeloupe and Martinique, as part of

two expeditions on board the research vessels *Cape Florida* and *Columbus Iselin*, August 1985 and July 1986. A scheme illustrating the extraction and quantification methods is presented in Fig. 1. Only individual gorgonian colonies were used, thus avoiding mixed species collections or intraspecific variation of secondary metabolite composition. In the case of two readily identifiable gorgonians, *Pseudopterogorgia acerosa* (Pallas) and *P. rigida* (Bielschowsky), three individual colonies were extracted and tested separately. Otherwise, each gorgonian colony was distinct from every other colony by one or several morphological characteristics, including growth form, polyp size, color, texture, mucus production, etc. Colonies were labelled, frozen, and small voucher samples set aside for later identification. Shortly after collection, 15 g of frozen tissue from each colony was stripped from the axial skeleton, chopped into small pieces and macerated in a mortar and pestle with addition of diethyl ether to form a thick paste.

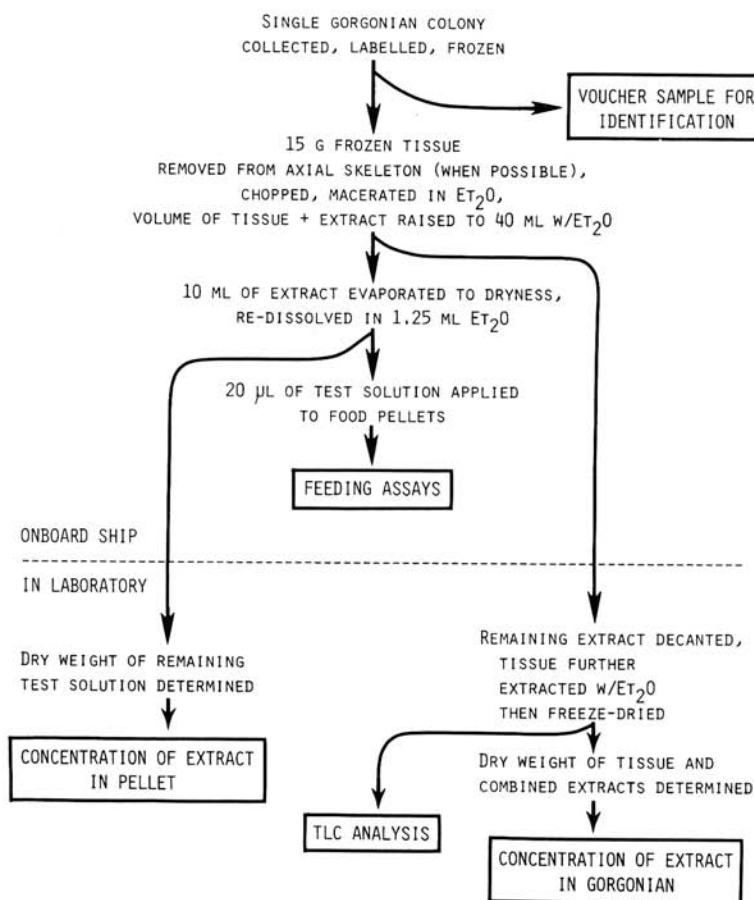


Fig. 1. Extraction and analysis scheme used in a preliminary survey of chemical defense among 37 types of gorgonian corals.

The macerated tissue was transferred to a graduated centrifuge tube, the total volume was increased with diethyl ether to 40 ml, and the graduated tube was vigorously agitated. The tube was centrifuged with a hand-operated centrifuge and 10 ml of the ether supernatant were evaporated to dryness in a glass vial. The remaining tissue/extract mixture was tightly sealed and stored below freezing for later laboratory weight determination and chemical analysis. The dry extract was redissolved in 1.25 ml of diethyl ether, and 20  $\mu$ l portions of this test solution were applied to food pellets for assay experiments. Vials containing the test solutions which remained after the feeding assays were tightly sealed and stored below freezing for later weight determination.

The concentrations of extract in both the gorgonian and the food pellets were determined subsequently in the land-based laboratory where precise analytical instruments could be employed. The remaining supernatant extract was decanted from the graduated tubes and the tissue pellet was exhaustively extracted in diethyl ether as before. The combined extracts were dried and weighed, the tissue pellet was lyophilized and weighed and the concentration of the ether-soluble components as a percentage of the gorgonian dry tissue weight determined. It should be noted that these "dry weight" calculations included the weight of calcitic spicules which commonly pervade gorgonian soft tissues at levels of 25–40% dry weight. Hence, the weight determinations represent conservative estimates of the percentage concentration of lipid-soluble metabolites in the soft tissue. The remaining test solution was similarly dried and the concentration of the extract as a percentage of the food pellet dry weight determined. Thin-layer chromatography (TLC) was used to qualitatively assess the presence of secondary metabolites in gorgonian extracts (silica gel plates, diethyl ether mobile phase, visualized by exposure first to UV light and then by acid-charring). The presence of secondary metabolites was readily confirmed upon visualization of UV-absorbing spots or intensely colored spots on the TLC plate after acid charring. Many compounds were readily identified on the basis of their unique colorations,  $R_f$  values, and by comparison with authentic standards isolated during earlier investigations.

#### BIOLOGICAL ASSAYS

The fish chosen for laboratory assays was the carnivorous wrasse *Thalassoma bifasciatum*, one of the most abundant Caribbean reef fishes (Randall, 1967). Fish were captured with hand nets and glass jars in 2–3 m water off the Les Saintes Islands, Guadeloupe and off St. Annes, Martinique. Approximately 35 fish (approximate ratio of one to four dominant males to yellow-phase individuals) were placed in a 120 l aquarium supplied with flowing sea water at ambient temperature. The fish quickly acclimated and began accepting food pellets (freeze-dried krill, *Euphausia* sp., San Francisco Bay Brand,  $\cong$  4 mg each). The same group of fish remained healthy and was used in all subsequent feeding assays.

In preliminary assays of gorgonian extracts, test solutions were applied so as to saturate food pellets following methods described in Pawlik *et al.* (1986). Control

pellets were treated with diethyl ether alone. Five pairs of treated and control pellets were offered, one at a time, to fish at the top of the aquarium. The order in which control or treatment pellets were offered was varied haphazardly. A food pellet was considered rejected if three or more fish sequentially accepted the pellet into the mouth cavity and then spat it out; the pellet was considered eaten if swallowed by one of the first three fish to encounter it.

Seven moderately to highly unpalatable extracts were chosen for repeated assay, with serial dilutions of the extract applied to food pellets. As before, vials of the remaining diluted test solutions were tightly sealed and later weighed in the laboratory. Four wrasses (one adult male, three yellow-phase individuals) were added to each of eight 60 l aquarium compartments. Sea water at ambient temperature flowed sequentially through opaque dividers from one compartment to the next. Eight pairs of treated and control pellets were prepared as before and one pair offered to the fish in each compartment (except the 1/2 dilution assay of *Plexaura* sp., voucher 29, for which only seven pairs of pellets were assayed). The order in which a control or treatment pellet was offered was determined by coin toss and the order in which the compartments were assayed was varied haphazardly. Assays were scored as previously described. Data were arranged in  $2 \times 2$  contingency tables and analyzed with the Fisher exact test of independence (one-tailed test; Zar, 1984).

## RESULTS

Results of the preliminary fish feeding assays of the lipid-soluble extracts of 37 types of Caribbean gorgonians are presented in Table I, along with data on the concentration of lipid-soluble extracts in both the gorgonian tissue and in the assay pellets. TLC evidence for the presence of secondary metabolites is also tabulated. All five control food pellets (treated with solvent alone) were eaten by fishes in every assay. Food pellets treated with extracts of 19 gorgonian types were highly unpalatable (51% of 37 types surveyed), with zero or one out of five food pellets eaten, four types (11%) were moderately unpalatable (two or three out of five pellets eaten) and 14 types (38%) were palatable (four or five out of five pellets eaten) to assay fish. Overall, assay pellets contained an average 4.2-fold greater concentration of extract than corresponding gorgonian tissue, or a 2.8-fold mean difference as  $\approx 1/3$  of the dry weight was attributable to the mass of calcitic spicules. Percentage dry weight of extract on pellets exceeded that of corresponding gorgonian tissue by a mean of 5.2-fold for palatable extracts, 3.9-fold for highly unpalatable extracts and 2.3-fold for moderately unpalatable extracts. In only one instance did the concentration of extract in the gorgonian tissue exceed that of the assay pellet: for the highly unpalatable *Eunicea* (*Euniceopsis*) sp., voucher 33, extract concentration on the pellet was half that of corresponding gorgonian tissue. There was TLC evidence of secondary metabolites in 14 of 19 types (74%) of the highly unpalatable gorgonians, two of four types (50%) of the moderately palatable gorgonians and six of 14 types (43%) of the palatable gorgonians assayed.

TABLE I

Preliminary survey of the palatability of gorgonian extracts to the reef fish *Thalassoma bifasciatum*. TLC, thin-layer chromatographic evidence of secondary metabolites: -, no evidence; +, positive evidence; ?, no reliable conclusions possible.

Voucher number	Family	Species identification <sup>a</sup>	Percentage (dry wt) of extract in:		
			Gorgonian	Pellet	TLC
Group I. Highly unpalatable (zero or one of five pellets eaten)					
23	Anthothelidae	<i>Erythropodium caribaeorum</i>	2.6	6.3	+
7	Briareidae	<i>Briareum asbestinum</i> (erect)	4.3	17.0	+
24		<i>Briareum</i> sp. (encrusting)	5.2	20.0	+
12	Gorgoniidae	<i>Pseudopterogorgia acerosa</i>	6.3	8.4	+
38		<i>Pseudopterogorgia acerosa</i> <sup>b</sup>	8.0	10.2	+
39		<i>Pseudopterogorgia acerosa</i> <sup>b</sup>	10.0	12.2	+
22		<i>Pseudopterogorgia rigida</i>	15.0	34.0	+
40		<i>Pseudopterogorgia rigida</i> <sup>b</sup>	10.0	12.0	+
41		<i>Pseudopterogorgia rigida</i> <sup>b</sup>	12.5	8.0	+
1		<i>Pterogorgia anceps</i>	6.7	27.0	—
9		<i>Pterogorgia citrina</i>	2.1	6.9	—
15		<i>Pterogorgia guadalupensis</i>	11.0	42.0	—
5	Plexauridae	<i>Eunicea laciniata</i>	6.0	21.0	+
19		<i>Eunicea mammosa</i>	5.5	43.0	+
4		<i>Eunicea tournefortii</i>	1.7	9.2	+
6		<i>Eunicea tournefortii</i>	2.0	16.0	+
34		<i>Eunicea tournefortii</i>	0.9	5.5	—
11		<i>Eunicea</i> sp.	7.2	29.0	+
25		<i>Eunicea</i> sp.	3.5	21.0	+
26		<i>Eunicea</i> sp.	7.2	32.0	+
33		<i>Eunicea</i> ( <i>Euniceopsis</i> ) sp.	2.2	1.1	+
3		<i>Muriceopsis flavida</i>	2.1	4.0	—
29		<i>Plexaura</i> sp.	11.0	14.0	+
Group II. Moderately unpalatable (two of three of five pellets eaten)					
2	Gorgoniidae	<i>Gorgonia ventalina</i>	6.1	15.0	+
10	Plexauridae	<i>Muricea muricata</i>	4.0	12.0	+
31		<i>Muricea</i> sp.	1.3	3.2	—
8		<i>Plexaura flexuosa</i>	0.9	1.1	—
Group III. Palatable (four or five of five pellets eaten)					
37	Gorgoniidae	<i>Pseudopterogorgia americana</i>	6.4	39.0	+
21		<i>Pseudopterogorgia</i> sp.	11.5	48.0	+
32	Plexauridae	<i>Muricea</i> sp.	0.6	4.6	—
13		<i>Plexaura flexuosa</i>	1.0	3.3	—
20		<i>Plexaura flexuosa</i>	1.3	6.1	—
27		<i>Plexaura flexuosa</i>	1.4	10.0	—
36		<i>Plexaura flexuosa</i>	2.3	11.0	—
30		<i>Plexaura</i> sp.	15.6	58.0	+
35		<i>Plexaura</i> sp.	13.6	34.0	+
28		<i>Plexaurella dichotoma</i>	4.2	27.0	—
18		<i>Plexaurella nutans</i>	1.6	14.0	—
16		<i>Plexaurella</i> sp. ( <i>fusifera</i> ?)	5.5	35.0	?
17		<i>Plexaurella</i> sp. ( <i>fusifera</i> ?)	3.7	21.0	+
14		<i>Pseudoplexaura porosa</i>	5.0	8.0	+

<sup>a</sup> Refer to Bayer (1961) for taxonomic authorities.

<sup>b</sup> Replicate assay.

The results of repeated assays of pellets containing sequentially lower concentrations of extracts from seven unpalatable gorgonians are presented in Table II. The extracts were unpalatable to assay fish at concentrations near or below the levels at which they occur in gorgonian soft tissue. Extracts of two species, *Erythropodium caribaeorum* and *Pseudopterogorgia rigida*, were unpalatable at concentrations below 1/10th of those found in the soft tissues of the corresponding gorgonians.

## DISCUSSION

The bluehead wrasse, *Thalassoma bifasciatum* (Bloch), proved to be a particularly amenable assay organism for the purposes of this study. The species is one of the most abundant on reefs throughout the Caribbean, is a generalist carnivore, and has been observed to pick at gorgonian corals (Feddern, 1965; Randall, 1967). In addition, *T. bifasciatum* was easily acclimated to small aquaria and readily consumed untreated food pellets, without satiation, throughout the course of feeding assays.

Major difficulties arise in assessing chemical defense in the Gorgonacea, because of the morphological plasticity within the group and the subjective nature of gorgonian classification and identification (Bayer, 1961). Several of the colonies collected for this study could not be unambiguously identified at the species level, and, although all of the 37 types subjected to feeding assays were morphologically distinct, some of these were subsequently placed within the same species. Regardless of taxonomic difficulties,

TABLE II

Assay of extracts of seven unpalatable gorgonian species at sequentially diminished concentrations.  
\*  $P < 0.05$ , Fisher exact test.

Voucher no.	Species identification (% extract in gorgonian)	Treated pellets eaten/control pellets eaten (% extract in pellet)						
		Dilution: 1	1/2	1/4	1/10	1/40	1/80	1/160
23	<i>Erythropodium caribaeorum</i> (2.6)	0/8* (6.3)	0/8* (3.8)	0/8* (1.9)	3/8* (0.8)	3/8* (0.2)	5/8 (0.09)	—
2	<i>Gorgonia ventalina</i> (6.1)	0/8* (15.0)	3/8* (9.4)	5/8 (4.9)	—	—	—	—
12	<i>Pseudopterogorgia acerosa</i> (6.3)	0/8* (8.4)	3/8* (5.0)	7/8 (2.6)	—	—	—	—
22	<i>Pseudopterogorgia rigida</i> (15.0)	0/8* (34.0)	0/8* (23.0)	0/8* (13.0)	0/8* (5.6)	2/8* (1.5)	3/8* (0.7)	5/8 (0.4)
19	<i>Eunicea mammosa</i> (5.5)	0/8* (43.0)	0/8* (32.0)	4/8* (19.0)	2/8* (8.7)	6/8 (2.3)	—	—
3	<i>Muriceopsis flavida</i> (2.1)	0/8* (4.0)	3/8* (2.3)	7/8 (1.2)	—	—	—	—
29	<i>Plexaura</i> sp. (11.0)	5/8 (14.0)	4/7 (8.8)	7/8 (4.6)	—	—	—	—



Fig. 2. Chemical structures of some secondary metabolites previously identified from Caribbean gorgonian corals. References to original descriptions are cited in the text. (a) Erythrolide A from *Erythropodium caribaeorum*; (b) ancepsenolide from *Pterogorgia anceps*; (c) furoventalene from *Gorgonia ventalina*; (d) curcuquinone from *Pseudopterogorgia rigida*; (e) pseudopterolide from *Pseudopterogorgia acerosa*; (f) asperdiol from *Eunicea asperula* and *E. tournefortii*.



Assays of extracts of gorgonians of the genus *Pseudopterogorgia* gave mixed results. *Pseudopterogorgia acerosa* and *P. rigida* were among the most unpalatable while *P. americana* (Gmelin) and an unidentified *Pseudopterogorgia* sp. (voucher 21) were readily consumed. *P. rigida* yielded the second most active extract assayed, an effective feeding deterrent at 0.7% dry weight of food pellet. *P. acerosa* and *P. rigida* were among the few gorgonians which could be reliably identified in the field, and replicate analyses ( $n = 3$ ) were completed with individual colonies of both species. Within each species, virtually identical results were obtained in replicate bioassays, and TLC analysis of each colony revealed identical secondary metabolite compositions. *P. rigida* is known to contain curcuquinone (Fig. 2d) and derivatives, compounds which possess potent antimicrobial and cytotoxic activities (McEnroe & Fenical, 1978). Indeed, the presence of curcuquinone and its related metabolites was confirmed in the extract of *P. rigida* by TLC analysis. Similarly, the extract of *P. acerosa* was found to contain pseudopterolide (Fig. 2e), a rearranged diterpenoid of a novel class (Bandurraga *et al.*, 1982). Although pseudopterolide is a highly bioactive compound, it has not been shown to produce the deterrent effects found in the crude extract of the gorgonian. Each of the palatable *Pseudopterogorgia* species appeared to contain, by TLC analysis, previously undescribed secondary metabolites.

Although gorgonians within the family Plexauridae were widely divergent in their palatability to assay fish, the data were sharply delineated at the generic level. All *Eunicea* types proved to be highly unpalatable, and TLC data revealed that eight of the nine types contained secondary metabolites. Gorgonians of the genus *Eunicea* are among the most chemically-replete groups within the Caribbean and are known to produce diterpenoids such as asperdiol (Fig. 2f; Weinheimer *et al.*, 1977), and related compounds of the cembrene class (Faulkner, 1984; 1986), as well as diterpenoids of the elemane (Gopichand & Schmitz, 1978), dolabellane (Look & Fenical, 1982) and cubitane classes (Look *et al.*, 1984b). But, as in the previous cases, these compounds have yet to be assessed for ecologically-relevant properties.

Gorgonians of the related genera *Plexaura*, *Plexaurella* and *Pseudoplexaura* were generally palatable and, for the most part, appeared chemically depauperate by TLC analysis. Although palatable to assay fish, vouchers 17, 30 and 35 appeared to contain metabolites which have not been previously identified. Two of the three *Plexaura* types (vouchers 30 and 35) were palatable to the assay fish at a more than two-fold greater concentration of extract in the food pellets than in the corresponding gorgonian soft tissue. The extract of *Plexaura* sp. (voucher 29) was unpalatable in the preliminary assay (Table I) and TLC analysis of this extract revealed the presence of complex esterified prostaglandins. However, this extract did not significantly deter fish feeding in the serial dilution assay (Table II). Gerhart (1984) performed feeding assays with yellowhead wrasses and reported regurgitation of food pellets coated with the unesterified acids of both 15 (S) and 15 (R) – prostaglandin A<sub>2</sub> derived synthetically from *P. homomalla*. Under natural conditions, however, prostaglandins of *P. homomalla* occur exclusively as esters in the tissue of the gorgonian (Schneider *et al.*, 1977), and the defensive role

of these esters has yet to be assessed. Contrary to Gerhart's observations, Lasker (1985) found that *P. homomalla* was the preferred prey of the butterflyfish *Chaetodon capistratus* Linnaeus at a site in the San Blas Islands, Panama. The results of the present study suggest that the naturally-occurring prostaglandin esters found in some *Plexaura* species may be only marginally effective at deterring predation by some carnivorous fishes.

Most of the unpalatable extracts subjected to assays at serially-diluted concentrations on food pellets were minimally active at levels remarkably close to those found within the gorgonian soft tissues. Energetic constraints would tend to dictate that defensive metabolites be sequestered at minimally effective concentrations (Faulkner & Ghiselin, 1983). It remains to be seen whether potential gorgonian predators other than *Thalassoma bifasciatum* have different tolerance thresholds for the noxious metabolites of gorgonians. A considerably higher tolerance would be expected for ovulid gastropods, particularly *Cyphoma gibbosum* which preys specifically on the tissues of a variety of Caribbean gorgonians (Birkeland & Gregory, 1975). It seems likely that these molluscs retain defensive secondary metabolites which are then used in their own defense, a situation analogous to that found among dorid nudibranchs which prey on chemically noxious sponges (Faulkner & Ghiselin, 1983). Dorid nudibranchs are frequently brightly colored and conspicuous so as to advertise their distastefulness to potential predators. Similarly, *Cyphoma gibbosum* displays a brightly colored mantle which covers its shell when the snail is feeding.

An important outcome of this study is the direction it can provide for further research into the chemical ecology of the Gorgonacea. In the past, programs of marine natural products isolation have focused on the identification of novel classes of compounds, with little or secondary regard to the biological function of the metabolites. This survey provides a base from which to identify specific, ecologically-relevant compounds in a bioassay-directed fashion. Future research can now be directed toward the characterization of gorgonian metabolites that play an important role in affecting the distribution and abundance of the species comprising this conspicuous component of the Caribbean coral reef biota.

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