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Title: Chemical Defenses in Three Species of *Sinularia* (Coelenterata, Alcyonacea): Effects Against the Predator *Chaetodon unimaculatus* (Perciformes).

Approved: ____
Valerie J. Paul, Chairman, Thesis Committee

The butterflyfish *Chaetodon unimaculatus* feeds selectively on the soft corals *Sinularia* sp., *S. polydactyla*, and *S. maxima* located on a Cocos Lagoon patch reef in Guam. *Sinularia* sp. is the most preferred, while *S. maxima* is the least preferred. Secondary metabolites, functioning as feeding deterrents, were hypothesized as a major determinant of *C. unimaculatus* feeding preferences. Types and concentrations of terpenoid secondary metabolites varied among the 3 species of soft corals. Organic extracts were not deterrent at whole colony concentrations (3-7% dry wt.); however, all extracts were feeding deterrents at concentrations of 20% dry weight and the *S. polydactyla* extract was deterrent at 10% dry weight. Actual extract concentrations in the tips of *Sinularia* sp. (12% dry wt.) were lower than the concentration which causes feeding deterrence, while the extract concentration in *S. maxima* tips (28% dry wt.) was higher. Chemical

feeding deterrents help to explain preferences for these 2 soft corals but not for *S. polydactyla*. Comparisons between extracts of any 2 species showed no significant differences in deterrence when tested at the same concentrations. No significant differences in deterrency were found between grazed and ungrazed colony extracts. Nonpolar terpenoid hydrocarbon fractions were also not deterrent at whole colony or tip concentrations. A major cembranoid diterpene isolated from *S. maxima* was deterrent only at the concentration found in the tips (12% dry wt.). Sclerites as structural defenses appear to be of little importance in determining the soft coral preferences of *C. unimaculatus*. *Sinularia* secondary metabolites were very effective in deterring other fish predators in the field. Thus, *C. unimaculatus* seems to have an unusual tolerance to the secondary metabolites of *Sinularia* enabling this fish to exploit a food source unavailable to most other marine organisms.

CHEMICAL DEFENSES IN THREE SPECIES OF SINULARIA (COELENTERATA, ALCYONACEA): EFFECTS AGAINST THE PREDATOR CHAETODON UNIMACULATUS (PERCIFORMES)

BY

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INTRODUCTION

A few species of butterflyfishes (Chaetodontidae) have been reported to feed on alcyonaceans (soft corals) and gorgonians (Hobson, 1974; Randall, 1974; Anderson et al., 1981; Birkeland & Neudecker, 1981; Harmelin-Vivien & Bouchon-Navaro, 1981, 1983; Tursch & Tursch, 1982; Lasker, 1985). This foraging behavior is intriguing since many octocorals are immune from predators (Bakus, 1981; Coll et al., 1982, 1983; Gerhart, 1984; LaBarre et al., 1986b; Pawlik et al., 1987; Sammarco et al., 1987). Many octocorals produce toxic secondary compounds which are thought to function in predator defense, competition for space, antifouling, and reproduction (Tursch et al., 1978; Bakus, 1981; Coll et al., 1982; Coll & Sammarco, 1983; Sammarco et al., 1983, 1985, 1987; Gerhart, 1984, 1986; Bowden et al., 1985; Bakus et al., 1986; LaBarre et al., 1986a, b; Pawlik et al., 1987). Octocoral coenenchyme can also be heavily embedded with calcium carbonate sclerites which provide an additional defense mechanism (Harvell et al., in press).

Toxicity and feeding deterrence represent two forms of chemical defense employed by octocorals (Coll et al., 1982; LaBarre et al., 1986b). These defenses have been proposed to play a critical role in the behavior and ecological interactions of predators and their prey, with little experimental evidence to support these hypotheses. Much of the known information on the toxic and feeding deterrent roles of alcyonacean secondary metabolites have come from experiments in which the freshwater mosquitofish *Gambusia affinis* (Baird & Girard) was the test organism (Coll *et al*., 1982; LaBarre *et al*., 1986b). Although these experiments did not use potential predators of soft corals, they did provide clues as to why octocorals have few predators in an environment known for intense predation pressures (Sammarco, 1980; Bakus, 1981; Grigg *et al*., 1984; Huston, 1985).

Little is known about the chemical interactions involved in the feeding activities of chaetodontid predators which have circumvented the chemical defenses of their octocoral prey. Lasker (1985) studied the feeding preferences of the butterflyfish *Chaetodon capistratus* Linnaeus which feeds mainly on exposed gorgonian polyps. The feeding rates were related to gorgonian spawning activities which in turn suggested that changes in chemical composition influenced the fish's preferences. Pawlik *et al*. (1987) tested gorgonian secondary metabolites against the wrasse *Thalassoma bifasciatum* (Bloch) and found that 51% of the extracts tested were highly unpalatable. The use of *T. bifasciatum* as an assay organism provided a more ecologically relevant experiment since this fish is common on Caribbean reefs and has been observed to pick at gorgonian corals (Pawlik *et al*., 1987).

In some areas, *Chaetodon unimaculatus* Bloch feeds mostly on scleractinian corals and invertebrates (Talbot, 1965; Hobson, 1974; Sano *et al*., 1984; Cox, 1986), yet in other areas it has been observed to feed on soft corals of the Order Alcyonacea (Anderson *et al*., 1981; Tursch & Tursch, 1982). *C*. *unimaculatus* feeds on soft corals by tearing large (1-2 mm) pieces of coenenchyme (Motta, 1985) containing high amounts of secondary metabolites and sclerites.

In this study, I relate the patterns of feeding by *C*. *unimaculatus* on three species of the soft coral *Sinularia* to the secondary chemistry found in the prey. I asked the following questions: 1) does *C*. *unimaculatus* prefer some species of *Sinularia* over others?, 2) if so, does the secondary chemistry or structural defenses of *Sinularia* species affect these preferences?, 3) can the feeding-deterrent properties of the lipid-soluble organic extracts affect the preferences of *C*. *unimaculatus*?, and 4) do the lipid-soluble organic extracts act as feeding deterrents towards other carnivorous fishes?

MATERIALS AND METHODS

STUDY SITES AND COLLECTIONS

Feeding observations of *Chaetodon unimaculatus* were conducted in Cocos Lagoon, Guam on a patch reef located 1 km southeast of the Merizo boat launch. The 35 m X 60 m patch reef was chosen because of the relatively high *C. unimaculatus* density and the abundance of 3 soft coral species, *Sinularia* sp., *Sinularia maxima* Verseveldt, and *Sinularia polydactyla* (Ehrenberg).

Field bioassays toward generalist carnivorous fishes were conducted at Fingers Reef, Apra Harbor, Guam. Laboratory bioassays with *C. unimaculatus* were conducted at the University of Guam Marine Laboratory. Soft corals used for extractions were collected at the Cocos Lagoon study site and adjacent patch reefs. *C. unimaculatus* used in bioassays were captured in Cocos Lagoon away from the study site.

STUDY SITE COMPOSITION

Species composition of the Cocos Lagoon patch reef was determined by a random quadrat sampling method. A sixteen-point 0.0625-m^2 quadrat was placed at 1-m intervals on randomly distributed transect lines. Items falling directly beneath the points were recorded. A random numbers table was consulted to yield compass bearings for transect line directions. Transect lines began at the boat anchor which was haphazardly thrown from the boat towards the patch reef. Seven transects were performed in June 1987 and another 7 transects were done in December 1987. The data were pooled for later analysis since species composition was similar on both dates. This yielded an analysis of over 500 quadrats and 8600 points.

FIELD OBSERVATIONS

Chaetodon unimaculatus feeding observations were conducted between June 1987 and May 1988. All observations were made between 1000 and 1500 h. Individual *C. unimaculatus* (Approx. 7-15 cm in total length) were followed for 5-minute periods and the numbers of bites taken from each food item were recorded. Since soft coral preferences were the focus of this study, the numbers of bites taken from the lobes (polyp-bearing portion) or the base of each of the 3 *Sinularia* species were recorded. A total of 203 5-minute observations were made of approximately 20 individual *C. unimaculatus* that inhabited the study site reef.

The numbers of bites taken of each prey item and the relative abundance of prey items in the foraging area were analyzed by three common electivity indices which are reviewed by Lechowicz (1982). I calculated Ivlev's Electivity Index, Jacob's Log Q, and Chesson's Alpha

(Ivlev, 1961; Jacobs, 1974; Chesson, 1978). The range of values varies with each index used so that Ivlev's E varies between -1 and 1 (negative values indicate avoidance and positive values indicate preference), Jacob's Log Q varies between plus to minus infinity, and Chesson's Alpha ranges between 0 and 1. The preference data were used to compare diets of *C. unimaculatus* with the feeding deterrent properties of the secondary chemistry of each *Sinularia* species.

CHEMICAL EXTRACTION AND ANALYSIS

Soft corals were collected and, within one hour, extracted in a 1:1 mixture of dichloromethane and methanol by blender homogenation. The extracts were filtered and evaporated under vacuum leaving an oily organic extract. Whole colonies, bases, lobes, and tips (top 3-10 cm of the lobe) were extracted separately for each *Sinularia* species. Lipid-soluble extract yields were calculated as the dry weight of extract divided by the total dry weight of soft coral and the extract. Soft corals were dried for 5-7 days at 58°C in a drying oven. Colonies were weighed when completely dry (no weight loss over 2 consecutive days). Concentration estimates were conservative since extraction was probably never complete (see Hay *et al*., 1987). Replicate extractions were made on separate, individual corals in order to estimate natural concentrations.

Intra- and interspecific variation in concentrations among _Sinularia_ colony parts were analyzed by a two-way analysis of variance (ANOVA) which was carried out with the BMDP statistical programs. Normality and homoscedasticity was achieved by arc sine transformation of the data. The Student-Newman-Keuls test (SNK) was used to make multiple comparisons (BMDP 5D & 7D).

A major cembranoid diterpene from _S_. _maxima_ (Fig. 1) and nonpolar terpenoid fractions from all 3 _Sinularia_ species were isolated by silica gel flash-column chromatography with hexane and dichloromethane in differing concentrations. Extracts from the tips of each _Sinularia_ species were chromatographed by flash-column to determine the concentration of isolated fractions.

Thin-layer chromatography (TLC) was used to qualitatively analyze the presence of secondary metabolites in extracts of each species of _Sinularia_. TLC plates were developed in 3 solvent systems: 100% hexane for nonpolar metabolites, 100% dichloromethane, and 50% ethyl acetate:hexane for more polar metabolites. The presence of secondary metabolites was confirmed visually by ultraviolet activity under a UV lamp and by color reactions after the TLC plates were charred in 50% sulfuric acid (Norris & Fenical, 1985).

Grazed and ungrazed colonies of _Sinularia_ sp. and _S_. _polydactyla_ were extracted and chemically analyzed.

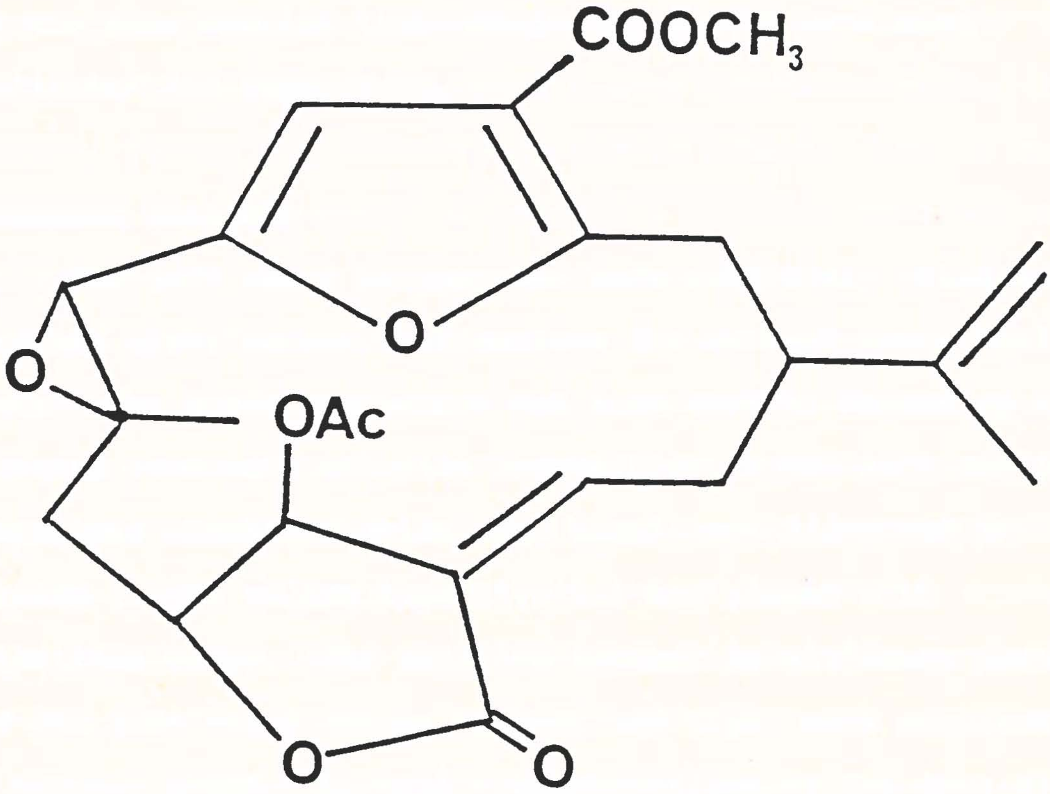

Fig. 1. Chemical structure of the major cembranoid diterpene isolated from *Sinularia maxima*.

Individual C. unimaculatus were followed to determine which colonies were preferentially eaten. Tips of the preferred colonies were collected and the tips of the nearest colony of the same species that remained ungrazed were also collected for comparison.

SCLERITE ANALYSIS

The soft coral homogenates were completely dried and weighed after solvent extractions for extract concentration data. The dried homogenate was also used to determine the calcium carbonate sclerite composition in the soft corals. Replicate chunks of dried soft coral were dissolved in bleach (5.25% sodium hypochlorite) and rinsed with distilled water. The sclerite content in different parts of the soft coral (bases, lobes, and tips) were compared by a two-way ANOVA. Several transformations of the data did not reduce heteroscedasticity so a Brown-Forsythe ANOVA (BMDP 7D) was used. A log transformation was used since it was the most effective in providing normality (BMDP 5D). The SNK test was used to make comparisons among means (BMDP 7D).

EFFECTS OF EXTRACTS TOWARDS GENERALIST CARNIVORES

The feeding-deterrent properties of lipid-soluble extracts from each Sinularia species and the major cembranoid diterpene from S. maxima were assessed by field assays towards an array of generalist carnivores. SCUBA was used to conduct assays on Fingers Reef in Apra Harbor, Guam. Thin strips of squid (2 cm X 2 cm) were coated with extracts and the isolated diterpene dissolved in ether to yield natural soft coral whole colony concentrations. Four control (ether only) and four treated pieces of squid were attached by paper clips to each of 2 separate 3-stranded polypropylene ropes and placed on the reef as a matched pair (0.25-0.5 m apart). Eleven to 16 pairs were used in each trial, and each pair was placed several meters apart. When at least four pieces of squid were consumed, the numbers of control and treated pieces eaten were recorded. Trials lasted approximately 15-20 minutes, and the extract remained on the squid throughout the bioassay as confirmed by TLC after the assays, although some material was likely lost in the seawater. The numbers of squid pieces eaten on control and treated ropes were compared by Wilcoxon's sign-ranks test, a nonparametric test for paired comparisons.

EFFECTS OF EXTRACTS TOWARDS C. UNIMACULATUS

Individual C. unimaculatus (7-8 cm TL) were placed in twelve 50-l flowing seawater indoor aquaria. Each aquarium was separated by black plastic so that influences of the other fish would not affect the assays. The flow rate and temperature were kept between 2-2.5 l/minute and 27.2-27.8°C. An artificial lighting system maintained a day-night cycle in accordance with Guam's day-night cycle. The fish were allowed to acclimate 3-7 days and quickly began to feed on freeze-dried *Tubifex* worm cubes attached by a rubberband to a Syracuse watch glass. Fish which appeared to be unhealthy or abnormal in behavior after several weeks were replaced with healthy specimens.

Freeze-dried *Tubifex* worm cubes (90-150 mg pieces) were saturated with *Sinularia* extracts (dissolved in diethyl ether) at natural *Sinularia* concentrations as determined by chemical analyses. Control pieces of *Tubifex* were coated only with diethyl ether. The extracts are lipid soluble and absorb into the *Tubifex* cubes after the ether evaporates. TLC was used to confirm that the extracts remained in the *Tubifex* throughout the assay. Some material was probably lost in the seawater, but this tended to make the results more conservative.

One control and one treated piece of *Tubifex* were attached (approx. 3-4 cm apart) to a watch glass and placed into the aquarium. The bites taken on the treated and control pieces during 5-minute periods were tallied by 2 hand-held counters. The fish were observed from approximately 2 m away from the aquaria, and the 5-minute period began when the fish took its first bite on the *Tubifex*. Twelve individual *C. unimaculatus* were used in this test. All assays were conducted between 0800 and 1100 h, and only one assay was performed per day. The numbers of bites taken from control and treated Tubifex pieces were compared by Wilcoxon's sign-ranks test.

Bioassays with actual Sinularia tips (2-3 cm long) were also conducted in a similar manner to compare each species of Sinularia against the others.

FECAL ANALYSIS OF C. UNIMACULATUS

Secondary metabolite composition of the 3 soft coral species were compared to those of the feces produced by C. unimaculatus feeding on the corals. Several C. unimaculatus (8-13 cm TL) were kept in 50-l flowing seawater outdoor aquaria, starved for 2 days, and then allowed to graze on individual species of soft corals for 1-3 days. The feces were collected every day and extracted in 1:1 mixture of dichloromethane and methanol. TLC was used to compare the soft coral and fecal extracts.

RESULTS

STUDY SITE COMPOSITION

The substrate composition of the patch reef at Cocos Lagoon is presented in Table 1. Several types of algae, mainly *Dictyota*, *Halimeda*, *Caulerpa*, and blue-greens, dominated the reef. The soft coral *Sinularia* sp. was the dominant benthic invertebrate, while *S. maxima* and *S. polydactyla* covered considerably less substrate area. Living scleractinian corals (mostly *Acropora*) were rare.

FIELD OBSERVATIONS

Chaetodon unimaculatus averaged 14 bites during each 5 minute observation period. The expected frequency of bites were calculated based on prey (living cover) availability and compared to the observed frequency of bites by *C. unimaculatus* which indicated a nonrandom feeding pattern ($X^2=13165$, df=4, $p<0.001$). *C. unimaculatus* took most of its bites from the dominant soft coral, *Sinularia* sp. (Table 1). Bites on *S. maxima* accounted for the lowest percentage of total bites on soft corals. During July 1987 and October 1987, a bloom of the red alga *Ceramium matzatlanense* Dawson occurred which accounted for the high percentage of bites on algae. No other algae were observed to be consumed by *C. unimaculatus*.

All 3 electivity indices yielded similar results. The rank order of preferences did not change with the different

TABLE 1. Relative percentages of substrate at the Cocos Lagoon study site and total bites taken by *Chaetodon unimaculatus* during 203 five minute observation periods. The values for Ivlev's Electivity (E) Index, Chesson's Alpha, and Jacob's Log Q are shown for each potential prey item.

Substrate	% of all substrates	% of living cover	Number of bites (%)	Ivlev's E Index	Chesson's Alpha	Jacob's Log Q
SOFT CORALS						
Sinularia sp.	8.1	11.8	2184 (83.4)	0.75	0.52	1.58
S. polydactyla	0.8	1.2	71 (2.7)	0.39	0.17	0.36
S. maxima	0.9	1.3	51 (1.9)	0.19	0.11	0.17
OTHER						
Live coral (*Acropora*)	0.3	0.4	28 (1.1)	0.46	0.20	0.44
Algae	58.5	84.9	284 (10.9)	-0.77	0.01	-1.66
Other animals (sponges, bryozoans, anemones etc.)	0.3	0.4	0	-1.0	0	$-\infty$
Coral rubble	31.1	---	---	---	---	---

indices. The indices indicated that Sinularia sp. was the most preferred soft coral and S. maxima was the least preferred soft coral while algae were avoided (Table 1). The lumping of C. matzatlanense with all the other algae masked C. unimaculatus' selectivity for this alga. When C. matzatlanense blooms it is opportunistically consumed by C. unimaculatus; however, all other algae were avoided.

C. unimaculatus fed mostly on the lobes rather than the bases of Sinularia sp. and S. polydactyla; however, all bites on S. maxima were taken from the bases. Basal bites accounted for only 2.8% of the total bites on all 3 Sinularia species.

CHEMICAL EXTRACT AND SCLERITE ANALYSIS

Each of the three Sinularia species contained a complex mixture of terpenoid secondary metabolites based upon TLC and proton nuclear magnetic resonance (NMR) analyses. TLC indicated that Sinularia sp. contained 6-7 metabolites of varying polarity. The extract of S. polydactyla was similar to Sinularia sp. and contained 7-8 metabolites of different polarities. The S. maxima extract contained 5-6 metabolites but was unique because one cembranoid diterpene, a rearranged isomer of 13-alpha-acetoxy-pukalide (Bowden et al., in press), was the major metabolite in this extract. This major metabolite was isolated and tested in the bioassays. The other extracts did not contain any single major metabolites and the structures of these other terpenoids were not determined.

Lipid-soluble extract concentrations in each soft coral species and isolated coral parts are compared in Figure 2. Whole colonies of *Sinularia* sp. and *S. polydactyla* contained approximately 4% extract on a dry-weight basis. *S. maxima* contained a higher concentration (7% extract). Extracts in the bases are lower than in the lobes (p<.05, SNK). Base concentrations ranged between 2-4% while lobe concentrations ranged between 6-20%. *S. maxima* contained the highest tip extract concentration (28%) which was significantly different (p<.05, SNK) from *Sinularia* sp. but not from *S polydactyla*. *S. polydactyla* and *Sinularia* sp. contained tip concentrations of approximately 21% and 12% respectively. Concentrations of nonpolar terpene hydrocarbons in whole colonies ranged from 0.1% for *S. polydactyla*, to 0.4% for *S. maxima*, and to 1% for *Sinularia* sp. Nonpolar terpene concentrations in the tips were 0.2% for *S. polydactyla*, 1% for *S. maxima*, and 2% for *Sinularia* sp. The major cembranoid diterpene (Fig. 1) isolated from *S. maxima* occurred at 2% in the whole colony and 12% in the tips.

Sclerite content for each soft coral is shown in Figure 3. Sclerite content shows an inverse relationship to extract concentration in the 3 species of *Sinularia* (Fig. 4). The sclerite content in *S. maxima* tips differed

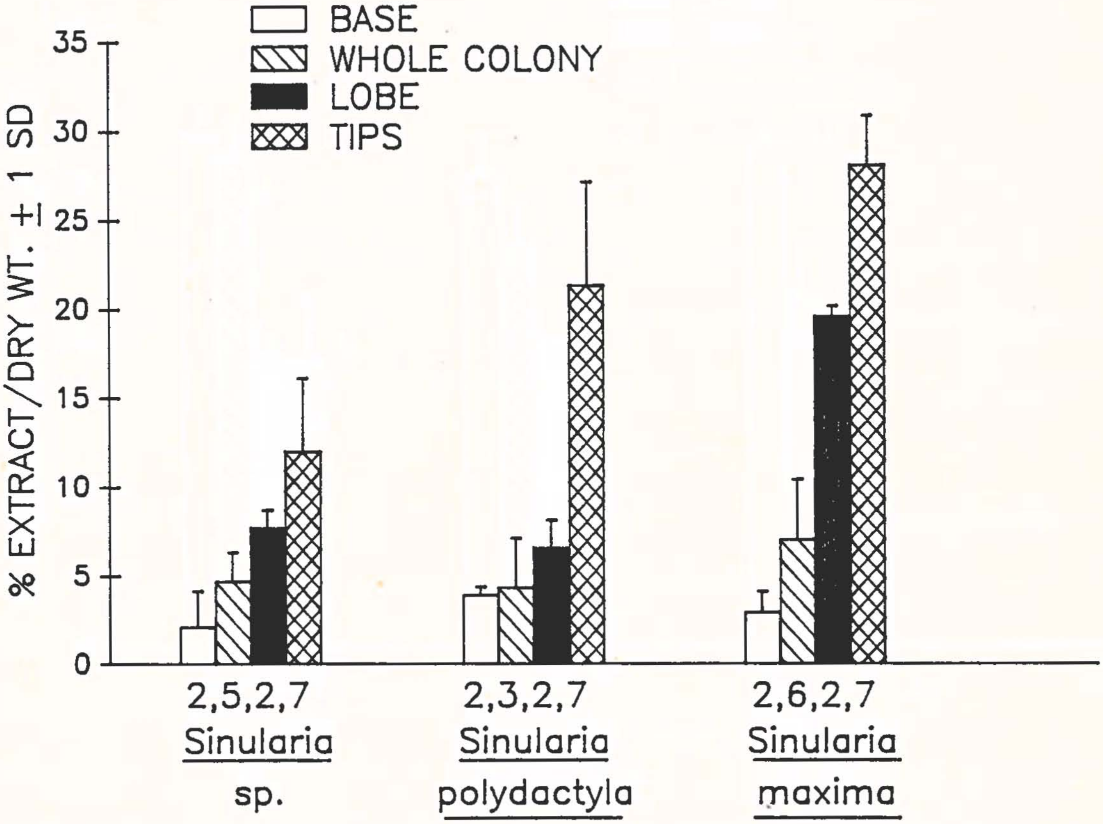

Fig. 2. Lipid-soluble extract/dry weight percentages from each <u>Sinularia</u> species and the colony parts. Numbers on the X axis correspond to the number of colonies used for analysis.

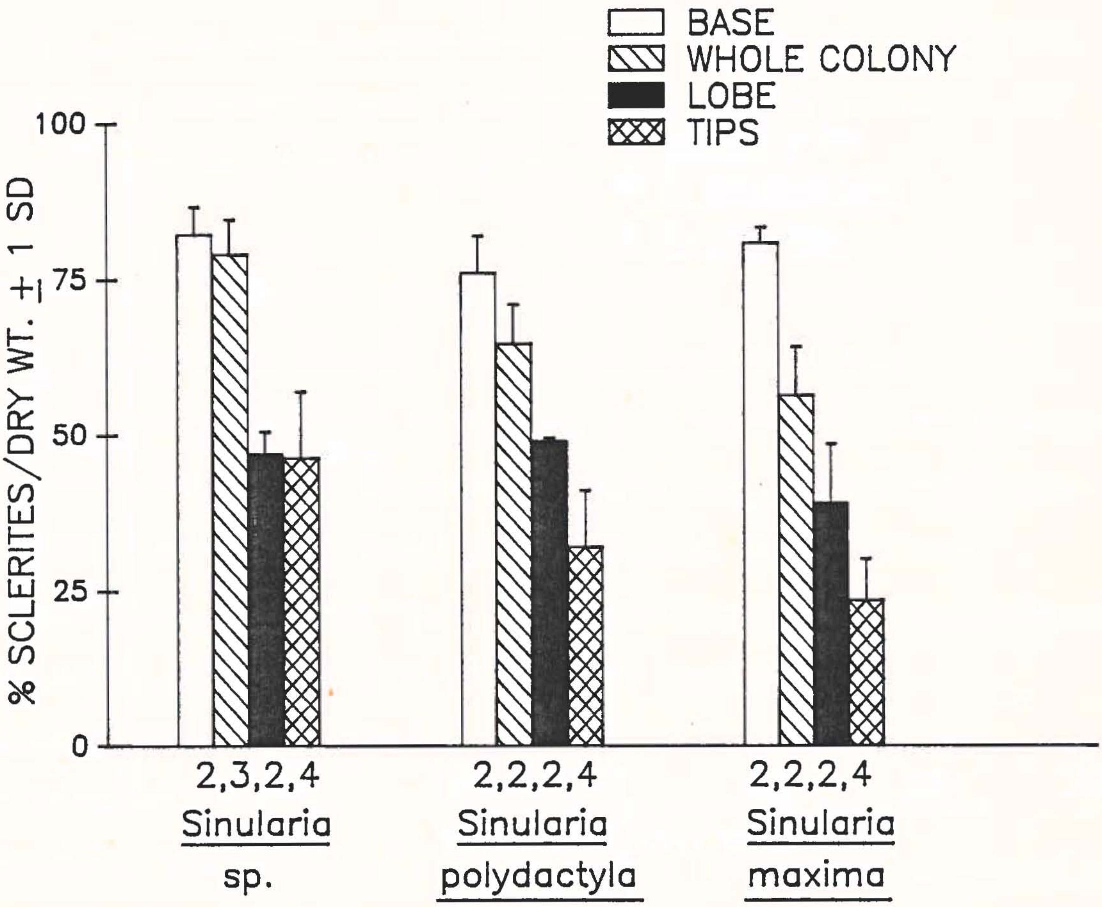

Fig. 3. Calcium carbonate sclerite/dry weight percentages found in each _Sinularia_ species and the colony parts. Numbers on the X axis correspond to the number of colonies used for analysis.

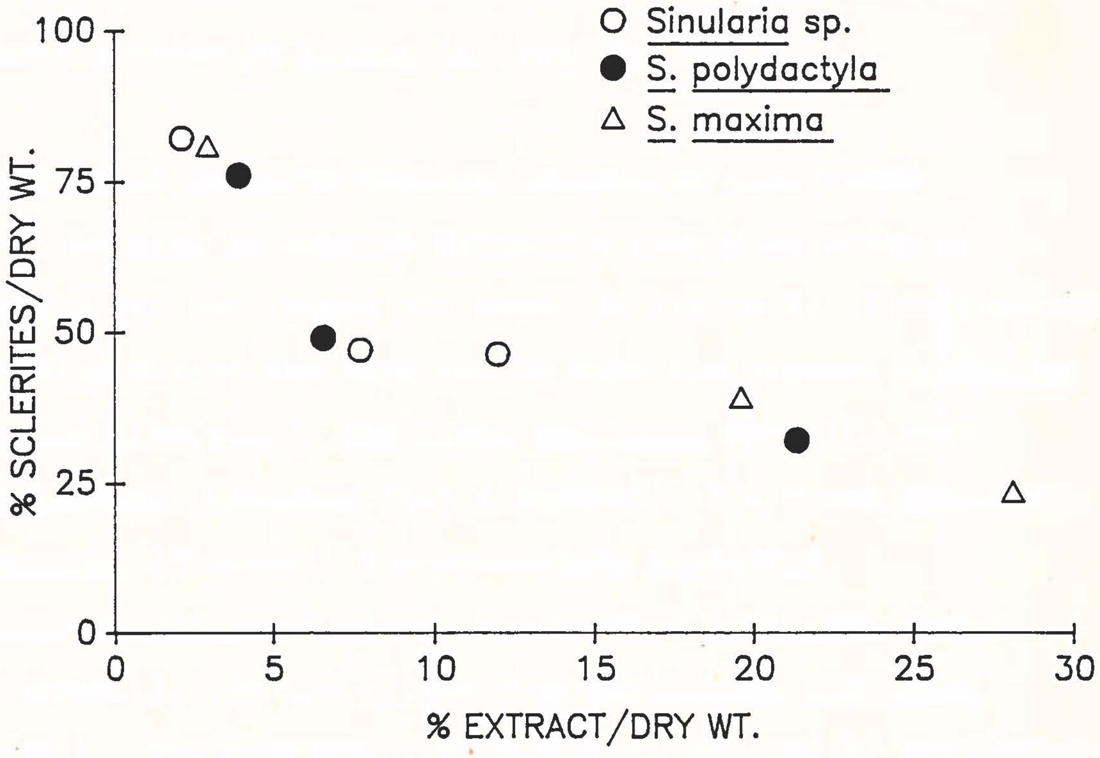

Fig. 4. Relationship between extract percentages and sclerite percentages. Data were taken from figures 2 and 3 and only tip, lobe, and base data were included.

significantly (p<.05, SNK) from *Sinularia* sp. but not from *S. polydactyla* (Fig. 3). The spicules (sclerites that are sharp and elongated) in the tips of *Sinularia* sp. and *S. polydactyla* were large (1-3 mm) while spicules in the tips of *S. maxima* were approximately 0.1-0.2 mm (Gawel, 1977). Sclerite concentrations were highest in the bases of all three species of *Sinularia* (Fig. 3).

EFFECTS OF EXTRACTS TOWARDS GENERALIST CARNIVORES

Results of extract bioassays toward an array of generalist carnivores are shown in Figure 5. Fish observed feeding during the experiments were the wrasses *Gomphosus varius* (Lacepède), *Cheilinus undulatus* Rüppell, and *Thalassoma lutescens* (Lay & Bennett), the bream *Pentapodus macrurus* (Bleeker), the damselfish *Abudefduf septemfasciatus* (Cuvier), the emperor *Lethrinus harak* (Forsskål), and the triggerfish *Balistapus undulatus* (Park). The extracts from *Sinularia* sp. and *S. polydactyla* were deterrent at 3% dry weight of squid (.01>p>.005 and p=.009 respectively, Wilcoxon's), and the *S. maxima* extract was deterrent at 6% dry weight of squid (.025>p>.01, Wilcoxon's). The cembranoid diterpene from *S. maxima* was deterrent at 2% dry weight of squid (.05>p>.025, Wilcoxon's). There was no significant difference when the extract of *Sinularia* sp. was compared to that of *S. maxima* at the same concentration of 3% dry weight (p>.05,

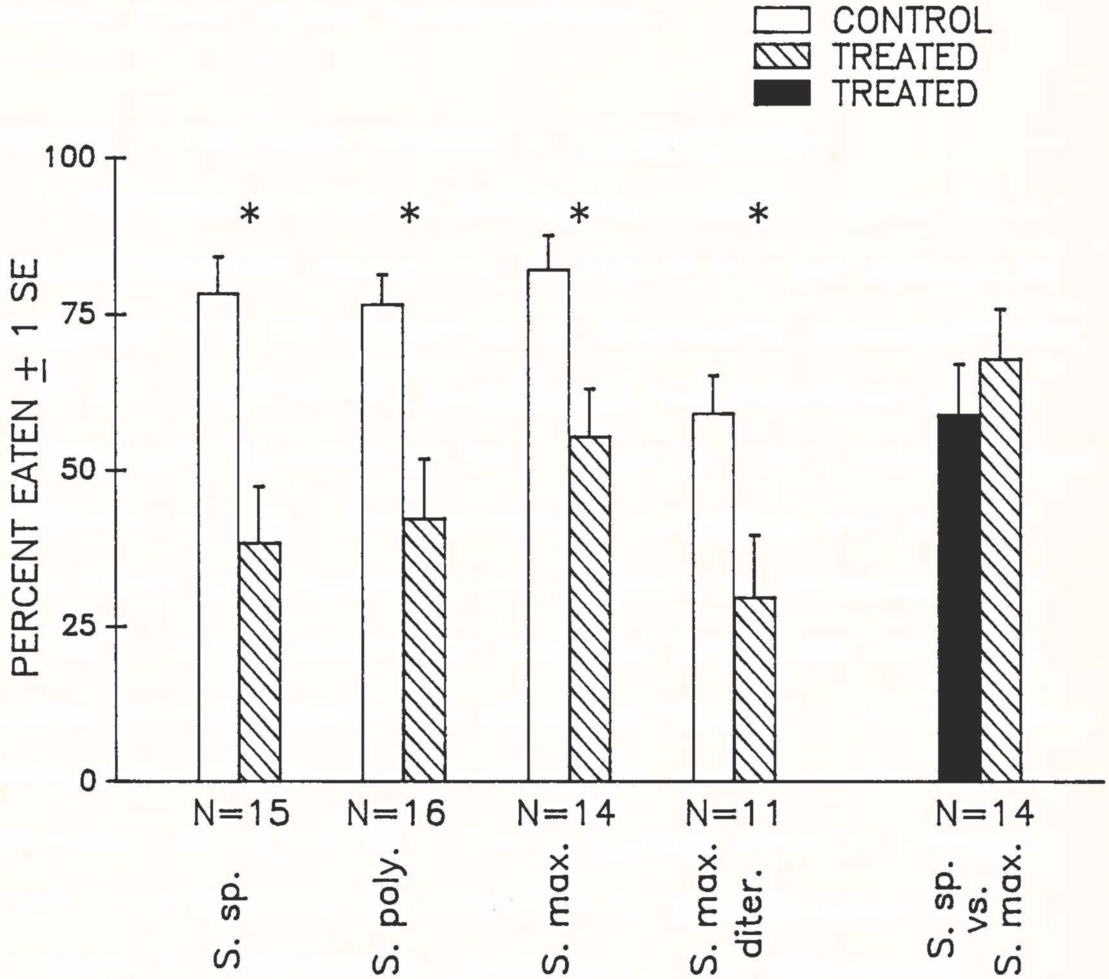

Fig. 5. Results of field assays with squid strips coated with Sinularia extracts and one isolated metabolite and offered to carnivorous fishes. S. sp. = Sinularia sp.; S. poly. = Sinularia polydactyla; S. max. = Sinularia maxima; diter. = pure cembranoid diterpene; N = number of trials; * = significant feeding deterrent (Wilcoxon's).

Wilcoxon's, 2-tailed). Crude extract concentrations used in assays were lower than the average concentration for whole colonies. The *S. maxima* diterpene was tested at natural whole colony concentration.

EFFECTS OF EXTRACTS TOWARDS *C. UNIMACULATUS*

Bioassay results of *Sinularia* extracts tested on *C. unimaculatus* are summarized in Figure 6. Two species of *Sinularia* were not deterrent at dry-weight concentrations up to 10%; however, *S. polydactyla* was deterrent at 10% (.05>p>.025, Wilcoxon's) but not at 9%. All extracts were deterrent at 20%. The extract of *S. maxima* was a significant attractant at 6% (.025>p>.01, Wilcoxon's, 2-tailed). Assay results of nonpolar fractions and the *S. maxima* pure diterpene are shown in Figure 7. All nonpolar terpene fractions of each *Sinularia* species were not deterrent at 1% and 5%. The *S. maxima* nonpolar terpene fraction significantly attracted *C. unimaculatus* at 1% (.025>p>.01, Wilcoxon's, 2-tailed). The cembranoid diterpene from *S. maxima* was not deterrent at 3% and 10% but was deterrent at 12%. Extract concentrations used in assays represent a range of natural concentrations found in whole colonies and the different colony parts.

Results of assays comparing the extracts of lobes vs. bases or grazed vs. ungrazed colonies for each *Sinularia* species are shown in Figures 8 and 9 respectively. All

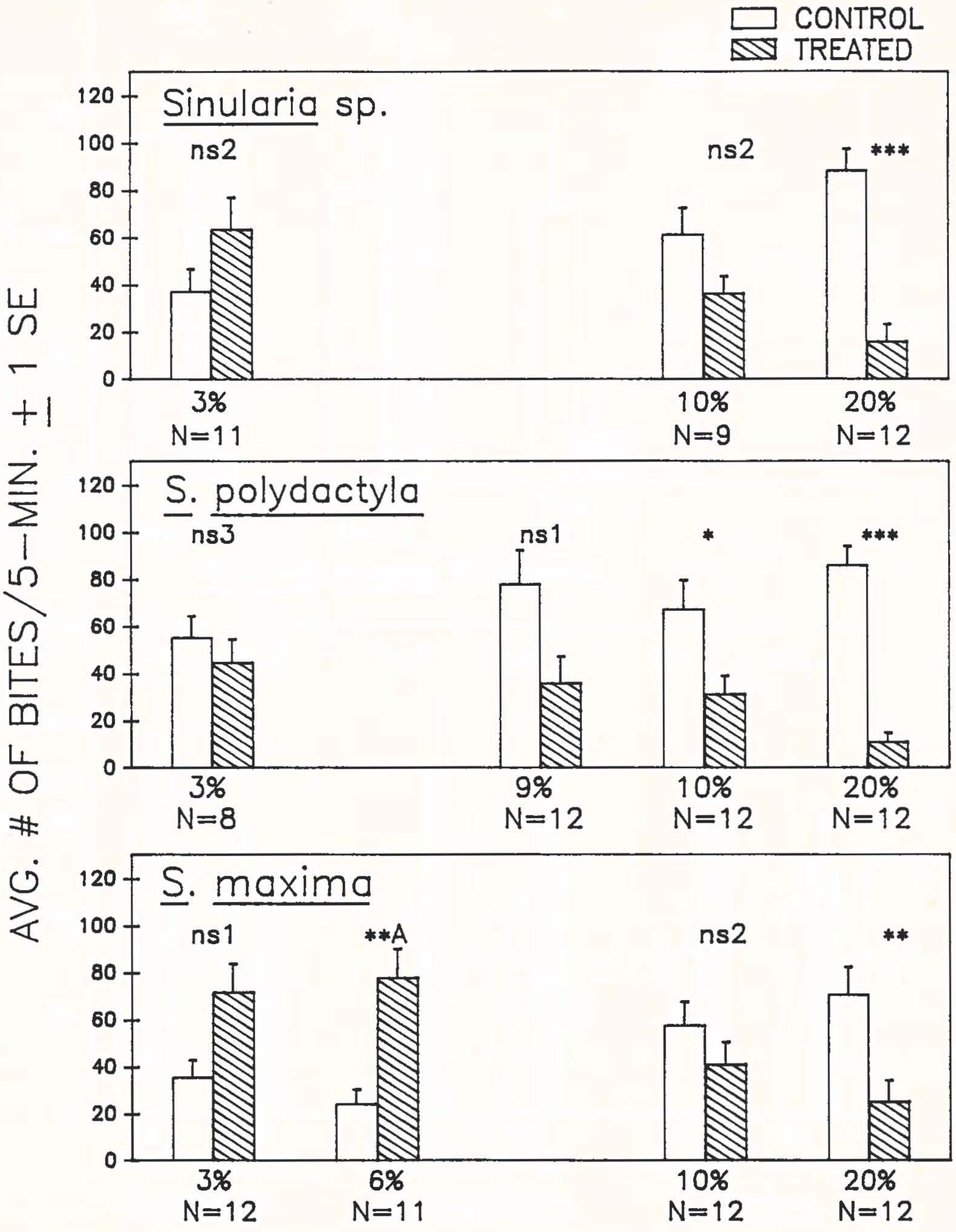

Fig. 6. Results of laboratory assays with crude _Sinularia_ extracts. _Tubifex_ cubes were coated with extracts and offered to _C_. _unimaculatus_. Percentages on X axis represent final dry weight concentrations of extract on the _Tubifex_. N = number of trials; ns1 = not significant (.10>p>.05); ns2 = .25>p>.10; ns3 = p>.25; * = significant deterrent (.05>p>.025); ** = .025>p>.01; *** = .005>p>.001; A = attractant (Wilcoxon's).

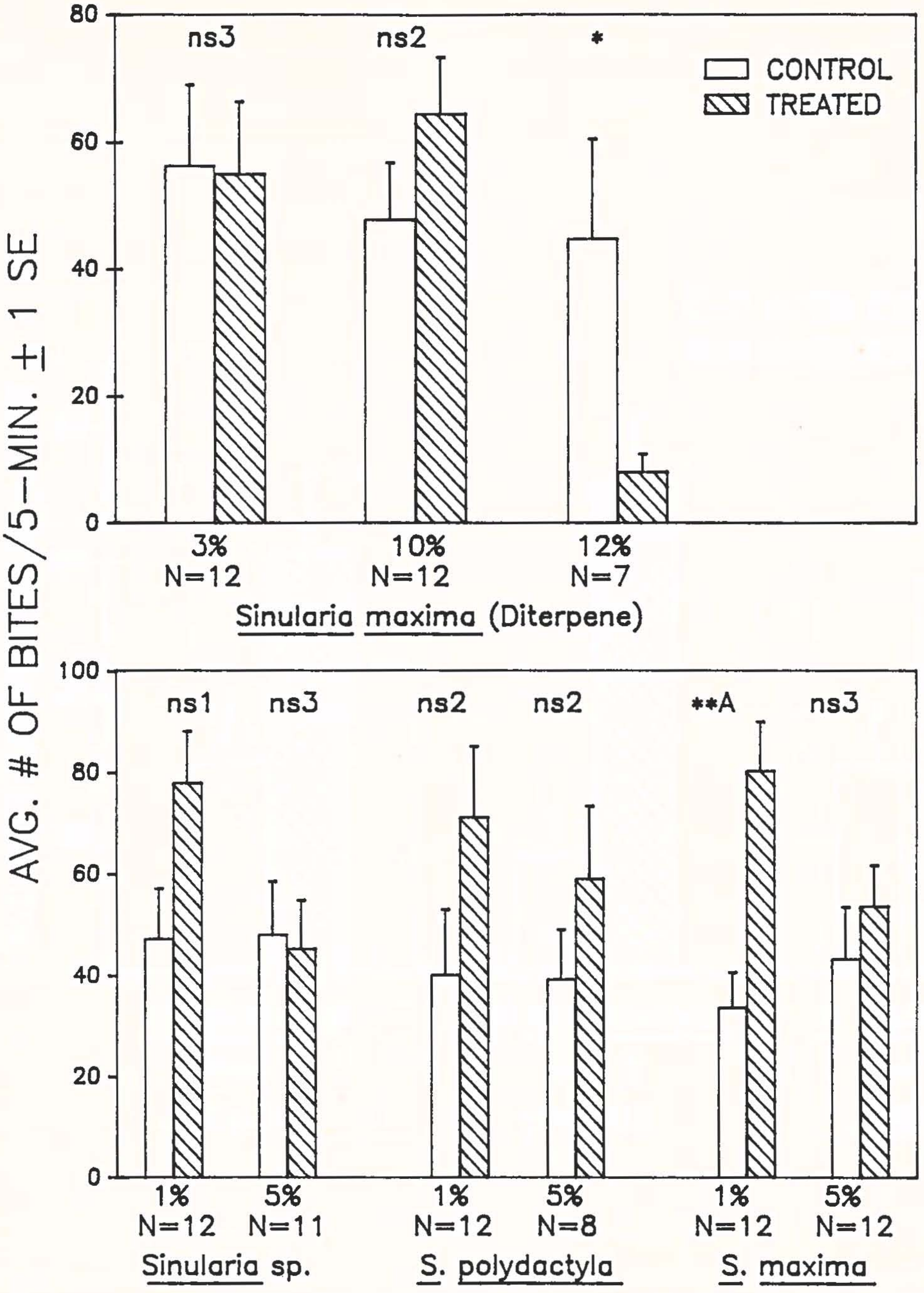

Fig. 7. Results of laboratory assays with *Sinularia* terpenoid hydrocarbon fractions and the pure *S. maxima* diterpene. *Tubifex* cubes were coated with metabolites and offered to *C. unimaculatus*. Percentages, N, and p-values are defined in Fig. 6.

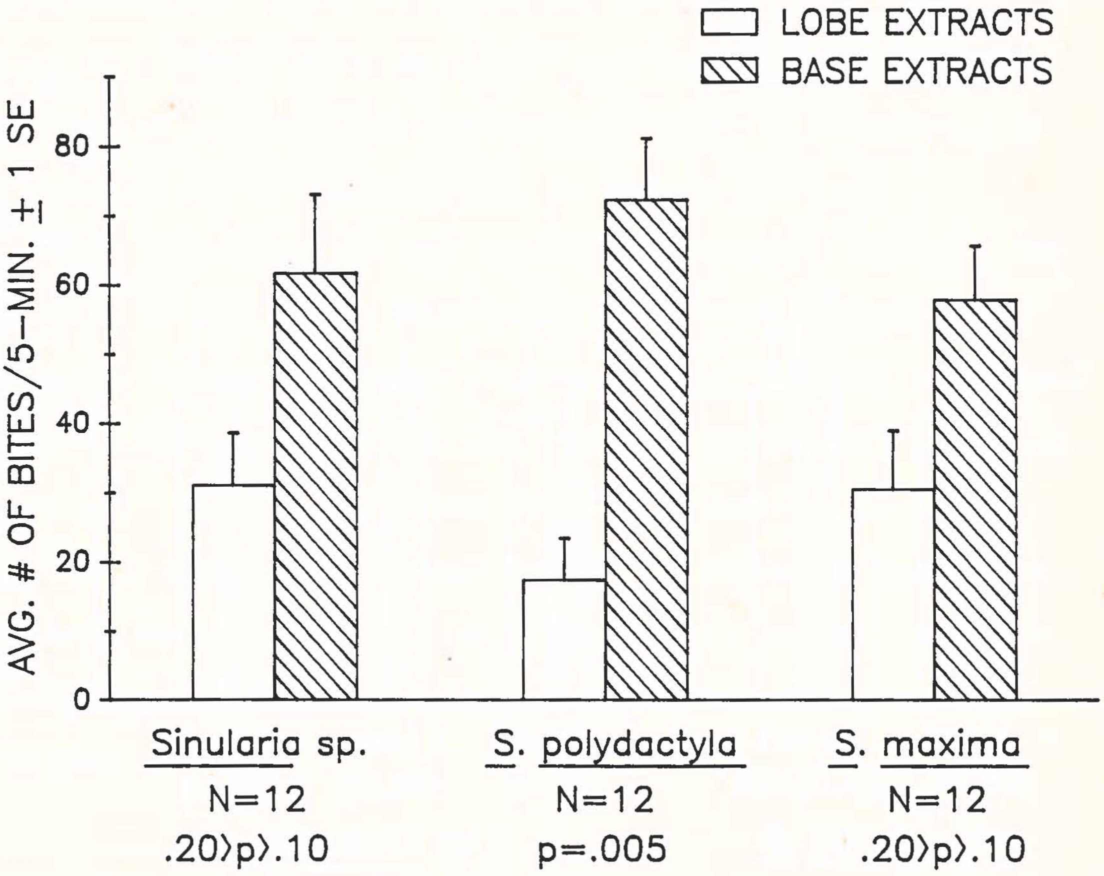

Fig. 8. Results of laboratory assays comparing Sinularia lobe and base extracts. Tubifex cubes were coated with extracts at 10% dry weight and offered to C. unimaculatus. N = number of trials. P-values determined by Wilcoxon's (2-tailed).

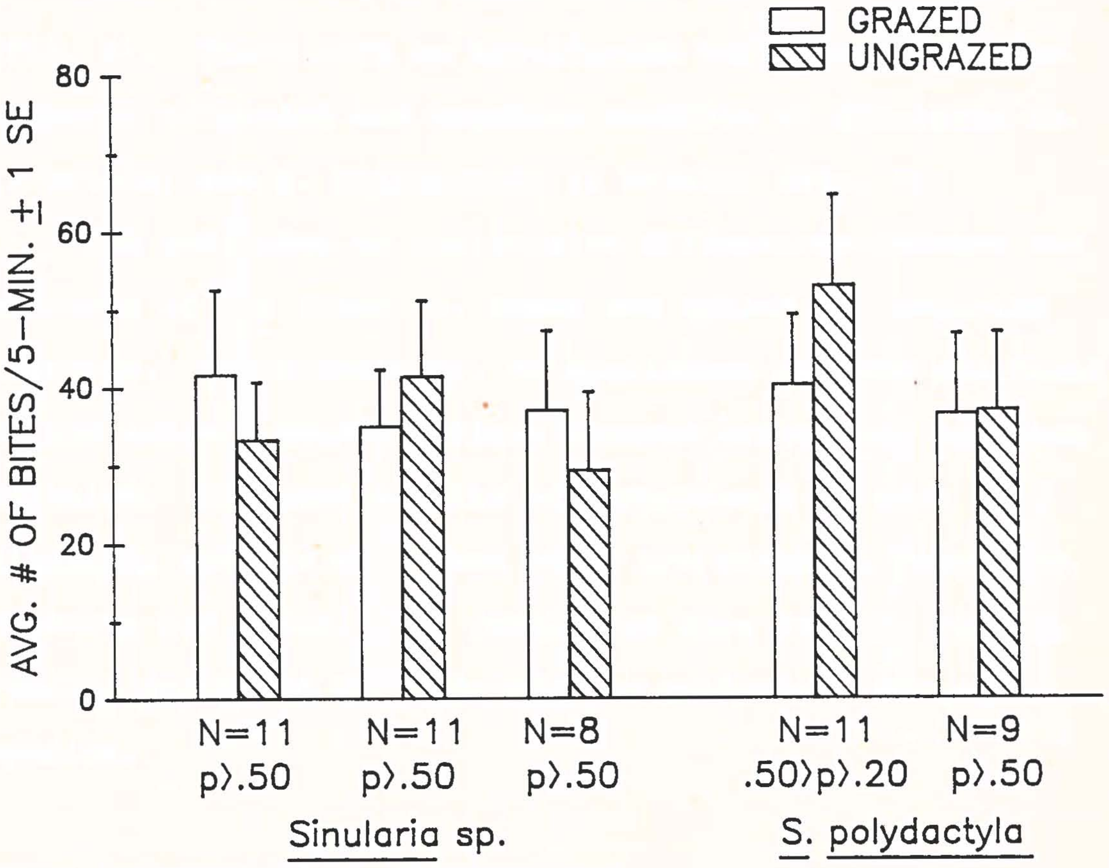

Fig. 9. Results of laboratory assays comparing grazed and ungrazed *Sinularia* extracts. *Tubifex* cubes were coated with extracts at 10% dry weight and offered to *C. unimaculatus*. N = number of trials. Three separate assays were performed on *Sinularia* sp. and two assays for *S. polydactyla*. P-values determined by Wilcoxon's (2-tailed).

extracts were tested at 10% dry weight. *C. unimaculatus* was significantly attracted to the basal extract of *S. polydactyla* (p=.005, Wilcoxon's, 2-tailed) but showed no preference for lobes or bases in the other two species (Fig. 8). There were no significant differences between extracts of grazed and ungrazed colonies of *Sinularia* sp. (3 trials) and *S. polydactyla* (2 trials) (Fig. 9).

TLC indicated no qualitative differences between the extracts of the lobes vs. bases and grazed vs. ungrazed colonies. Quantitatively, no consistent pattern was found in the extract concentrations between grazed and ungrazed colonies. Bioassay results of comparisons of each *Sinularia* species with each other are shown in Figure 10. Extracts and actual soft coral tips were tested, and all results indicated no difference or preference for any species.

FECAL ANALYSIS OF *C. UNIMACULATUS*

The analysis by TLC of *C. unimaculatus* fecal material after the fish were allowed to graze on single species of *Sinularia* showed no qualitative differences from the soft coral extract. Apparently, secondary metabolites were not degraded or converted to other compounds as they passed through the digestive system.

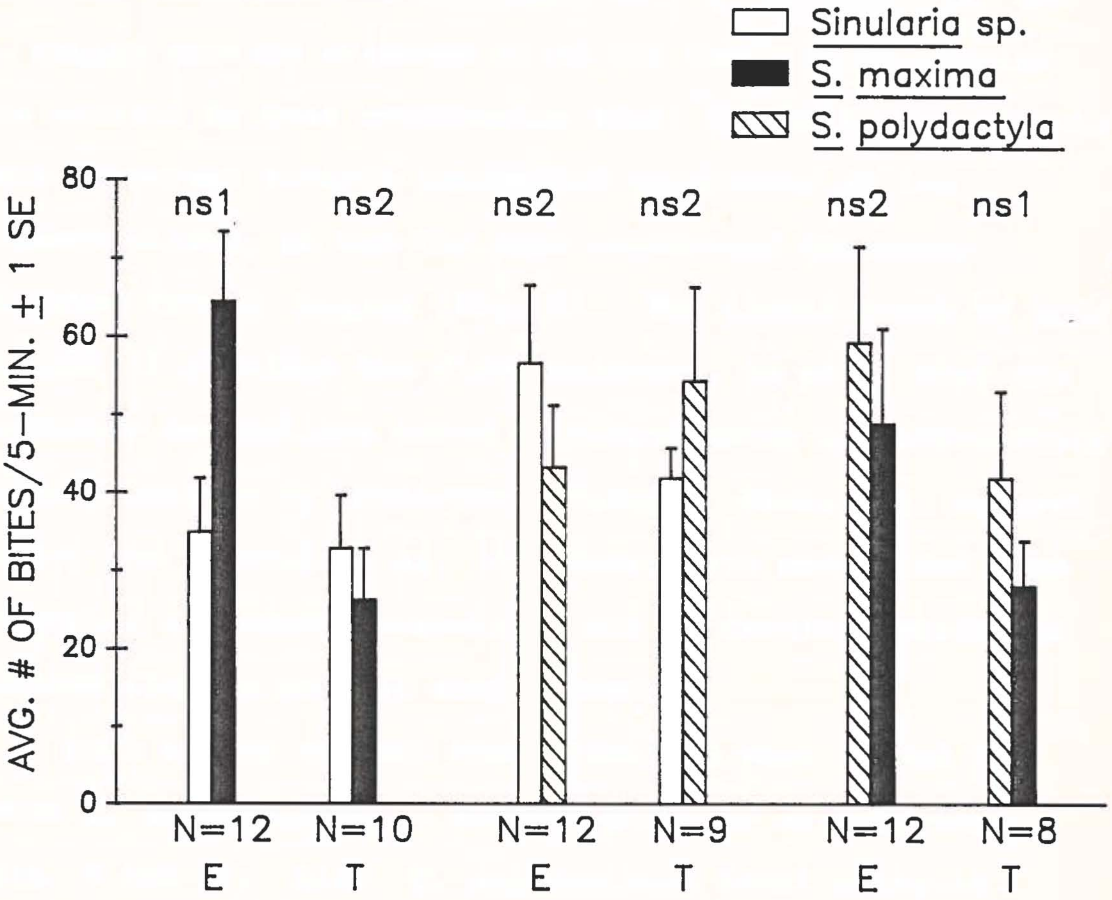

Fig. 10. Results of laboratory assays comparing *Sinularia* extracts against each other and actual *Sinularia* tips against each other. Extracts were coated at 10% dry weight of the *Tubifex* cubes and actual tips were 2-3 cm in length. Assays were performed with *C*. *unimaculatus*. N = number of trials; E = trials with crude extracts; T = trials with actual soft coral tips; ns1 = .50>p>.20; ns2 = p>.50 (Wilcoxon's, 2-tailed).

DISCUSSION

The feeding deterrent effects of lipid-soluble extracts isolated from Sinularia sp., S. polydactyla, and S. maxima provide evidence as to why these three species are avoided by most carnivorous fish; all of these extracts were deterrent towards generalist carnivores at low concentrations, as was the pure cembranoid diterpene isolated from S. maxima (Fig. 5). The significance of this is that it provides the first evidence, in situ, that the secondary metabolites found in alcyonaceans can effectively deter predation by carnivorous fishes. Similarly, Gerhart (1984) and Harvell et al. (in press) have shown that some gorgonian secondary metabolites act as feeding deterrents towards fish in natural environments.

Much higher extract concentrations were needed to deter C. unimaculatus, a natural predator of soft corals (Fig. 6 and 7). Also, C. unimaculatus fecal analysis indicated that the terpenoid metabolites contained in Sinularia extracts are passed through the digestive system without any apparent breakdown or conversion to other metabolites. In contrast, the gastropod Ovula ovum (Linnaeus), which also feeds on soft corals, transforms the major terpene in Sarcophyton into a less toxic compound (Coll et al., 1983).

Tursch & Tursch (1982) in their soft coral survey of a sheltered reef in Papua New Guinea noticed that 4 out of 18 species of chaetodontids fed on soft corals. Although they did not quantitatively assess their feeding preferences, they suggested that *C. unimaculatus* fed indiscriminately upon all soft coral species. However, my observations in Guam show that although *C. unimaculatus* feeds upon several different species of *Sinularia*, distinct preferences are evident (Table 1).

The feeding deterrent effects of extracts from *S. maxima* and *Sinularia* sp. appear to be major determinants of the feeding preferences of *C. unimaculatus*. The deterrent effects of *S. maxima* extracts occur at much lower concentrations than found naturally in the soft-coral tips, while the extracts of *Sinularia* sp. are deterrent only at much higher concentrations (compare Fig. 2 and Fig. 6). The observed high preference for *Sinularia* sp. and the low preference for *S. maxima* could be related to the feeding deterrence of their secondary metabolites. In contrast, the extracts of *S. polydactyla* are deterrent even at concentrations which are lower than those occurring naturally in the tips. In the field, however, *S. polydactyla* was not avoided by *C. unimaculatus*, and colonies frequently showed bite marks. LaBarre *et al*. (1986b) found that the extract of *S. polydactyla* collected from the Great Barrier Reef in Australia was one of the most palatable extracts that they tested.

The secondary metabolites, and thus the feeding deterrent potential, of conspecific soft corals can differ with location. Braekman et al. (1980) isolated a major nonpolar sesquiterpene hydrocarbon called africanene from S. polydactyla collected at Laing Island in Papua New Guinea. However, the colonies of S. polydactyla collected in Cocos Lagoon, Guam did not contain africanene but instead contained other nonpolar terpene hydrocarbons. S. polydactyla collected in Australia was found to contain at least six highly functionalized diterpenes (Bowden et al., in press) including the major metabolite found on Guam in S. maxima but not in S. polydactyla (Fig. 1).

Deterrent effects of the Sinularia extracts appear to result from the more polar, functionalized terpenes present in the extracts. The nonpolar terpene hydrocarbon fractions from each of the 3 Sinularia species showed no deterrent effects towards C. unimaculatus, even at concentrations higher than those naturally occurring in the colonies (Fig. 7). In fact, the nonpolar terpene fraction from S. maxima actually increased feeding by C. unimaculatus. The more polar cembranoid diterpene isolated from S. maxima (Fig. 1) was a deterrent at the concentration (12% dry weight) found in the tips.

Laboratory comparisons revealed no significant differences in deterrence between Sinularia species (Fig. 10). This is interesting since C. unimaculatus rarely fed upon *S. maxima* in the field, and then only on the basal portions. In the laboratory, however, *C. unimaculatus* fed upon *S. maxima* tips as readily as on the tips of *Sinularia* sp. and *S. polydactyla*. This may indicate that *C. unimaculatus* can tolerate the high concentration of extract in *S. maxima* tips if necessary. When offered *S. maxima* in the laboratory, *C. unimaculatus* consumed it without apparent harm.

Quantitative and qualitative assessment of extracts from grazed and ungrazed colonies did not provide evidence for intraspecific prey preferences by *C. unimaculatus*. The extracts from grazed and ungrazed colonies of the same species contained identical secondary metabolites (based on TLC) in similar concentrations. There was no preference for extracts from ungrazed colonies over those from grazed colonies when both were offered to *C. unimaculatus* in aquarium assays (Fig. 9). Gerhart (1986) also found that qualitative differences in chemical composition between grazed and ungrazed gorgonians could not account for the preferences of the gastropod *Cyphoma gibbosum* (Linnaeus). I was not able to determine if the reproductive state of the soft corals affected the feeding of *C. unimaculatus* as Lasker (1985) found with *C. capistratus* and gorgonians, since during the entire study only 1 reproductive colony was observed. Bowden *et al*. (1985) found differences in terpenoid content between reproductive and nonreproductive alcyonacean colonies which could conceivably affect feeding patterns. If *C. unimaculatus* is selecting specific colonies within *Sinularia* populations, secondary chemistry does not appear to explain this selection.

C. unimaculatus takes most of its bites from the lobes of the colony where extract concentrations are the highest. The tree-like morphology rather than the chemistry of the *Sinularia* probably accounts for this behavior. *Sinularia* lobes support large finger-like tips which can render the basal sections inaccessible to predation by fishes. However, the coral tips are exposed and easy to attack. The lobes also support the polyps which may be the actual food goal. *C. unimaculatus* took bites on the base only when the base was exposed. TLC indicated no qualitative differences between base and lobe extracts, although large differences occurred in extract concentrations (Fig. 2). Difference in feeding-deterrence of base and lobe extracts were found only for *S. polydactyla* where the lobe extract was more effective as a feeding deterrent than the base extract (Fig. 8).

Harvell *et al*. (in press) suggested that a more balanced approach in analyses of octocoral defense should consider both chemical and structural defense adaptations. Sammarco *et al*. (1987) found that mineralization of *Sinularia* coenenchyme was negatively associated with toxicity. I found a similar relationship between the amount of sclerites and extract concentration (Fig. 4); however, the high amounts of sclerites or large spicules in the tips of each *Sinularia* species do not seem to influence the preferences of *C. unimaculatus*. The most preferred species *Sinularia* sp. contained higher amounts of sclerites than either *S. maxima* or *S. polydactyla* and had very large and sharp spicules in contrast to the powder-like spicules found in the tips of *S. maxima*. In contrast, Harvell *et al*. (in press) found that gorgonian sclerites could effectively deter the feeding of fishes.

The results of this study show that *C. unimaculatus* has a high tolerance for the secondary metabolites present in several species of *Sinularia*. *C. unimaculatus* also appears to be unaffected by the sclerites embedded in the *Sinularia* coenenchyme. These tolerance thresholds appear to play an important role in determining preferences of *C. unimaculatus* for alcyonaceans. *C. unimaculatus* has definite preferences among species of *Sinularia*, and the feeding deterrence of secondary metabolites appears to play a significant role in determining these preferences. An important outcome of *C. unimaculatus* tolerances to the defenses of various species of soft corals is that this fish is able to exploit a food source which is unavailable to most other carnivorous fish.

Literature Cited

Anderson, G. R. V., A. H. Ehrlich, P. R. Ehrlich, J. D. Roughgarden, B. C. Russell, and F. H. Talbot. 1981. The community structure of coral reef fishes. Am. Nat. 117:476-495.

Bakus, G. J. 1981. Chemical defense mechanisms on the Great Barrier Reef. Science 211:497-499.

Bakus, G. J., N. M. Targett, and B. Schulte. 1986. Chemical ecology of marine organisms: an overview. J. Chem. Ecol. 12:951-987.

Birkeland, C., and S. Neudecker. 1981. Foraging behavior of two Caribbean chaetodontids: Chaetodon capistratus and C. aculeatus. Copeia 1981:169-178.

Braekman, J. C., D. Daloze, B. Tursch, S. E. Hull, J. P. Declercq, G. Germain, and M. Van Meerssche. 1980. Chemical studies of marine invertebrates. XXXVIII. 9(15)-Africanene, a new sesquiterpene hydrocarbon from Sinularia polydactyla (Coelenterata, Octocorallia, Alcyonaceae). Experientia 36:893.

Bowden, B., J. Coll, D. Tapiolas, and R. Willis. 1985. Some chemical aspects of spawning in alcyonacean corals. Proc. Fifth Int. Coral Reef Symp. 4:325-329.

Bowden, B. F., J. C. Coll, and A. D. Wright. in press. Studies of Australian soft corals. XLIV. New diterpenes from Sinularia polydactyla (Coelenterata, Anthozoa, Octocorallia). Aust. J. Chem.

Chesson, J. 1978. Measuring preference in selective predation. Ecology 59:211-215.

Coll, J. C., S. La Barre, P. W. Sammarco, W. T. Williams, and G. J. Bakus. 1982. Chemical defences in soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef: a study of comparative toxicities. Mar. Ecol. Prog. Ser. 8:271-278.

Coll, J. C., and P. W. Sammarco. 1983. Terpenoid toxins of soft corals (Cnidaria, Octocorallia): their nature, toxicity, and ecological significance. Toxicon, Suppl. 3:69-72.

Coll, J. C., D. M. Tapiolas, B. F. Bowden, L. Webb, and H. Marsh. 1983. Transformation of soft coral (Coelenterata: Octocorallia) terpenes by *Ovula ovum* (Mollusca: Prosobranchia). Mar. Biol. 74:35-40.

Cox, E. F. 1986. The effects of a selective corallivore on growth rates and competition for space between two species of Hawaiian corals. J. Exp. Mar. Biol. Ecol. 101:161-174.

Gawel, M. J. 1977. The common shallow-water soft corals (Alcyonacea) of Guam. M. Sc. thesis, University of Guam, 201 pp.

Gerhart, D. J. 1984. Prostaglandin A_2: an agent of chemical defense in the Caribbean gorgonian *Plexaura homomalla*. Mar. Ecol. Prog. Ser. 19:181-187.

Gerhart, D. J. 1986. Gregariousness in the gorgonian-eating gastropod *Cyphoma gibbosum*: tests of several possible causes. Mar. Ecol. Prog. Ser. 31:255-263.

Grigg, R. W., J. J. Polovina, and M. J. Atkinson. 1984. Model of a coral reef ecosystem. III. Resource limitation, community regulation, fisheries yield and resource management. Coral Reefs 3:23-27.

Harmelin-Vivien, M. L., and Y. Bouchon-Navaro. 1981. Trophic relationships among chaetodontid fishes in the Gulf of Aqaba (Red Sea). Proc. Fourth Int. Coral Reef Symp. 2:537-544.

Harmelin-Vivien, M. L., and Y. Bouchon-Navaro. 1983. Feeding diets and significance of coral feeding among chaetodontid fishes in Moorea (French Polynesia). Coral Reefs 2:119-127.

Harvell, C. D., W. Fenical, and C. H. Greene. in press. Chemical and structural defenses of Caribbean gorgonians (*Pseudopterogorgia* spp.) I: development of an *in situ* feeding assay. Mar. Ecol. Prog. Ser.

Hay, M. E., J. E. Duffy, C. A. Pfister, and W. Fenical. 1987. Chemical defense against different marine herbivores: are amphipods insect equivalents? Ecology 68:1567-1580.

Hobson, E. S. 1974. Feeding relationships of Teleostean fishes on coral reefs in Kona, Hawaii. Fish. Bull. 72:915-1031.

Huston, M. A. 1985. Patterns of species diversity on coral reefs. Ann. Rev. Ecol. Syst. 16:149-177.

Ivlev, V. S. 1961. Experimental ecology of the feeding of fishes. Yale University Press, New Haven, Connecticut, 302 pp.

Jacobs, J. 1974. Quantitative measurement of food selection. Oecologia 14:413-417.

La Barre, S. C., J. C. Coll, and P. W. Sammarco. 1986a. Competitive strategies of soft corals (Coelenterata: Octocorallia): III. Spacing and aggressive interactions between alcyonaceans. Mar. Ecol. Prog. Ser. 28:147-156.

La Barre, S. C., J. C. Coll, and P. W. Sammarco. 1986b. Defensive strategies of soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef. II. The relationship between toxicity and feeding deterrence. Biol. Bull. 171:565-576.

Lasker, H. R. 1985. Prey preferences and browsing pressure of the butterflyfish *Chaetodon capistratus* on Caribbean gorgonians. Mar. Ecol. Prog. Ser. 21:213-220.

Lechowicz, M. J. 1982. The sampling characteristics of electivity indices. Oecologia 52:22-30.

Motta, P. J. 1985. Functional morphology of the head of Hawaiian and Mid-Pacific butterflyfishes (Perciformes, Chaetodontidae). Env. Biol. Fish. 13:253-276.

Norris, J. N., and W. Fenical. 1985. Natural products chemistry: uses in ecology and systematics. In, Handbook of phycological methods, ecological field methods: macroalgae, edited by M. M Littler and D. S. Littler, Cambridge University Press, Cambridge, New York, pp. 121-145.

Pawlik, J. R., M. T. Burch, and W. Fenical. 1987. Patterns of chemical defense among Caribbean gorgonian corals: a preliminary survey. J. Exp. Mar. Biol. Ecol. 108:55-66.

Randall, J. E. 1974. The effect of fishes on coral reefs. Proc. Second Int. Coral Reef Symp. 1:159-166.

Sammarco, P. W. 1980. Diadema and its relationship to coral spat mortality: grazing, competition, and biological disturbance. J. Exp. Mar. Biol. Ecol. 45:245-272.

Sammarco, P. W., J. C. Coll, and S. La Barre. 1985. Competitive strategies of soft coral (Coelenterata: Octocorallia). II. Variable defensive responses and susceptibility to scleractinian corals. J. Exp. Mar. Biol. Ecol. 91:199-215.

Sammarco, P. W., J. C. Coll, S. La Barre, and B. Willis. 1983. Competitive strategies of soft corals (Coelenterata: Octocorallia): allelopathic effects on selected scleractinian corals. Coral Reefs 1:173-178.

Sammarco, P. W., S. La Barre, and J. C. Coll 1987. Defensive strategies of soft corals (Coelenterata: Octocorallia) of the Great Barrier Reef. III. The relationship between ichthyotoxicity and morphology. Oecologia 74:93-101.

Sano, M., M. Shimizu, and Y. Nose. 1984. Food habits of Teleostean reef fishes in Okinawa Island, Southern Japan. Univ. Mus., Univ. Tokyo, Bull. 25:1-128.

Talbot, F. H. 1965. A description of the coral structure of Tutia reef (Tanganyika territory: East Africa) and its fish fauna. Proc. Zool. Soc. London 145:431-470.

Tursch, B., J. C. Braekman, D. Daloze, and M. Kaisin. 1978. Terpenoids from coelenterates. In, Marine natural products: chemical and biological perspectives, vol. 2, edited by P. J. Scheuer, Academic Press, New York, pp. 247-296.

Tursch, B., and A. Tursch. 1982. The soft coral community on a sheltered reef quadrat at Laing Island (Papua New Guinea). Mar. Biol. 68:321-332.