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Biogeographic comparisons of chemical and structural defenses of the Pacific gorgonians *Annella mollis* and *A. reticulata*

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ABSTRACT: Compared to other areas of chemical ecology, biogeographic comparisons of the chemical defenses associated with benthic marine organisms are few. This study addresses geographic differences in the chemical and structural defenses of 2 Pacific gorgonians (*Annella mollis* and *A. reticulata*), found at 2 islands: Guam, Micronesia, and Lizard Island, Australia. Crude extracts and sclerites extracted from the mid-axis and tips of colonies were assayed against natural assemblages of reef fishes at Western Shoals, Guam, and Mermaid's Cove, Lizard Island. Reciprocal feeding assays clearly demonstrated that crude extracts from *Annella* spp. were unpalatable to natural assemblages of reef fishes at Western Shoals and Mermaid's Cove and sclerites have little or no role as generalist predator defenses. Sclerites from the tips of *A. reticulata* were only effective as feeding deterrents at high concentrations. Variation in the palatability of the mid-axis extracts of *A. mollis* suggested that chemical defenses are more concentrated at the tips of the colonies from Guam and Lizard Island. When assayed at concentrations similar to and higher than the tips the mid-axis extracts did not deter fish feeding at Mermaid's Cove.

KEY WORDS: Biogeography · Chemical defenses · Marine chemical ecology · *Annella mollis* · *Annella reticulata* · Indo-pacific · Gorgonian

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INTRODUCTION

Biogeographic comparisons of defenses in marine plants and invertebrates have evaluated qualitative and quantitative differences in secondary metabolites (Steinberg 1989, 1992, Targett et al. 1992, Harvell et al. 1993, Pavia & Aberg 1996, Van Alstyne et al. 1999) or directly tested the responses of generalist consumers to organic extracts or plant materials (Van Alstyne & Paul 1990, Bolser & Hay 1996, Cronin et al. 1997, Pennings et al. in press). In comparisons between temperate and tropical species, higher levels of predator-deterrent compounds have been shown to be more common in tropical species (Bakus & Green 1974, Bakus 1981, Bolser & Hay 1996), although there is evidence to suggest that this is not always the case (Steinberg 1992, Targett et al. 1992). The higher instance of defenses in marine organisms from tropical regions is proposed to be a consequence of increased predation and competition at lower latitudes (Bakus & Green 1974, Bolser & Hay 1996). This hypothesis is supported by comparisons made at the consumer level between temperate and tropical sea urchins and fish (Bolser & Hay 1996, Cronin et al. 1997). Overall, consumers from North Carolina and the Bahamas preferred temperate algal species in paired assays when offered side-by-side with their congeners from the tropics (Bolser & Hay 1996). In assays with sea urchins and fish from North Carolina and Guam, lower concentrations of pure metabolites from the brown alga *Dictyota acutiloba* were needed to deter the temperate herbivores com-

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pared to the tropical species (Cronin et al. 1997). Cronin et al. (1997) proposed that tropical herbivores (i.e. in Guam) are more resistant to seaweed chemical defenses.

Comparisons among species of brown algae from geographically different regions show that polyphenol (phlorotannins) concentrations can exhibit considerable latitudinal and local variation (Steinberg 1989, 1992, Steinberg & Van Altena 1992, Pavia & Aberg 1996, Van Alstyne et al. 1999). On the global scale, brown algae from Australia and New Zealand were shown to produce higher concentrations of polyphenols compared to their North American counterparts (Steinberg 1989, 1992). On the more local scale, Pavia & Aberg (1996) reported considerable variability in polyphenol concentrations between 2 areas in the North Atlantic separated by >1000 km. Site-to-site comparisons in kelp and rockweeds along the north Pacific coast of the United States showed that only 25% of the kelps and 3 of 4 rockweeds exhibited geographic variation in polyphenol concentrations, suggesting that localized selection or phenotypic plasticity may be phylogenetically constrained (Van Alstyne et al. 1999). More recently, Pennings et al. (in press) addressed variability in herbivore defenses of marsh plants along the Atlantic coast of the USA with a variety of consumers. Reciprocal feeding assays clearly demonstrated that fresh marsh plant material from Rhode Island and Maine was more palatable to consumers from both northern and southern regions than plants from Florida and Georgia. Local variation of herbivore communities within temperate regions is suggested to select for these latitudinal trends (Steinberg 1992, Pennings et al. in press). Pavia & Aberg (1996) point out the importance of local herbivores with a limited habitat range in explaining the differences in defenses between local communities. Early work on coral reefs suggested that almost all of the common, exposed coral reef invertebrates are chemically and/or structurally defended from predators (Bakus & Green 1974, Green 1977, Bakus 1981). Because chemical and structural defenses are prevalent in tropical benthic marine organisms (Hay 1996) few studies have addressed local variation in defenses between tropical regions (Harvell et al. 1993) compared to tropical versus temperate and temperate versus temperate comparisons.

Gorgonian corals are conspicuous members of coral reef communities (Kinzie 1970, Yoshioka & Yoshioka 1989). Except for a few specialist predators, coral reef fish and invertebrates do not readily consume sea fans and whips (Lasker 1985, Harvell & Suchanek 1987, Lasker & Coffroth 1988, Ruesink & Harvell 1990, Van Alstyne & Paul 1992, Vrolijk & Targett 1992, Cronin et al. 1995, Slattery 1999). Gorgonians produce chemical (i.e. secondary metabolites) and/or structural (i.e. sclerites) defenses against predation (Pawlik et al. 1987, Harvell et al. 1988, 1996, Fenical & Pawlik 1991, Pawlik & Fenical 1992, Van Alstyne & Paul 1992, West 1997, 1998). Some species exhibit intra-colony variation in secondary metabolite and sclerite concentrations suggesting that different parts of the colony rely more upon chemical defenses and others rely upon structural defenses (Harvell & Fenical 1989, Van Alstyne & Paul 1992). For example, the Caribbean gorgonians *Pseudopterogorgia* spp. have higher concentrations of metabolites at the tips of colonies and more sclerites in the base (Harvell et al. 1988, Harvell & Fenical 1989). In other species, crude extract and sclerite concentrations are uniform throughout the colony (Van Alstyne & Paul 1992, Slattery 1999). *Gorgonia ventalina*, also found in the Caribbean, exhibited no quantitative variation in crude extract and sclerite concentrations in the upper portions of the colony (Van Alstyne & Paul 1992). However, Kim et al. (2000) have shown that crude extracts from the tips of healthy *Gorgonia* spp. colonies are more resistant to the fungal pathogen *Aspergillus sydowii* than extracts from other parts of the colony.

For some species, a variety of different secondary metabolites have been reported from colonies collected from discrete regions (Faulkner 1999 and references cited within). A geographic comparison of colonies of the Caribbean gorgonian *Briareum asbestinum* from the Bahamas and St. Croix showed that the 2 populations produced different classes of diterpenes as chemical defenses (Harvell et al. 1993). Further, in shallow habitats where colonies are exposed to higher levels of predation, *B. asbestinum* produced smaller sclerites at higher densities as structural defenses (West et al. 1993).

Biogeographic comparisons are thought to be essential to the understanding of the evolution and ecology of coral reef communities (Hay 1996, Sammarco 1996). In this study we address quantitative differences in crude extract and sclerite concentrations within colonies (base, mid-axis, tips) of 2 Pacific gorgonians, *Annella mollis* and *A. reticulata*, and among sites at 2 islands, Guam (GU) and Lizard Island (LI). We assay crude extracts and sclerites from the mid-axes and tips of the colonies against natural assemblages of reef fishes at: (1) their island of origin and (2) the other island. Previous studies of gorgonian corals on Caribbean reefs suggest that there is considerable variability in the production of chemical and structural defenses among species (Pawlik et al. 1987, Harvell & Fenical 1989), making predictions of intra-colony variation in *Annella* spp. difficult. However, a broad survey of gorgonian crude extracts demonstrated that the extracts from species with small sclerites were usually unpalatable to fish (Pawlik et al. 1987, Harvell & Fenical 1989). Small, colorless sclerites are characteristic of

sea fans in the genus *Annella* (Chen & Chang 1991); therefore we expected the crude extracts to be unpalatable to natural assemblages of reef fish. GU and LI are tropical, and predation should be intense at both islands (Hay 1996), suggesting that populations of *Annella* spp. will be under similar or equal pressure to produce defenses against generalist predators. This hypothesis predicts no differences in chemical and/or structural defenses among sites or between islands. Alternatively, local predators associated with different collection sites can result in differences in defenses (Pavia & Aberg 1996).

METHODS AND MATERIALS

Study organisms. The *Annella* spp. (formerly *Subergorgia* spp., Family: Subergorgiidae) (Grassoff 1999) are azooxanthellate gorgonians common in the Indo-Pacific from the northern Red Sea to the central Pacific. They are the largest and most conspicuous sea fans on the reefs of GU, growing to 2–3 m at depths of >20 m. At LI, *Annella* spp. can be found on reefs as shallow as 5 m and at depths greater than 25 m. Sea fans in this genus have branches closely anastomosed as a network. The sclerites are small and colorless (Chen & Chang 1991). *A. mollis* (Nutting) is typically brown or orange with oblong cells. 'Double-wheel' sclerites characteristic of this species are found densely packed in the coenenchyme (Grassoff 1999). *A. reticulata* (Ellis & Solander) is pink to orange with small cells characterized by 'double-head' sclerites. The larger, oblong cells of *A. mollis* and small cells of *A. reticulata* are often found in the same colony. In addition, the 'double-heads' and 'double-wheels' can be found in the same colony (Phil Alderslade pers. comm.). Colonies used in this study were identified by sclerite analysis and limited to those specimens that contained either the 'double-heads' or the 'double-wheels'.

Collection. Sea fans were collected by SCUBA from sites around GU (144° 45′ E, 13° 30′ N) between March and July 1998 (Fig. 1) and sites around LI (145° 28′ E, 14° 41′ S) between January and February 1999 (Fig. 2). GU is a high island with fringing reefs. LI is a continental island on the Great Barrier Reef approximately 35 km off the coast of Queensland, Australia. *Annella mollis* was collected from Blue Hole, Cocos Wall and Hospital Point, GU, between 30 and 40 m. Collections at LI were made at Mac Gillivray's Reef, North Point and Pidgin Point between 10 and 20 m. *A. reticulata* was collected from the wall between the Blue Hole and Crevice and at Hospital Point, GU, between 30 and 40 m, and from Bird Islets, Mac Gillivray's Reef and Pidgin Point, LI, between 10 and 15 m. Voucher specimens were preserved in 70% ethanol or 10% formalin and air-dried depending upon what was available on site. A representative of each species was deposited in the collections at the University of Guam Marine Lab (UOGML), Museum and Art Gallery of the Northern Territories, Darwin, and/or Australian Museum of Natural History (all Australian samples), Sydney.

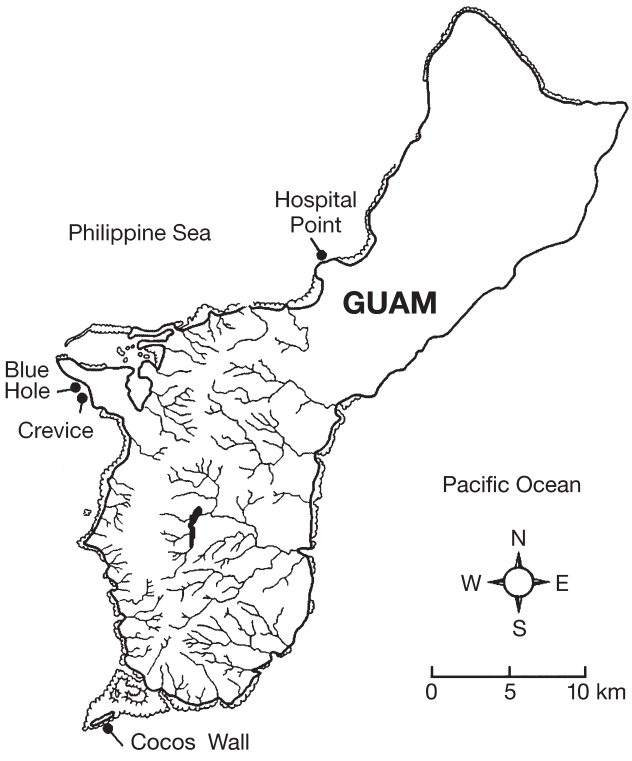

Fig. 1. Collection sites at Guam

Between 2 and 6 individual colonies were removed from each site by cutting at the base of the sea fan with underwater shears. Colonies were separated into base (lowest 10 cm of colony), mid-axis (the center of the colony between the base and tip) and tip (top 5 cm of colony around the outer edges) sections (Harvell & Fenical 1989, Van Alstyne & Paul 1992). When possible, colonies were extracted immediately at the UOGML or LI Research Station (LIRS); otherwise, colonies were frozen at 0°C and freeze-dried for transport to the University of Mississippi, Oxford, or the University of New South Wales, Sydney, for extraction.

Crude extract, gorgonin and sclerite concentrations. A 3 × 3 cm square piece of fresh or freeze-dried animal tissue was weighed and exhaustively extracted in 1:1 dichloromethane/methanol or 1:1 ethanol/ethyl acetate over 72 h. The remaining tissue was dried in an oven for 24 h at 64°C. After determining the dry mass, samples were dissolved in 5.25% sodium hypochlorite (bleach) solution to obtain the sclerites and the gor-

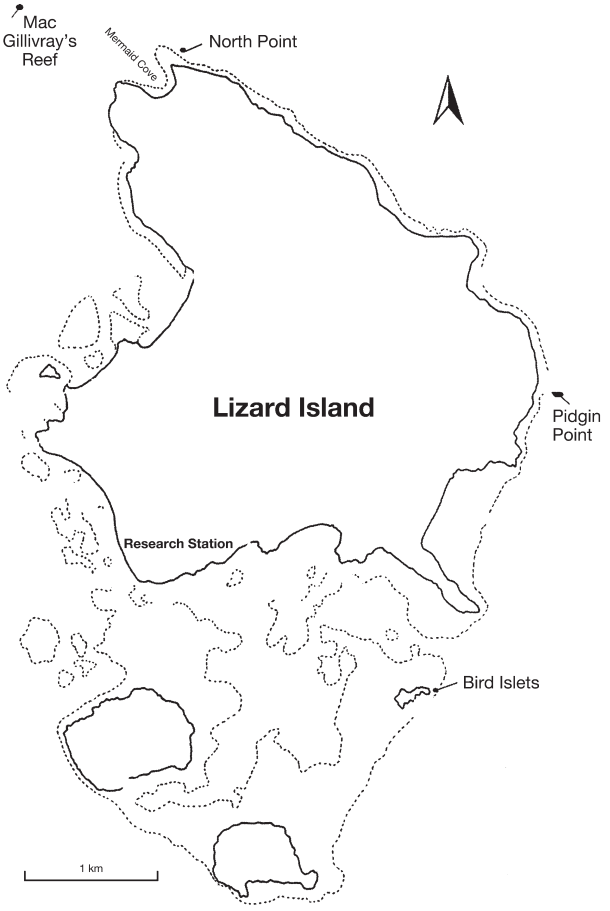

Fig. 2. Collection sites at Lizard Island

gonin skeleton. These were rinsed in fresh water and dried in an oven for 24 h at 64°C. Crude extracts were dried down under reduced pressure (when available) and then weighed. All extracts were stored at 0°C and transported frozen to study sites. The concentration of sclerites was determined as a proportion of the entire colony (dividing by the total dry mass and multiplying by 100) and as a proportion of the soft tissue as described below for the crude extracts.

$$\text{Yield (\%)} = \frac{\text{crude extract mass}}{(\text{dry mass} - \text{gorgonin skeleton mass})} \times 100$$

Data did not meet the requirements for parametric analysis. Therefore, differences in base, mid-axis and tip yields among sites and within individuals were calculated with a 2-way Kruskal-Wallis test (Sokal & Rohlf 1981). The factors were site and part of colony. Differences between the GU and LI populations were calculated by a Mann-Whitney *U*-test. Differences between sites were also calculated by a Mann-Whitney *U*-test with α adjusted for the number of analyses. In addition, the soft tissue concentrations of sclerites and crude extracts were compared by a simple regression to determine if there was a relationship between these variables. All statistical analyses were generated with Statview 5.0 for Macintosh (Abacus Concepts Inc.).

Protein concentration. The protein concentrations of the base, mid-axis and tips were determined by a modified Bradford protein assay (Slattery et al. 1995, Karentz et al. 1997). Approximately 50 mg of tissue was removed from the gorgonin skeleton and digested for 12 h in 5 ml of 1 N NaOH. Five ml of 1 N HCl was added to neutralize the solution. Of each sample 200 µl was diluted with 5 ml of Bio-Rad protein solution and the absorbance (λ = 595 nm) was recorded on a DU-65 Beckman spectrophotometer. Protein concentrations were calculated with a calibration curve generated from standards with bovine serum albumin that were prepared following the same digestion procedure described above. Data did not meet the requirements for parametric analysis even after transformation. Differences in the protein concentration of base, mid-axis and tip among sites and within individuals were calculated with a 2-way Kruskal-Wallis test and post-hoc comparisons with a Mann-Whitney *U*-test as described above (Sokal & Rohlf 1981). Mean protein concentration did not differ significantly among parts (base: 26.43 ± 4.322; mid-axis: 37.30 ± 17.506; tips 32.27 ± 8.608); ($p = 0.0696$) or among sites ($p = 0.6862$) for colonies of *Annella mollis*. There was a significant difference between the mean protein concentration of the base (20.59 ± 1.435) and the mean protein concentration of the mid-axis (27.79 ± 3.557) and tips (27.02 ± 4.909) in colonies of *A. reticulata* ($p = 0.0023$) but none among sites ($p = 0.3323$). The artificial diets were prepared to approximate the mean protein concentration of the mid-axes and tips of the colonies.

Feeding assays. Feeding experiments were conducted at Mermaid Cove, LI, in May 1999 and Western Shoals, GU, in June and July 1999. Enough material was available to test the crude extracts and sclerites from the mid-axes and tips, but not from the bases. Extracts and sclerites from the *Annella mollis* colonies collected from Hospital Point, GU, and Pidgin Point, LI, were selected for the feeding assay studies. We used extracts and sclerites from *A. reticulata* colonies collected at Blue Hole, GU, and Mac Gillivray's Reef, LI. To eliminate possible biases, the sources of the extracts and sclerites were chosen at random without prior knowledge of secondary metabolite composition.

Because we were unable to obtain the same diet at GU and LI we used 2 products that report similar nutritional qualities. In feeding assays conducted at GU, the artificial diet was prepared with 5.0 g Kruses™ Brand

catfish food, 2.5 g carrageenan (Type 1) and 80 ml water. In assays at LI, 5.0 g Atlantic Salmon Starter Crumbles was substituted for the catfish food and 3.75 g of carrageenan was added to adjust the protein concentration. The carrageenan was stirred into a 250 ml beaker containing the water and microwaved on high until the mixture boiled, approximately 2 min. The fish food and extract (dissolved in ethyl acetate) or sclerites were stirred into the beaker after the carrageenan had cooled for approximately 30 s. One ml of ethyl acetate was added to the control cubes for the crude extract assays. Twenty-five 1 × 1 cm food cubes were prepared by pouring the mixture into a partitioned tray. At LI, the mixture was poured into a tray without partitions and 1 × 1 cm cubes were cut with a wire.

A snorkeler or diver offered 1 control cube paired with 1 treatment cube to natural assemblages of fishes on the reef. The first cube that was completely eaten and not regurgitated within 15 s was scored as eaten and the other cube was scored as uneaten. This was usually very clear because the less palatable food cubes accumulated on the reef at the assay site. Fish were randomly offered food prepared with the crude extract from one part of the colony (mid-axis or tips) collected at GU or LI paired with a control. By testing the GU and LI extracts in the same assay, we eliminated any effects (i.e. learned aversion) due to the prior exposure which might occur if the fishes were exposed to one set of extracts before the other. We followed the same procedure for the sclerite assays. Only 2 sets of assays were conducted per day at a site. Data from the feeding assays were analyzed by a chi-square analysis for a 2 × 2 contingency table to compare the number of eaten and uneaten food cubes (Sokal & Rohlf 1981).

An informal survey of the fish assemblages we encountered at Western Shoals, GU, and Mermaid's Cove, LI, suggests that there were considerable feeding guild differences. In feeding assays on GU we encountered schools of scissor-tail sergeant *Abudefduf sexfasciatus*, staghorn damsel *Amblyglyphidodon curacao*, juvenile parrotfishes *Scarus schlegeli* and *S. sordidus*, the occasional wrasses *Cheilinus fasciatus* and *Thalossoma hardwickii*, surgeonfishes *Naso vlamengii*, *N. literatus* and *Acanthurus triostegus*, and the butterflyfish *Chaetodon auriga*. At LI more species were present in fewer numbers during the assays. These included the butterflyfish *C. citrinellis*, *C. lunula*, *C. vagabundus* and *C. unimaculatus*, the angelfish *Pomacentrus imperator* and *Centropyge flavissimus* and the wrasses *Cheilinus fasciatus*, *C. trilobatus*, *Epibulus insidiator*, *Heliochoeres trimaculatus*, *Thalossoma lutescens*, *T. purpurea* and *T. quinquevittatum*. Also present were many species of damselfishes, goatfishes, and parrotfishes.

RESULTS

Crude extract, gorgonin, and sclerite concentrations

The gorgonin concentrations in colonies of *Annella mollis* and *A. reticulata* collected from sites around GU and LI were highest in the bases and decreased significantly ($p < 0.001$) approaching the tips (Table 1). Colonies of *A. mollis* did not exhibit differences in gorgonin concentrations among sites ($p = 0.6019$) or between islands ($p = 0.4729$). For *A. reticulata*, the overall gorgonin concentrations in the colonies collected from LI were significantly higher than from GU ($p = 0.0166$) but there were no significant differences among sites ($p = 0.0618$).

As a proportion of the whole colony dry mass, the sclerite concentrations (Table 1) were lowest in the bases and increased significantly ($p < 0.001$) approaching the tips in colonies of both species. There were no differences among sites ($p = 0.1235$) or between islands ($p = 0.0648$) in colonies of *Annella mollis*. Colonies of *A. reticulata* from GU had higher concentrations of sclerites than colonies from LI ($p = 0.0166$) but there were no significant differences among sites ($p = 0.0618$).

Overall the sclerite and crude extract concentrations in the soft tissue (coenenchyme and polyps) of *Annella mollis* (Table 1a) were significantly higher in colonies collected from GU ($p < 0.001$ and $p = 0.0164$, respectively), but there were no significant differences among parts ($p = 0.2053$ and $p = 0.2813$, respectively). Colonies from Cocos Wall and Hospital Point, GU, had higher sclerite concentrations than the other 4 sites ($p = 0.0011$), and colonies from Hospital Point, GU, and Mac Gillivray's Reef, LI, had lower extract concentrations compared to the other 3 sites ($p < 0.001$). There were no correlations between the soft tissue sclerite and extract concentrations for colonies from GU ($r^2 = 0.008$, $p = 0.2711$), LI ($r^2 = 2.46 \times 10^{-4}$, $p = 0.9288$) or the combined data set ($r^2 = 0.021$, $p = 0.1251$).

Soft tissue sclerite concentrations did not differ significantly among parts of *Annella reticulata* colonies ($p = 0.2254$), collection sites ($p = 0.2144$) or between islands ($p = 0.0783$) (Table 1b). There were significant differences among sites ($p = 0.0013$) in crude extract concentration (Table 1b) but no overall significant difference among parts ($p = 0.0779$) or between the islands ($p = 0.7102$). Extract concentrations were higher at Hospital Point, GU, and Mac Gillivray's Reef, LI compared to the other 3 sites. This species also showed no correlation between the soft tissue sclerite and crude extract concentrations for colonies from GU ($r^2 = 0.016$, $p = 0.5191$), LI ($r^2 = 0.021$, $p = 0.4246$) or the combined data set ($r^2 = 0.001$, $p = 0.7661$).

Table 1. Mean gorgonin, coenenchyme sclerite*, whole animal sclerite** and crude extract concentrations (%) with the standard error for the base, mid-axis and tips of (a) *Annella mollis* and (b) *A. reticulata* collected from Guam (GU) and Lizard Island (LI). n = number of colonies samples. P = part, B = base, M = mid-axis and T = tips

Site	n	P	Gorgonin concentration (%)	Sclerite concentration (%)*	Sclerite concentration (%)**	Crude extract concentration (%)
(a) *Annella mollis*						
GU						
Blue Hole	3	B	83.63 ± 2.67	59.39 ± 9.43	9.97 ± 2.92	12.48 ± 1.51
		M	70.63 ± 4.92	74.48 ± 0.47	21.83 ± 3.52	9.71 ± 0.19
		T	<1	56.40 ± 7.86	56.40 ± 7.86	20.05 ± 5.51
Cocos Wall	5	B	87.59 ± 1.03	66.31 ± 5.45	8.31 ± 1.15	15.59 ± 1.86
		M	74.14 ± 4.79	66.91 ± 8.64	17.38 ± 4.12	16.22 ± 0.79
		T	20.86 ± 3.78	75.49 ± 4.57	59.08 ± 0.78	31.80 ± 5.07
Hospital	3	B	71.40 ± 6.27	86.17 ± 6.89	23.75 ± 6.43	10.81 ± 4.92
Point		M	52.63 ± 12.90	70.09 ± 2.50	33.21 ± 9.08	7.28 ± 0.12
		T	18.04 ± 4.57	70.03 ± 5.66	57.26 ± 5.16	6.71 ± 1.08
LI						
Mac	5	B	85.74 ± 0.64	43.73 ± 5.95	7.11 ± 1.29	4.63 ± 0.41
Gillivray's		M	73.89 ± 3.21	69.52 ± 5.98	20.32 ± 5.44	7.86 ± 3.32
Reef		T	24.78 ± 7.17	72.41 ± 7.42	50.12 ± 3.88	5.23 ± 2.01
North Point	3	B	82.69 ± 1.79	58.45 ± 6.23	10.15 ± 1.46	8.81 ± 1.76
		M	77.96 ± 2.25	59.90 ± 16.11	17.81 ± 8.71	9.35 ± 1.34
		T	26.27 ± 2.51	89.38 ± 0.62	74.3 ± 3.10	15.77 ± 4.22
Pidgin	4	B	78.52 ± 4.55	26.23 ± 4.57	26.23 ± 4.57	11.21 ± 3.25
Point		M	77.56 ± 4.63	57.61 ± 8.38	13.22 ± 2.56	19.43 ± 1.13
		T	22.85 ± 3.91	33.41 ± 12.25	20.46 ± 10.69	13.72 ± 4.10
(b) *Annella reticulata*						
GU						
Blue Hole	5	B	87.52 ± 1.65	73.79 ± 2.96	9.23 ± 1.34	11.03 ± 1.32
		M	70.21 ± 5.22	71.26 ± 2.03	21.06 ± 3.52	10.24 ± 1.59
		T	6.38 ± 1.97	82.68 ± 2.20	78.18 ± 3.39	12.80 ± 1.46
Hospital	5	B	85.16 ± 3.06	88.55 ± 1.61	24.42 ± 3.36	5.70 ± 0.93
Point		M	36.26 ± 6.98	83.91 ± 3.88	61.36 ± 12.95	3.39 ± 0.21
		T	10.91 ± 2.49	71.46 ± 0.43	62.64 ± 8.06	9.4 ± 0.84
LI						
Bird Islets	6	B	85.66 ± 0.64	40.56 ± 2.04	5.53 ± 0.56	6.03 ± 1.09
		M	77.31 ± 4.09	54.86 ± 3.72	3.11 ± 3.30	13.52 ± 2.91
		T	21.04 ± 3.28	64.38 ± 2.20	50.63 ± 1.58	11.43 ± 2.61
Mac	2	B	88.22 ± 0.90	37.63 ± 0.78	4.42 ± 0.24	5.14 ± 0.40
Gillivray's		M	81.29 ± 1.33	51.52 ± 1.96	9.78 ± 1.07	3.67 ± 2.21
Reef		T	36.70 ± 1.52	48.94 ± 6.15	31.13 ± 4.65	8.64 ± 1.92
Pidgin	3	B	84.8 ± 2.51	32.30 ± 0.66	5.11 ± 0.71	10.61 ± 1.04
Point		M	74.94 ± 2.06	53.68 ± 2.93	13.56 ± 1.62	11.87 ± 0.87
		T	42.31 ± 17.52	45.59 ± 1.66	38.40 ± 4.34	14.53 ± 7.91

Feeding assays

The crude extracts from the mid-axes and tips of *Annella mollis* colonies deterred feeding by natural assemblages of fishes at Western Shoals and Mermaid's Cove ($p < 0.001$) with 2 exceptions (Fig. 3a,c). The mid-axis extracts from GU and LI were not unpalatable to fish at Mermaid's Cove ($p = 0.2037$ and $p = 0.4056$, respectively). Sclerites do not appear to serve as structural defenses for this species (Fig. 3b,d).

Crude extracts from the mid-axes and tips of *Annella reticulata* colonies deterred feeding by fish ($p < 0.001$) (Fig. 4a,c), and some evidence suggests that sclerites can deter predators at high concentrations ($p < 0.001$) (Fig. 4b,d). At Western Shoals, the LI mid-axis extract was palatable to reef fish ($p = 0.1524$) (Fig. 4a). Sclerites from the tips of *A. reticulata* from GU deterred feeding at Western Shoals ($p = 0.05$) and Mermaid's Cove ($p < 0.001$) when incorporated into the diet at 78.18% (Fig. 4d). Low concentrations of sclerites from the tips of

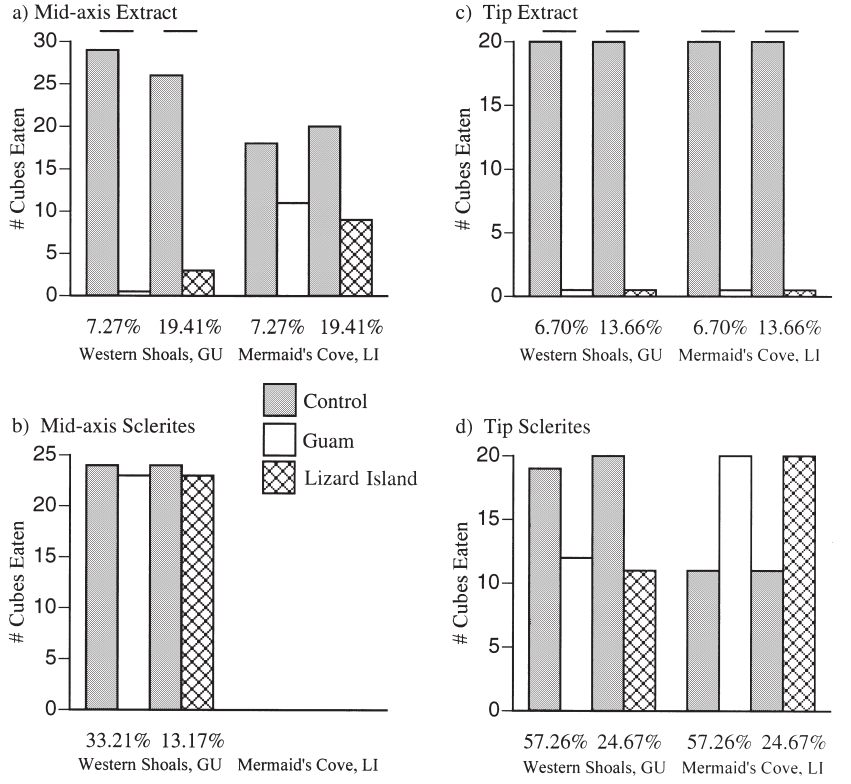

Fig. 3. Feeding assay results for (a) mid-axis crude extracts (n = 29) and (b) sclerites (n = 47), and (c) tip crude extracts (n = 20) and (d) sclerites (n = 31) from *Annella mollis* collected at Pidgin Point, Lizard Island (LI) and Hospital Point, Guam (GU). Crude extract and sclerite concentrations are indicated below each bar. A significant difference ($p < 0.05$) between the palatability of the control and treated cubes is indicated by a line drawn above the 2 corresponding bars. n = number of replicate pairs in each assay

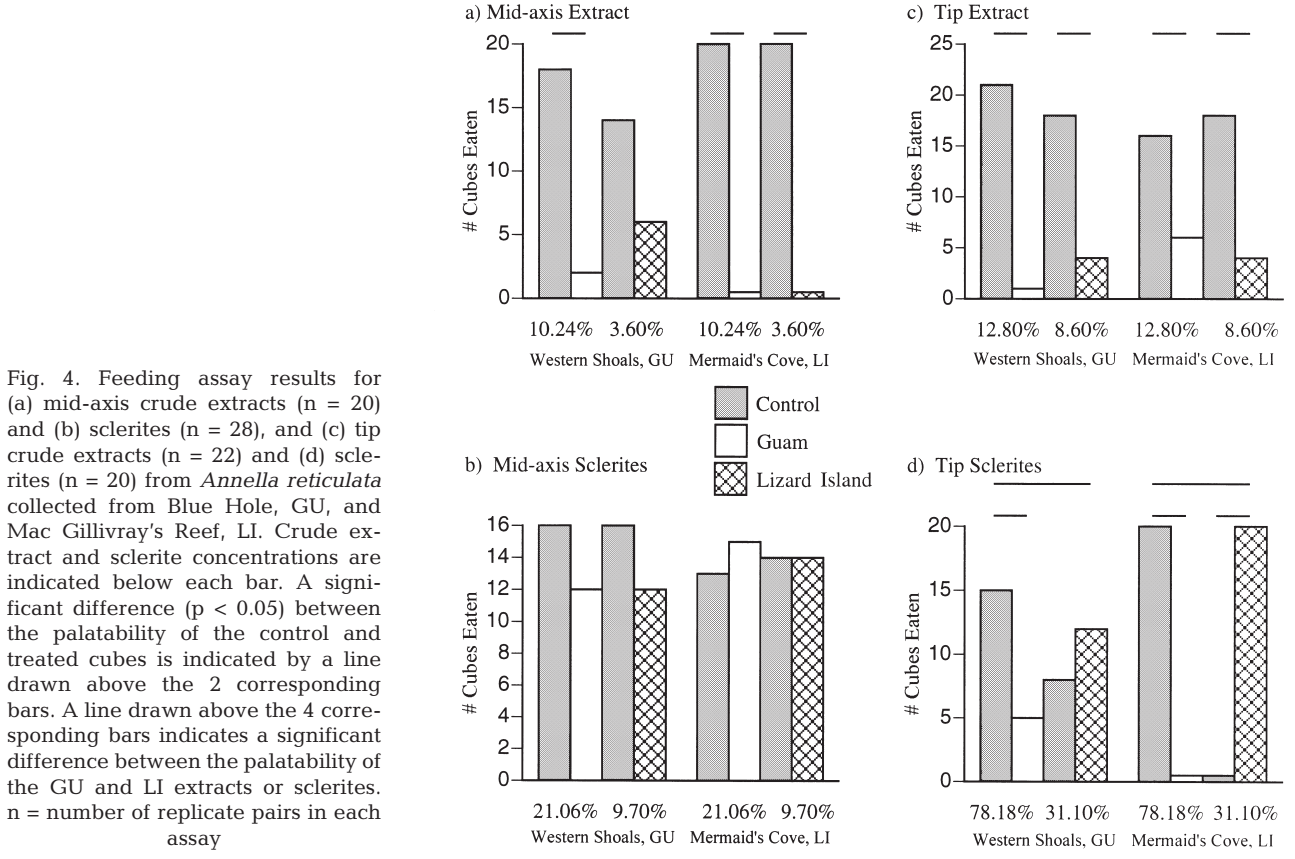

Fig. 4. Feeding assay results for (a) mid-axis crude extracts (n = 20) and (b) sclerites (n = 28), and (c) tip crude extracts (n = 22) and (d) sclerites (n = 20) from *Annella reticulata* collected from Blue Hole, GU, and Mac Gillivray's Reef, LI. Crude extract and sclerite concentrations are indicated below each bar. A significant difference ($p < 0.05$) between the palatability of the control and treated cubes is indicated by a line drawn above the 2 corresponding bars. A line drawn above the 4 corresponding bars indicates a significant difference between the palatability of the GU and LI extracts or sclerites. n = number of replicate pairs in each assay

A. reticulata colonies collected at LI were preferred over the control cubes at Mermaid's Cove ($p < 0.001$).

DISCUSSION

The reciprocal feeding assays conducted in this study clearly show that the crude extracts from *Annella mollis* and *A. reticulata* are unpalatable to natural assemblages of reef fishes at Western Shoals and Mermaid's Cove (Figs. 3 & 4). Sclerites from some Caribbean species have been shown to be unpalatable to fish when incorporated into artificial diets at high concentrations (Harvell et al. 1988, Van Alstyne & Paul 1992, West 1998, Slattery 1999). We observed similar results for sclerites from the tips of *A. reticulata*. These were only effective feeding deterrents at Western Shoals and Mermaid's Cove when assayed at 78.18% (Fig. 4d). In fact, at 31.10%, sclerites from the tips of *A. reticulata* collected at LI were preferred over the control cubes by fish at Mermaid's Cove. While some species, such as *Gorgonia ventalina*, invest in the production of chemical and structural defenses (Van Alstyne & Paul 1992, Slattery 1999), gorgonians in the genus *Annella* specialize in the production of chemical defenses against generalist predators (Harvell & Fenical 1989).

Unlike the tip extracts, the mid-axis extracts from *Annella mollis* and *A. reticulata* did not always deter feeding by natural assemblages of fishes. The mid-axis extracts of *A. mollis* from GU and LI did not deter fish feeding at Mermaid's Cove when assayed at concentrations similar to and higher than the tip extracts. These results suggest that the chemical defenses of *A. mollis* are more concentrated at the tips of the colonies. Similar patterns of intracolony variation have been reported for the Caribbean gorgonians *Pseudopterogorgia rigida* (Harvell et al. 1988, Harvell & Fenical 1989) and *Gorgonia* spp. (Kim et al. 2000). Higher concentrations of predator-deterrent secondary metabolites were found at the tips of *P. rigida* colonies (Harvell et al. 1988, Harvell & Fenical 1989). Crude extracts from the colony edges of *Gorgonia* spp. exhibited greater fungal resistance against the pathogen *Aspergillus sydowii* (Kim et al. 2000). It is not surprising to find the tips of the colonies better defended against generalist predators. Optimal defense theory would predict that chemical defenses would be greatest in new growth. This pattern has been observed for many marine organisms (Paul & Van Alstyne 1988, Harvell & Fenical 1989, Van Alstyne et al. 1994, Becerro et al. 1998, Kim et al. 2000). During vegetative reproduction new polyps are added to the outer colony edges (Szmant-Froelich 1974).

In feeding assays at Mermaid's Cove, the mid-axis extracts of *Annella reticulata* from LI and GU were both unpalatable to reef fishes (Fig. 4a), but at Western Shoals the LI extract did not deter fish feeding. The natural concentration of the mid-axis extract from GU (10.24%) was approximately 3-fold higher than the natural concentration of the LI mid-axis extract (3.60%). The results of these assays suggest that fishes in GU may have a higher tolerance to the mid-axis extracts at low concentrations. Since we used different diets in the feeding assays at Western Shoals and Mermaid's Cove we were not able to make direct comparisons between the results from the feeding assays at the 2 sites.

Differences in fish assemblages between the islands may account for some of the variability seen in the palatability of the mid-axis extracts for both *Annella mollis* and *A. reticulata* (Pavia & Aberg 1996). The Indo-Pacific, Indonesia and the Great Barrier Reef host a greater diversity of coral reef fishes (Thresher 1991). Informal surveys of the fish assemblages we encountered at Wester Shoals and Mermaid's Cove suggest that there were considerable feeding guild differences. On GU we mainly encountered schools of *Abudefduf sexfasciatus*, *Amblyglyphidodon curacao*, juvenile parrotfishes and the occasional wrasses, surgeonfishes and butterflyfishes. At LI more species were present in fewer numbers during the assays, including several species of butterflyfishes, angelfishes, wrasses, damselfishes, goatfishes, and parrotfishes.

Overall, the crude extract concentrations in the *Annella mollis* from GU were significantly higher than in colonies from LI (Table 1a). The natural concentrations of extracts collected at some sites around LI were approximately $^1/_2$ of those in the colonies from GU. And, crude extract concentrations did vary among sites at both islands for colonies of *A. reticulata* (Table 1b). However, palatability of the extracts does not appear to be highly correlated with crude extract concentration.

The *Annella* spp. had fairly consistent sclerite and crude extract concentrations in the soft tissue throughout the colony (Table 1). In this respect, *A. mollis* and *A. reticulata* are similar to the Caribbean gorgonian *Gorgonia ventalina* (Van Alstyne & Paul 1992, Cronin et al. 1995, Slattery 1999) but not the *Pseudopterogorgia* spp. (Harvell & Fenical 1989). Colonies of *Pseudopterogorgia* spp. exhibited an inverse relationship in crude extract and sclerite concentrations, where sclerite concentrations increased from tips to bases. We did not observe any correlation between the crude extract and sclerite concentrations for *A. mollis* or *A. reticulata*. Sclerites in cnidarians and spicules in other phyla, when densely packed, have been shown to increase rigidity and resist excessive deformation and tearing that could result from environmental stresses such as heavy currents (Koehl 1982, Lewis & Von Wallis 1991, West et al. 1993). In the basic architecture of *Annella* spp., the gorgonin skeleton com-

prised up to 90% of the dry mass at the bases but less than 1% of the total dry mass in the tips, while sclerites could comprise more than 70% of the dry mass at the tips (Table 1). A major role of sclerites in *Annella* spp. is probably the support of new tissues at the tips before an internal gorgonin skeleton can be deposited (Szmant-Froelich 1974).

Compared to other areas of chemical ecology, biogeographic comparisons of the predator/herbivore defenses associated with benthic marine organisms are few, mainly addressing differences between tropical and temperate species (Hay 1996, Sammarco 1996). This is the first study to address geographic differences in the palatability of crude extracts and sclerites of gorgonians collected from 2 distant tropical islands in the Pacific Ocean. The feeding experiments clearly demonstrated that *Annella mollis* and *A. reticulata* from GU and LI are chemically rather than structurally defended against natural assemblages of coral reef fishes, thus supporting the hypothesis that extracts from species with small sclerites are usually unpalatable to fish (Pawlik et al. 1987, Harvell & Fenical 1989). With a few exceptions, the crude extracts from the mid-axes and tips of both species collected from GU and LI were unpalatable to fishes at both Western Shoals and Mermaid's Cove. The production of chemical defenses by *Annella* spp. appears to be ubiquitous over a broad range in the Pacific. In other geographic comparisons of predator defenses in octocorals, the production of chemical defenses was reported to be highly conserved (Coll & Sammarco 1988). Harvell et al. (1993) also demonstrated that, while colonies of *Briareum asbestinum* from the Bahamas and St. Croix are both chemically defended against predation (Pawlik et al. 1987), the 2 populations produced different classes of secondary metabolites. Further studies are needed to determine if *A. mollis* and *A. reticulata* from GU and LI produce similar predator-deterrent compounds.

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