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Fungal disease resistance of Caribbean sea fan corals (*Gorgonia* spp.)

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Abstract An on-going, Caribbean-wide epizootic affecting sea fan corals (*Gorgonia* spp.) is caused by the fungus *Aspergillus sydowii* (Thom et Church). We examined the role of crude extracts in resistance of two species of sea fans, *Gorgonia ventalina* (L.) and *G. flabellum* (L.), against *A. sydowii* and a bacterial pathogen of fish, *Listonella anguillarum* (MacDonell et Colwell). Sea fans were collected in January 1997 from San Salvador, Bahamas, and in June 1997 and January 1998 from Alligator Reef, Florida Keys, USA. Crude extracts from both species were tested to determine concentrations inhibiting germination of *A. sydowii* spores. Crude extracts from both species inhibited spore germination at concentrations as low as 1.5 mg ml⁻¹; most samples were active at 5 to 10 mg ml⁻¹. These concentrations are within the range estimated in living tissue and were higher in healthy colonies suggesting their role in mediating disease susceptibility. We also detected within-

colony gradients in antifungal activity, which varied with the disease state of the colony. In healthy sea fans, resistance was highest at colony edges and lowest in medial and central regions of the colony. Among sea fans with lesions in the colony center, resistance in tissue from proximal and medial regions was as high as tissue from the colony edge (i.e. distal region). The increase in antifungal activity suggests an inducible response by the coral host to the fungal pathogen. This response is most evident among sea fans with lesions in the colony center and not among colonies with lesions at the edge. Antibacterial activity of crude extracts against *L. anguillarum* was highest at the colony edge but did not vary with disease state or tissue location.

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Introduction

Natural populations can be vulnerable to the disruptions caused by the introduction of pathogens (Mangel and Tier 1994; Garnett and Holmes 1996; Gilbert and Hubbell 1996; Real 1996). Although the importance of pathogens in terrestrial systems has been recognized for some time, the role of diseases in marine communities has received far less attention (Peters 1993). This is surprising given recent cases of marine pathogens effecting dramatic shifts in community structure by the near elimination of species such as sea stars (Dungan et al. 1982), sea urchins (Miller and Colodey 1983; Lessios et al. 1984; Scheibling and Stephenson 1984; Lessios 1988), corals (Guzmán and Cortés 1984; Garzón-Ferreira and Zea 1992; Aronson and Precht 1997; Bruckner and Bruckner 1997), sea grasses (Rasmussen 1977), and coralline algae (Littler and Littler 1995). Even when accounting for the increase in awareness, the incidence and the impact of disease among tropical marine organisms appears to be on the rise (Epstein et al. 1998; Goreau et al. 1998; Harvell et al. 1999). And yet, there is insufficient information about both mechanisms of resistance to disease (Harvell et al. 1999) and the identities of many pathogens (Richardson 1998) to

evaluate whether the increase is due to compromised resistance and subsequent opportunistic infections or to the emergence of new pathogens (Levins et al. 1994).

An on-going, Caribbean-wide epizootic of sea fan corals (*Gorgonia ventalina* and *G. flabellum*) (Fig. 1; Nagelkerken et al. 1997, 1998) provides an unprecedented opportunity to study the dynamics of an emergent disease and the mechanisms of host resistance. This epizootic is caused by the fungus *Aspergillus sydowii* (Smith et al. 1996; Geiser et al. 1998), which appears to be an emergent pathogen because it is terrigenous and unable to complete its life cycle in the ocean (Smith unpublished data). Given that many aspergilli are notoriously opportunistic pathogens of stressed and immune-compromised hosts wherever they occur (Dixon and Walsh 1992; Moore-Landecker 1996), the current epizootic may have been facilitated by environmental stressors, such as elevated water temperature, which can compromise resistance in the corals.

In the Florida Keys and Bahamas, we have observed apparently "resistant" individuals and low incidences of whole colony mortality at some sites (Nagelkerken et al. 1998; Kim, Quirolo, and Harvell unpublished data). These observations suggest that the infection is self-limiting (i.e. the pathogen senesces or loses pathogenicity

while in the host) or that the sea fans possess some measure of resistance. One mechanism of disease resistance among gorgonian corals could involve the many secondary compounds they possess. The gorgonian corals, and octocorals in general, are a rich source of biologically active compounds which play a defensive role against predators (e.g. Pawlik et al. 1987; Sammarco et al. 1987; Harvell et al. 1988, 1996; Harvell and Fenical 1989; Pawlik and Fenical 1989; Fenical and Pawlik 1991; Paul 1992; Van Alstyne and Paul 1992; Van Alstyne et al. 1992), competitors (Sammarco et al. 1983; Rittschoff et al. 1985; La Barre et al. 1986), and microbes and fouling organisms (Targett et al. 1983; Bandurraga and Fenical 1984; Gerhart et al. 1988; Ciereszko and Guillard 1989; Kim 1994; Slattery et al. 1995; Jensen et al. 1996). Although secondary compounds are effective against a range of marine and non-marine microbes, none of the microbes previously tested are known to be pathogenic to corals.

Studies of coral diseases have been problematic due in large part to the difficulties in isolating, identifying, and culturing marine microbes. Thus, the recent identification of *Aspergillus sydowii* as a pathogen of sea fan corals provides an opportunity for examining the role of secondary compounds in disease resistance. In this study, we tested the hypotheses that: (1) sea fans possess antifungal compounds, (2) there are within-colony gradients in antifungal activity, (3) efficacies and patterns in the antifungal activity vary according to whether the host is diseased or healthy, and (4) antifungal and antibacterial activities are correlated.

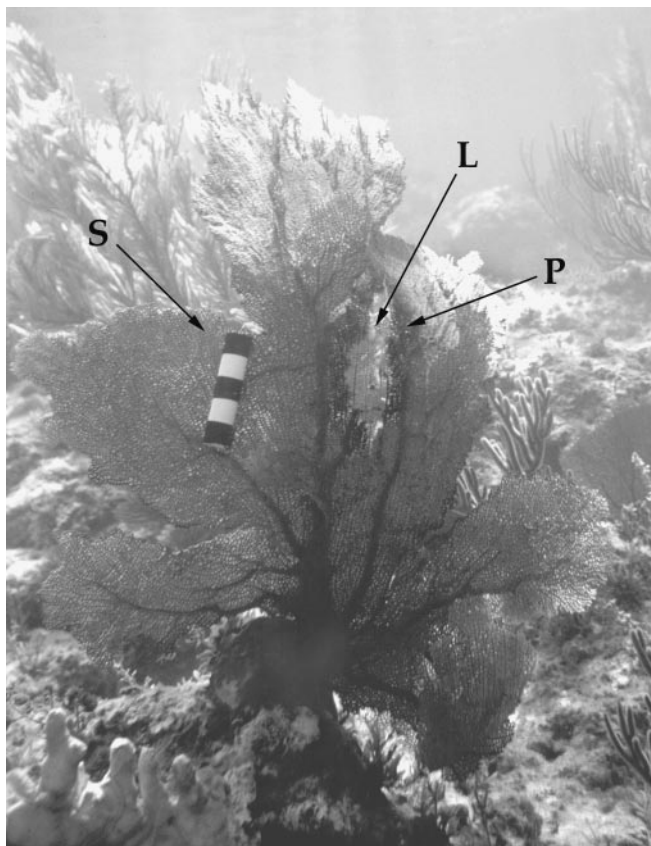


Fig. 1 *Gorgonia ventalina*. An *Aspergillus sydowii*-infected sea fan. The lesion (L) is surrounded by a halo of dark purple (P) coral tissue. Photograph taken on Rocky Point Reef (depth = 1.5 m), San Salvador, Bahamas. Scale bar (S) length from edge to edge = 10 cm

Materials and methods

Sample collection

Species comparison

Samples used for comparing differences in the antimicrobial (i.e. antifungal and antibacterial) properties of *Gorgonia ventalina* (L.) and *G. flabellum* (L.) were collected (January 1997) from three locations in San Salvador, Bahamas: Rice Bay, Linsey Reef, and Rocky Point; these areas are characterized by near-shore, shallow (< 3 m depth) patch-reefs dominated by the two sea fan species. From each reef, small fragments (5 × 5 cm) were collected from the edges of ten healthy colonies (i.e. no outward signs of infection) of both species.

Disease state and sample location

To examine whether within-colony variation in concentrations of predator deterrent compounds (Harvell and Fenical 1989; Van Alstyne and Paul 1992) were correlated with antifungal activity in the crude extracts, a set of samples, referred to as "center-to-edge," was collected from *Gorgonia ventalina* colonies on Alligator Reef near Long Key, Florida Keys, USA (June 1997). From 19 diseased and 15 healthy colonies, three tissue samples (2 × 5 cm) were collected: proximal (center of the colony), medial (3 to 5 cm away from the proximal), and distal (3 to 5 cm away from the medial and including the colony edge) (see Fig. 4A). In the diseased sea fans with lesions at the colony center, diseased proximal samples were therefore adjacent to the lesions. After finding elevated levels of

antifungal activity in these tissues, we collected "edge-to-edge" samples from sea fans with lesions on the colony edge to further evaluate the hypothesis of a localized antifungal response (Alligator Reef, January 1998; $n = 24$ healthy, 24 diseased; see Fig. 4B).

Antimicrobial assays

Samples collected for microbial assays were frozen shortly after collection and transported frozen on dry ice, then extracted in dichloromethane (DCM). DCM, in comparison to ethanol alone or DCM:ethanol (50:50 by volume), was an effective solvent in extracting antifungal activity (Kim et al. in preparation). Samples stored dry or in DCM did not lose activity over 2 months (Kim et al. in preparation). Samples were extracted in DCM for 24 h, transferred to a pre-weighed scintillation vial, and dried under a continuous flow of N_2 . Crude extract was weighed, then reconstituted in acetone to a concentration of 200 mg of extract ml^{-1} of solvent. Acetone was used as the carrier for the extracts because it is miscible in the fungal broth (unlike DCM) and less toxic to the fungus than ethanol.

Antifungal properties of crude extracts were tested against the fungal pathogen using minimum inhibitory concentration (MIC) assays. These assays are commonly used to examine the efficacy of drugs against filamentous fungi such as *Aspergillus* spp. by determining the lowest concentration at which hyphal growth or germination of fungal spores is inhibited (Rex et al. 1993); thus, a lower MIC is indicative of greater antifungal activity. In general, hyphal and spore MIC assays are well correlated (Guarro et al. 1997).

MIC assays were carried out in 96-microwell plates by exposing the pathogen to crude extract concentrations ranging from 1.5 to 24 mg ml^{-1} . For these assays, we used spores produced by *Aspergillus sydowii* (Thom et Church) isolated from diseased sea fans collected in San Salvador (Smith et al. 1996; Geiser et al. 1998). To each well on the plate, 75 μ l of sterile fungal broth and 25 μ l of spore suspension were added so that 20 000 to 25 000 spores were present in each well. Sea fan extracts were added to this suspension to make final concentrations ranging from 1.5 to 24 mg ml^{-1} . On each plate, the following controls were included: acetone only, spores without acetone or extract, and a known antifungal agent (hygromycin, 1 to 16 mg ml^{-1}). These controls were used to test the effects of using acetone as the carrier for the extract, to establish spore viability, and to compare to a known antifungal compound. The plates were incubated at 25 °C for 72 h, then examined for hyphal growth using a dissecting microscope. The absence of hyphal growth was taken as evidence of antifungal activity, and the lowest extract concentration inhibiting hyphal growth was determined as the MIC for that coral sample.

We also tested for antibacterial activity of the crude extracts from *Gorgonia ventalina* to investigate the relationship between antifungal and antibacterial activities. Extracts used in this assay were the same as those in the antifungal assays. We performed disk diffusion assays (see Kim 1994; Jensen et al. 1996) to determine the efficacy of the extract against the marine bacterium *Listonella* (= *Vibrio*) *anguillarum* (ATCC No. 19264). First, 1 mg of extract was loaded onto a sterile filter paper disk (6 mm in diameter), allowed to air dry for 1 h to remove traces of the carrier solvent (acetone), then placed on a newly spread lawn of bacteria. On each replicate plate, one disk treated only with acetone and another treated with tetracycline (30 μ g) were included as controls along with six disks treated with coral extracts. After incubation at 30 °C for 72 h, zones of inhibition (i.e. the distance from the edge of the filter paper disk to the growing edge of the bacteria) were measured to the nearest 0.5 mm using calipers. To correct for variation among plates that may have resulted from differences in the sizes of the bacterial inoculum during plating, zones of inhibition were normalized against the zone of inhibition produced by the tetracycline standard within each plate.

Sea fan composition

To determine the natural concentrations of crude extracts in sea fans, extract content in the tissue was quantified. For this analysis, we examined samples used from species comparison ($n = 1$ sample

per colony \times 10 colonies \times 3 reefs \times 2 species) and samples from the experiment comparing antifungal activity between disease states and among locations (center-to-edge samples only, 3 samples per colony \times 10 colonies \times 2 disease states). All coral samples, which had been extracted in DCM, were air dried for 48 h, weighed (accuracy ± 0.1 mg), and treated with a solution of hypochlorite and water (1:1 by volume) for ~ 40 min. This treatment dissolves the coral tissue but leaves the skeletal elements (sclerites and axial material) unaffected. The remaining skeletal elements were washed with distilled water (3 \times) and 100% ethanol (1 \times), air dried overnight, and weighed. Tissue content was taken as the whole sample mass minus the skeletal elements and crude extracts.

Statistical analyses

Because the MIC assays were carried out at set concentrations, the data were ordinal; therefore non-parametric tests were used (Siegel 1956). To test the hypothesis that there are natural within-colony gradients in antifungal activity, two separate Friedman tests were employed, one for each of the disease states (healthy or diseased). To test if antifungal activity varied as a function of disease state and tissue location within a colony, the Friedman tests were followed by pair-wise Wilcoxon signed rank comparisons among locations. Where appropriate, sequential Bonferroni corrections, which are more sensitive to false positives than the standard Bonferroni technique, were applied (see Rice 1989).

The *Listonella anguillarum* disk diffusion assay data were also analyzed using a repeated measures ANOVA. The repeated measures model is appropriate because each colony served as the source for all three of the tissue samples (proximal, medial, and distal; Fig. 4A). This analysis is analogous to a two-factor ANOVA (disease state \times location, both fixed) without replication. The data were first square-root transformed to equalize variances among all subgroups (disease state \times tissue location) (Bartlett's tests, $F = 0.5548$, $df = 5$, $p = 0.7348$). Spearman rank correlations (sequential Bonferroni corrected) were used to examine the relationship between antibacterial and antifungal activity.

Comparison of extract contents between *Gorgonia ventalina* and *G. flabellum* was carried out using a mixed model, two-factor ANOVA [species (fixed) \times reef (random)] on arcsine-transformed data (Bartlett's test of homogeneity of variance among all subgroups, $F = 1.608$, $p = 0.1540$). The effect of sample location on extract content was examined using a repeated measures ANOVA [disease state (fixed) \times sample location (fixed); Bartlett's test, $F = 0.461$, $p = 0.8055$).

Results

Species comparison

The crude extracts of both *Gorgonia ventalina* and *G. flabellum* possessed similar levels of antifungal activity, as indicated by the minimum inhibitory concentration (MIC) assays, with mean MICs ranging from 5.5 to 9.0 mg ml^{-1} (Fig. 2A). These two species did not differ in the quantities of crude extracts in their tissue (Fig. 2B; two-way ANOVA on arcsine-transformed data, $F_{1,2} = 5.90$, $p = 0.136$) although there was site-to-site variation ($F_{1,2} = 4.49$, $p = 0.016$; species \times site interaction, $F_{2,54} = 0.917$, $p = 0.4057$). Efficacy, determined as the percentage of colonies tested the extracts of which inhibited spore germination, was strongly concentration dependent (Fig. 3). At a concentration of 1.5 mg ml^{-1} , only one colony ($n = 60$) inhibited spore germination. Efficacy increased dramatically over a

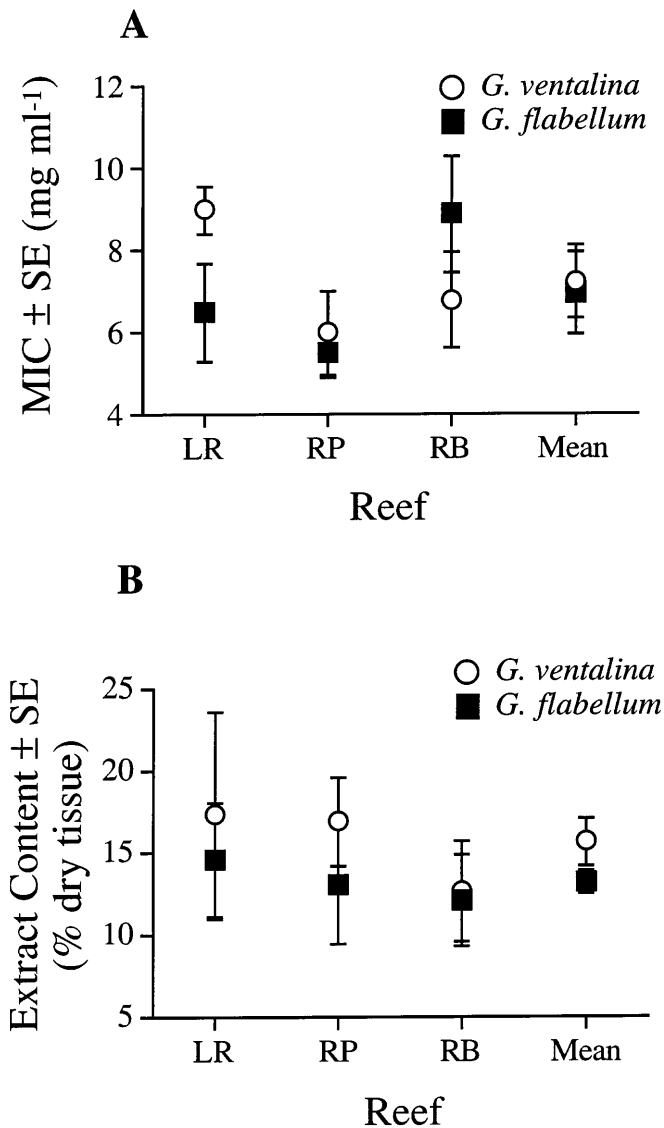


Fig. 2A,B *Gorgonia ventalina*, *G. flabellum*. Antifungal activities and crude extract contents. Samples ($n = 10$ colonies per species) were collected from each of three reefs located in San Salvador, Bahamas: Linsey Reef (LR), Rocky Point (RP), and Rice Bay (RB). **A** Antifungal activities given as MICs (i.e. minimum extract concentration inhibiting *Aspergillus sydowii* spore germination) \pm SE. Extracts with low MICs have high antifungal activities. **B** Extract content expressed as a percentage of dry tissue weight

narrow range of extract concentrations (5 to 10 mg ml⁻¹), and at concentrations > 15 mg ml⁻¹, all colonies tested inhibited spore germination.

Disease state and within-colony comparisons

The level of antifungal activity of extracts was dependent on both disease state of the colony and location within the colony. This was apparent in samples collected center-to-edge (Fig. 4A). There was significant within-colony variation in antifungal activity in both healthy (Friedman $X^2 = 17.03$, $df = 2$, $p < 0.001$) and

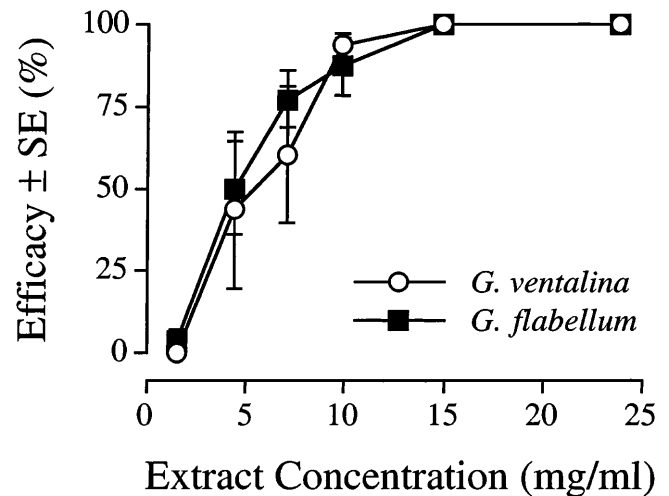


Fig. 3 *Gorgonia ventalina*, *G. flabellum*. Efficacy of crude extracts determined as the proportion of colonies tested showing antifungal activity at a given concentration. For each of three reefs (as listed in Fig. 2), ten colonies each of both species were assayed at six different concentrations. Data were averaged across reefs to give species means

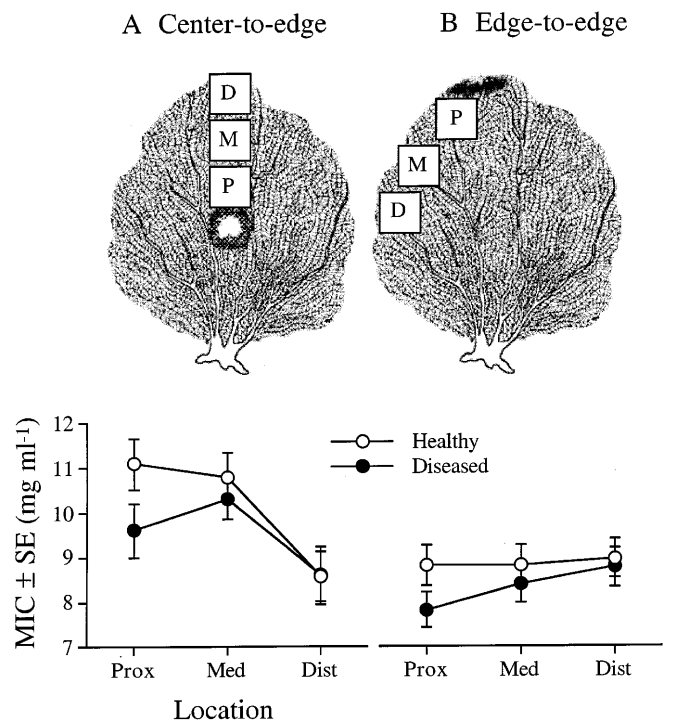


Fig. 4A,B *Gorgonia ventalina*. Antifungal activity of crude extracts from healthy and diseased colonies. Results are given for two sets of sampling schemes: **A** center-to-edge ($n = 19$ diseased, 15 healthy) and **B** edge-to-edge ($n = 24$ diseased, 24 healthy). Extracts with low MICs have high antifungal activities (P, Prox proximal; M, Med medial; D, Dist distal). In diseased colonies, proximal samples were taken adjacent to lesions

diseased colonies ($X^2 = 6.37$, $df = 2$, $p = 0.041$). In healthy colonies, antifungal activity tended to increase distally: pair-wise comparisons indicated that activity was higher in distal (edge) samples than either medial or

proximal (center) samples (Table 1-I). In diseased colonies, antifungal activity did not increase distally; in fact, antifungal activity in tissue proximal to a lesion was as high as in tissue at the colony edge (Table 1-III). The Bonferroni-corrected Wilcoxon rank test indicated that antifungal activity in medial tissue was similar to the colony edge; however, this is contrary to the results of the Friedman test (see above), suggesting the possibility of a Type II error resulting from the use of the more conservative ($\alpha = 0.0167$) Bonferroni correction. Comparisons between diseased and healthy colonies for each of the tissue locations indicated no significant differences in antifungal activity (Table 1). Antifungal activities of edge-to-edge samples were less variable. All edge-to-edge samples were similar in activity level regardless of location: healthy, Friedman $X^2 = 0.255$, $df = 2$, $p = 0.881$; diseased, $X^2 = 0.154$, $df = 2$, $p = 0.926$. Compositional determination of center-to-edge samples showed significant variation in crude extract content as a function of disease state and within-colony location (Fig. 5; Table 2). In general, crude extract content increased distally, but diseased colonies also had lower extract content than healthy colonies.

Relationship between antifungal and antibacterial activity

Extracts from *Gorgonia ventalina* at concentrations of 1 mg ml^{-1} inhibited growth of the marine bacterium *Listonella anguillarum* (Fig. 6). Zones of inhibition resulting from 1 mg of extract ranged from 0.5 to 13 mm (median = 3.1). In comparison, positive controls of $30 \mu\text{g}$ of tetracycline produced 10 to 12 mm zones of inhibition (median = 11). Patterns of antibacterial activity between disease states and among locations

Table 1 *Gorgonia ventalina*. Pair-wise comparisons of antifungal activity among samples taken proximal, medial, and distal to infection (corresponding to Fig. 4A). Within-colony comparisons were made using Wilcoxon paired signed rank tests and between-colony tests were made using Mann-Whitney *U*-tests. For each set of comparisons, * indicates significance at α based on sequential Bonferroni correction for multiple tests

Comparisons	Z-values	<i>p</i>
I. Within-colony tests: healthy colonies ($n = 15$; table-wide $\alpha = 0.0250$)		
Proximal vs Medial	-1.414	0.1573
Proximal vs Distal	-2.810	0.0050*
Medial vs Distal	-2.810	0.0050*
II. Within-colony tests: diseased colonies ($n = 19$; table-wide $\alpha = 0.0167$)		
Proximal vs Medial	-1.579	0.1144
Proximal vs Distal	-1.435	0.1514
Medial vs Distal	-2.269	0.0232
III. Between-colony tests (table-wide $\alpha = 0.0167$)		
Diseased proximal vs Healthy proximal	2.020	0.0433
Diseased medial vs Healthy medial	-0.263	0.7924
Diseased distal vs Healthy distal	-1.571	0.1161

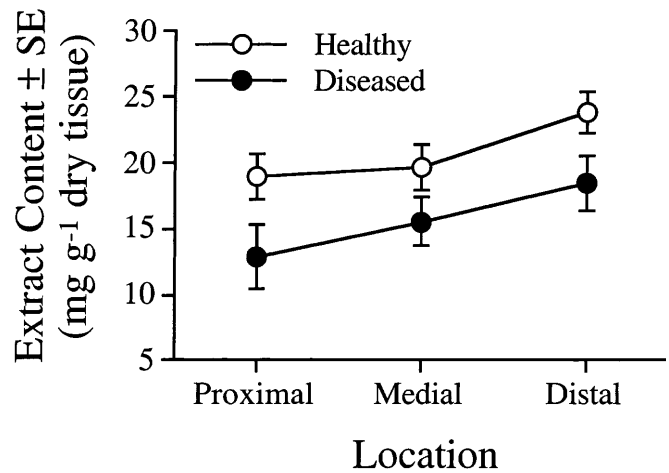


Fig. 5 *Gorgonia ventalina*. Crude extract content as a function of disease state and sample location ($n = 10$ diseased, 10 healthy)

Table 2 *Gorgonia ventalina*. Results of repeated measures ANOVAs comparing extract content (mg g^{-1} dry tissue) between diseased and healthy colonies ($n = 10$ each), and among within-colony locations (i.e. repeats: proximal, medial, and distal)

Source	<i>df</i>	MS	<i>F</i>	<i>p</i>	
State	1	405.4440	5.2688	0.0339	
Subject	18	76.9518	4.2339	0.0001	
Repeat	2	140.5090	7.7307	0.0016	
State \times Repeat	2	5.2787	0.2904	0.7497	
Error	36	18.1754			
Bonferroni post hoc tests					
			Difference	<i>p</i>	
			Dist-Med	3.52450	0.0384
			Dist-Prox	5.19150	0.0014
			Med-Prox	1.66700	0.5332

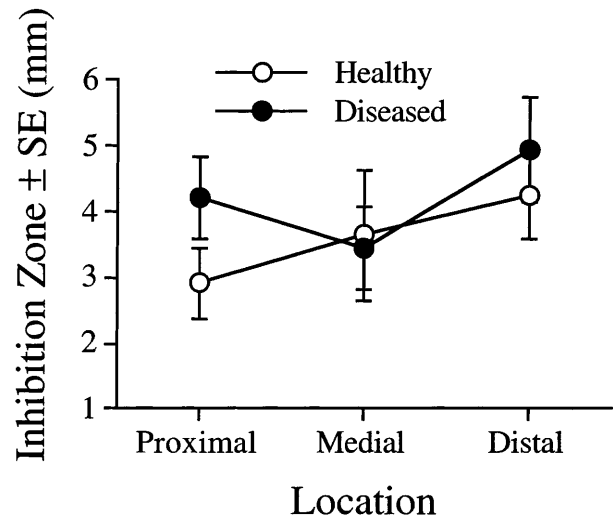


Fig. 6 *Gorgonia ventalina*. Antibacterial activity of crude extracts from center-to-edge samples reported as zones of inhibition (mean \pm SE) produced by 1 mg of extract against the marine bacterium *Listonella anguillarum* (cf. Fig. 4A)

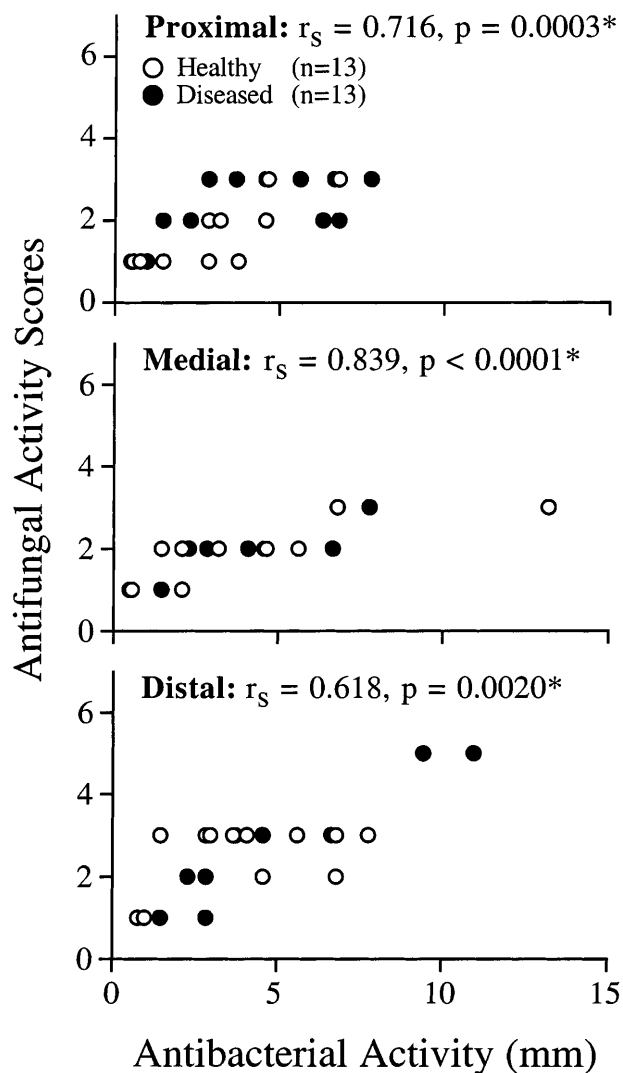


Fig. 7 *Gorgonia ventalina*. Relationship between antifungal and antibacterial activity as a function of colony disease state and tissue location within colony. Data for healthy and diseased colonies were combined within locations and analyzed using Spearman rank correlations (r_s). Antibacterial activity is given as the zone of inhibition (mm) produced by 1 mg of crude extract. Activity scores are based on MIC assays, where lower MICs are given higher activity scores. * indicates $p \leq 0.017$ (α based on sequential Bonferroni correction for multiple tests)

were similar to those observed for antifungal activity (cf. Fig. 4A). Repeated measures ANOVA on square-root-transformed data indicated that antibacterial activity did not differ between healthy and diseased individuals ($F_{1,24} = 0.727$, $p = 0.402$) but did vary according to location ($F_{2,48} = 4.9916$, $p = 0.0107$; interaction between disease state and location was not significant, $F_{2,48} = 1.1971$, $p = 0.3109$). Post hoc tests showed that extracts from distal regions were more active than those from both medial and proximal regions (Bonferroni contrasts: Distal–Medial, $p = 0.0151$; Distal–Proximal, $p = 0.0494$). There was a significant positive relationship between antifungal and antibacterial activity (Fig. 7).

Discussion and conclusions

The role of coral chemical defenses in resistance to potential pathogens has been evaluated only for bacteria, none of which were known to be pathogenic to corals (e.g. Kim 1994; Jensen et al. 1996; Koh 1997; and references therein). That fungi are potential pathogens of corals has been noted previously (Ramos-Flores 1983; Raghukumar and Raghukumar 1991; Carlton and Richardson 1995; Le Campion-Alsumard et al. 1995); however, it is only with the discovery of *Aspergillus sydowii* as a fungal pathogen of sea fans that we are able to examine the role of chemical defenses in disease resistance. This is the first attempt to examine resistance to a known fungal pathogen and to examine both antifungal and antibacterial responses together. Our study demonstrates that sea fans (*Gorgonia ventalina* and *G. flabellum*) possess chemical resistance against *A. sydowii*.

Crude extracts from both species of sea fans are active against *Aspergillus sydowii* spores at concentrations of 1.5 to 15 mg ml⁻¹ (Fig. 2). What do these MICs mean in situ? To understand the ecological relevance of our in vitro assays, we estimated the natural concentration of these compounds and began an assessment of how they are distributed within the organism. For instance, invading *A. sydowii* will experience different concentrations of the antifungal compounds depending on the disease state and location within the colony (Figs. 4, 5). Furthermore, efficacy of the crude extracts will depend on whether the active compounds are localized on the colony surface or distributed throughout the tissue. Because determining the exact distribution of these compounds in the coral tissue is difficult, in situ concentration has been estimated by normalizing extract weight by sample weight or volume (e.g. Van Alstyne and Paul 1992; Kim 1994; Pawlik et al. 1995; Chanas et al. 1996). Tissue volume was approximated by assuming that coral tissue is ~90% water (range = 70 to 90% for all living tissue; cf. Riccardi and Bourget 1998) and that specific gravity of sea water is ~1 g ml⁻¹; thus, 1 g of dry tissue has a living volume of 10 ml. Therefore, extract contents of 80 to 250 mg g⁻¹ of tissue (range, $n = 60$) represent natural concentrations of 8 to 25 mg ml⁻¹. This range of “in tissue” concentration approximates an effective range of 1.5 to 15 mg ml⁻¹ determined in MIC assays. This suggests that sea fans possess antifungal compounds and that these compounds occur at concentrations capable of inhibiting a fungal pathogen in nature. The difference in extract concentrations between diseased and healthy sea fan corals (Fig. 5) suggests a role for chemistry in mediating susceptibility to *A. sydowii*.

There also appear to be qualitative differences in the potencies of crude extracts. Although the crude extracts were assayed at standardized concentrations, in healthy colonies of *Gorgonia ventalina* antifungal activity increases distally from the colony center (Fig. 4) in a pattern similar to deterrence against predators (Harvell and Fenical 1989; Van Alstyne and Paul 1992). Thus, the

colony edge appears to be well defended against fungal infections by extracts which not only occur in higher concentrations, but are more potent (i.e. active at lower concentrations) than those near the colony middle. However, the center-to-edge activity gradient is not apparent among diseased colonies with lesions in the colony center. Instead, all areas appear to be equally active against the fungus (Fig. 4A; Table 1-II) resulting from an increase in potency, most notably in tissue near the infection site, rather than from a decrease at the colony edge (Table 1-III). Given that we did not observe a concomitant increase in crude extract concentration near the lesion (Fig. 5), infected sea fans appear to respond with increases in antifungal compounds rather than with increases in the production of all biologically active compounds. It should be noted, however, that the apparent increase in antifungal activity proximal to lesions is not statistically distinguishable from the same region in healthy colonies (Table 1-III). Nonetheless, it is clear that in diseased sea fans with lesions in the colony center, antifungal activity of tissue near that lesion is elevated relative to the colony edge. Although more studies are needed, the results of this study support the hypothesis that sea fans are capable of a chemically mediated, inducible response against *Aspergillus sydowii*. Our work also shows that the chemical response is limited, as it is not evident when infections occur on the colony edge (Fig. 4B). Antifungal activity is already the highest at the colony edges, and additional increases may not be possible due to autotoxicity or other physiological constraints.

Results of the antibacterial assays suggest that antifungal and antibacterial activities are correlated (Fig. 6). This finding was unexpected because of the fundamental biochemical differences between bacteria and fungi. However, it is plausible that the correlated activity resulted from a non-specific antimicrobial response, which includes the production of both antifungal and antibacterial compounds regardless of the nature of the invading agent. Because there are no prior studies of disease resistance in corals, the most useful model in developing hypotheses about how corals respond to pathogens is that of the plant-pathogen interaction. In plants, inducible defenses against pathogens are common and involve an impressive battery of compounds (Hammerschmidt and Kuc 1995; for marine algae, Cronin and Hay 1996; Hammerstrom et al. 1998; for terrestrial plants, Karban and Baldwin 1997). Challenge by a pathogen, particularly a fungal pathogen, results in structural changes (e.g. lignification or the formation of a cork layer) and a series of biochemical events known as the hypersensitive response. In the hypersensitive response, infected cells quickly die, and this, as well as the production of chemical defenses, limits spread of the pathogen. The diverse defensive chemicals from terrestrial plants include a group of low-molecular-weight antimicrobial compounds known as phytoalexins and a number of oxidants, lytic enzymes, and other antimicrobial proteins such as chitinases, glucanases,

proteases, and ribonucleases (Lamb et al. 1989; Kuc 1995; Lindsay et al. 1995). Typically, these compounds, having both antifungal and antibacterial properties, are induced as part of a general antimicrobial response. In contrast, there is a paucity of studies on the possible roles of secondary chemistry of corals in inhibiting pathogens. To our knowledge, there is no information on antifungal compounds from corals; antifungal compounds have been isolated from deep water sponges; these compounds include terpenes (Killday et al. 1993; Shigemori et al. 1994), alkaloids (Honma et al. 1995), and peptides (Kernan and Faulkner 1987; Kernan et al. 1988). Among plants, induced resistance is often localized at the infection site (Järemo et al. 1999), although systemic resistance, where the whole plant is protected against subsequent infections, can also occur (Kuc 1982; De Wit 1985; Sticher et al. 1997). In terrestrial plants, systemic resistance occurs as early as 7 h post-inoculation or as late as 2 to 3 weeks. In the marine alga *Dictyota menstrualis*, chemical defenses were locally induced by amphipod grazers and were maintained for at least several days, making subsequent attacks less likely (Cronin and Hay 1996).

To understand the role of diseases in the marine environment, it is important to characterize the mechanisms of host resistance. Only then can we begin to evaluate whether recent increases in disease frequency and impact have resulted from the emergence of novel pathogens, or lowered resistance of hosts due to environmental degradation, or a combination of the two. There is evidence that diseases are more common in stressed corals. Incidence of coral diseases appears to be sensitive to water temperature (Antonius 1981; Rützler et al. 1983; Feingold 1989) and other environmental stressors (Peters 1984; Glynn et al. 1989; Porter and Meier 1992), suggesting that pathogens only emerge when corals are stressed and unable to mount effective defenses. Our finding of antifungal activity among sea fans is the first evidence for chemical resistance to a pathogen in corals; however, it is also important to confirm the inducibility of this response. This has important implications for studies of the role of secondary compounds in coral-microbe interactions. For instance, assessing the susceptibility of a population to a potential pathogen by measuring natural levels of defense chemicals may not be appropriate if disease resistance is inducible. Conversely, examining only pathogen-impacted populations is also likely to result in an inaccurate evaluation of the role of chemistry in disease resistance because we do not know the history and the dynamics of the host-pathogen interaction. For example, if susceptibility is mediated by secondary compounds, diseased colonies may be those with lower natural levels of secondary compounds; however, once infected, an induced response may minimize differences in levels of defensive chemicals between diseased and healthy individuals. Or, if susceptibility is mediated by the rate at which chemical resistance is expressed, differences in antifungal activity between diseased and healthy

colonies are likely to be temporary and thus difficult to detect without knowing the timing of the infection process. In this regard, the sea fan–fungus system should provide a tractable model system for directly examining changes in antifungal activity following infection, and more generally, addressing a critical gap in our knowledge of factors affecting disease dynamics in marine systems.

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