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RESEARCH ARTICLE

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# Host range and resistance to aspergillosis in three sea fan species from the Yucatan

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**Abstract** Knowledge of host range and mechanisms of disease resistance is fundamental to predicting impacts and spread of marine diseases. Prevalence of signs of aspergillosis, caused by the terrestrial fungus *Aspergillus sydowii*, was assessed in the Yucatan among three species of sea fan: *Gorgonia ventalina*, *G. cf. mariae*, and *G. flabellum*. The Yucatan is unusual in that ranges of all three sea fan species overlap at many sites along a cline of increasing depth, allowing us to evaluate potential causes of differing prevalence among species. Signs of aspergillosis were observed in all the three species. However, the prevalence of infection in *G. cf. mariae*, a deep-water species, was consistently low even at sites where *G. ventalina* was common and had moderate levels of infection. Because *G. cf. mariae* is a relatively small-stature sea fan, we compared the prevalence of signs in *G. cf. mariae* to a subset of comparatively sized *G. ventalina*. *G. ventalina* had a significantly higher prevalence of aspergillosis, indicating that size does not explain the lower prevalence in *G. cf. mariae*. Prevalence of disease signs on the shallow-water *G. flabellum* was also significantly higher than *G. cf. mariae*, but did not differ from *G. ventalina*. To test the hypothesis that higher chemical resistance accounts for the low prevalence of disease in *G. cf. mariae*, we measured the response of *A. sydowii* in culture to antifungal extracts from each sea fan species. Significantly lower fungal growth rates on extracts of *G. cf. mariae* than *G. ventalina* support the hypothesis that *G. cf. mariae* is more chemically resistant to aspergillosis. When comparing sea fan disease across different regions of the Yucatan, we detected significantly higher prevalence in *G. ventalina* near Akumal than further north near Cozumel and Puerto Morelos. In Akumal, there was a strong positive correlation between sea fan size and disease, with the largest fans showing the highest prevalence and severity in all three species. In addition, prevalence of aspergillosis in *G. ventalina* was density-dependent in Ak.

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## Introduction

Despite a recent focus on emergent diseases and potential threats to terrestrial and marine biodiversity (Dobson and Foufopoulos 2001; Lafferty and Gerber 2002; Daszak et al. 2000), as well as the potential impacts on marine communities (Epstein et al. 1998; Goreau et al. 1998; Williams and Bunkley-Williams 1990; Harvell et al. 1999, 2004; Kim and Harvell 2004; Kim et al. 2004; Porter et al. 2001), little is known about the dynamics of infectious disease in the ocean. There is a dearth of quantitative data on the pathology and etiology of most coral diseases, as well as spatial and temporal variability in prevalence, host range, and local and geographic distribution (Weil 2002). Increasing numbers of epizootics of marine taxa (Ward and Lafferty 2004) have resulted in

recent mass mortalities of plants, invertebrates, and vertebrates and large shifts in community structure in the Caribbean, providing evidence that new diseases may impact marine biodiversity (Kim et al. 2004). From a conservation perspective, generalist pathogens that readily transmit among a broad host range are of particular concern (Lafferty and Gerber 2002). However, knowledge of pathogen–host interactions and disease resistance mechanisms is lacking in corals (Kim et al. 2004; Harvell et al. 1999, 2004). Understanding host range and resistance mechanisms is fundamental to predicting disease emergence and outbreak, especially in potentially warming tropical marine communities (Rosenberg and Ben-Haim 2002; Harvell et al. 2002).

An emergent infectious disease is broadly defined as one whose geographic range, host range, or prevalence has increased in recent years (Dobson and Foufopoulos 2001). Disease emergence occurs when a pathogen is introduced to and becomes established in a new host population (Morse 1995; Colwell 1996). Specificity and host range of a pathogen are important characteristics facilitating disease spread. While species-specific pathogens are unlikely to persist in small host populations (McCallum and Dobson 1995), pathogens with a wide host range infecting large host populations are responsible for virtually all recent disease outbreaks in endangered species (examples reviewed in Dobson and Foufopoulos 2001; Cleaveland et al. 2001; Daszak et al. 2000; Roelke-Parker et al. 1996; Heide-Jorgensen et al. 1992). These generalist pathogens capable of infecting multiple host species are a potentially rich source of emerging diseases known as the "zoonotic pool" (Morse 1995). In coral communities, examples of disease outbreaks caused by generalist pathogens include black band disease, reported on 42 species from 21 genera, white plague types I and II, collectively known to infect 22 species and 16 genera (reviewed in Green and Bruckner 2000; Richardson et al. 1998), and *Porites* ulcerative white spot disease, which affects at least seven species of *Porites* (Raymundo et al. 2003). The generalist nature of many coral diseases indicates that host shift may be a causative factor of emergent disease in coral communities (Weil et al. 2002).

Aspergillosis, an emergent disease in sea fan corals (*Gorgonia* spp.), was first documented in 1995 near Saba and detected throughout the Caribbean basin including the Florida Keys, USA (Nagelkerken et al. 1996). The pathogen is the terrestrial fungus *Aspergillus sydowii* (Smith et al. 1996; Geiser et al. 1998). Aspergilli commonly occur in soils, although they are successful opportunistic pathogens of a number of terrestrial taxa and marine mammals (Sweeney et al. 1976), causing diseases commonly referred to as aspergillosis. None were previously known to affect marine invertebrates. Signs of aspergillosis (lesions, tissue necrosis, galls, tumors, and a darkening of tissue pigmentation) have been observed on gorgonian corals throughout the Caribbean (Smith et al. 1998; Nagelkerken et al. 1996, 1997). This recent epizootic has been reported for *Gorgonia ventalina* and *G. flabellum* in the Bahamas, and has caused high population impacts for *G. ventalina* in the Florida Keys (Kim et al. 2000a, b; Smith et al. 1998; Nagelkerken et al. 1997) with Keys-wide losses of $>50\%$ of sea fan tissue from complete and partial mortality (Kim and Harvell 2004). Recently, signs of aspergillosis have been observed in *G. cf. mariae* in the Yucatan (personal observation) and other gorgonian species (Weil et al. 2002), indicating a potentially wide host range and generalist nature. The sea fan-aspergillosis pathosystem is a tractable system for the study of emergent marine disease ecology because the host is abundant in many reef systems, allowing quantitative spatial and epidemiological analyses (Jolles et al. 2002), and the pathogen is easily cultured in laboratory experiments (Kim et al. 2000b).

The ability to perform laboratory culture experiments with the pathogen *A. sydowii* provides an opportunity to examine how disease resistance mechanisms affect host range of a coral pathogen. Chemical resistance has been widely studied in plant–pathogen systems (Karban and Baldwin 1997; Harvell and Tollrian 1999), but has been poorly investigated in colonial invertebrates such as corals (Harvell et al. 1999, 2004). Extracts of many Caribbean gorgonian corals have antifungal activity (Kim et al. 2000b), and in aspergillotic sea fans, higher antifungal concentrations are localized in areas surrounding necrotic fungal lesions suggesting an induced response to infection (Kim et al. 2000a). Antifungal activity is negatively associated with size in *G. ventalina*, and prevalence and severity of visible disease signs increase as fan size increases (Dube et al. 2002; Jolles et al. 2002; Kim and Harvell 2002). This study assesses the ecological relevance of chemical defenses by comparing the response of morphologically similar and closely related species to the same pathogen. *G. cf. mariae* displays signs of aspergillosis, but is smaller in stature and is typically found in lower densities at greater depths ($>50$ ft) than the common *G. ventalina*. The overlapping presence of these species with *G. flabellum* in the Yucatan and the local emergence of aspergillosis offer a unique opportunity to examine the role of chemical defenses and disease dynamics in a tropical marine system (Kim et al. 2000a, b; Dube et al. 2002).

Levels of biochemical resistance vary not only with disease status within and among individual sea fans and between different species, but also between regions of the Caribbean (i.e., Florida Keys and Bahamas; Dube et al. 2002). Dube et al. (2002) hypothesized differences in resistance are driven by variation in disease pressure. In terrestrial communities, epidemics are more frequent and severe in genetically uniform plant communities (e.g., agricultural systems) than in managed semi-natural and undisturbed communities (reviewed in Burdon 1992; Burdon and Shatock 1980; Browning 1974). This supports the hypothesis that infectious disease in marine communities, coral reefs in particular, may have greater impacts where host density is high and host diversity is low, leading to local and regional variation. In plant communities, high-density, single-species stands are particularly vulnerable to attack by fungal pathogens

(Burdon 1982; Burdon and Chilvers 1975, 1976), and rate of infection of other plant diseases is positively correlated with the density of the host population (Jennersten et al. 1983; Harper 1977; Berger 1973; Van der Plank 1969). Understanding the effect of host density on prevalence and severity of aspergillosis is critical to better understand coral disease distribution patterns.

This study of aspergillosis tests the following hypotheses about patterns of disease distribution and host range:

1. Aspergillosis is present on all three species of sea fans from various regions of the Yucatan.
2. Disease prevalence and severity increase with host size for all three species.
3. Disease prevalence and severity increase with host density, as predicted by a simple neighborhood transmission model.
4. Antifungal activity is higher in species with low levels of disease prevalence and severity.

## Methods

### Field component

#### *Disease surveys*

Data were acquired using SCUBA in March 2000, June 2000, and March 2001 on 19 haphazardly selected reefs along the Yucatan Peninsula spanning three regions: Puerto Morelos (Pm), Akumal (Ak), and Cozumel (Cz) (Table 1 and Fig. 1). Site depths ranged from 0.5–7.5 m in Cz and Pm and 3.0–21.5 m in Ak. None of the deeper reefs in Pm or Cz had sea fans. Most reefs in Ak were surveyed at 2–3 different depths (noted in the annotation of the X axis of Fig. 3), and each depth was considered an independent site.

At each site, three parallel 25 m transects were deployed, separated by approximately 10–15 m. Divers measured the height and assessed disease status of each sea fan within one meter of each transect. All colonies were identified to species based on colony morphology, which was confirmed by sclerite morphology. Diseased colonies were characterized by spots, purpling, lesions, and tumors. Spots are small dark purple areas ($<1$ cm$^2$). Purpling is a darkly pigmented area ranging from 1 cm$^2$ to encompassing the entire sea fan that does not contain polyps. Often there is a thickening of the gorgonin associated with purpling. Lesions are areas of tissue necrosis (bare axis with no coenenchyme present) and obvious holes within the normally uniform planar surface of the fan, the edges of which are often purpled. Tumors are areas with extensive thickening of the affected gorgonin and may be darkly pigmented. For all diseased fans, observers recorded an estimate of percent area of the colony affected. To ensure consistent data collection between observers, sites and years, surveys were standardized among all divers ($n=6$) by recording for each sea fan species signs and severity of disease on the same transects, followed by a confirmation period in which questions and discrepancies were resolved. Inter-comparability checks were repeated until all divers were comparable.

Prevalence, percent of sea fans with disease signs, and severity, percent of a colony infected, were measured in *G. ventalina, G. cf. mariae*, and *G. flabellum*. Transects with three or fewer sea fans of any one species, or three or fewer sea fans per size class, were not included in the analyses. In the regional analysis, regions with fewer than three transects on which a species occurred were not included. Dead sea fans (i.e., standing skeletons) were

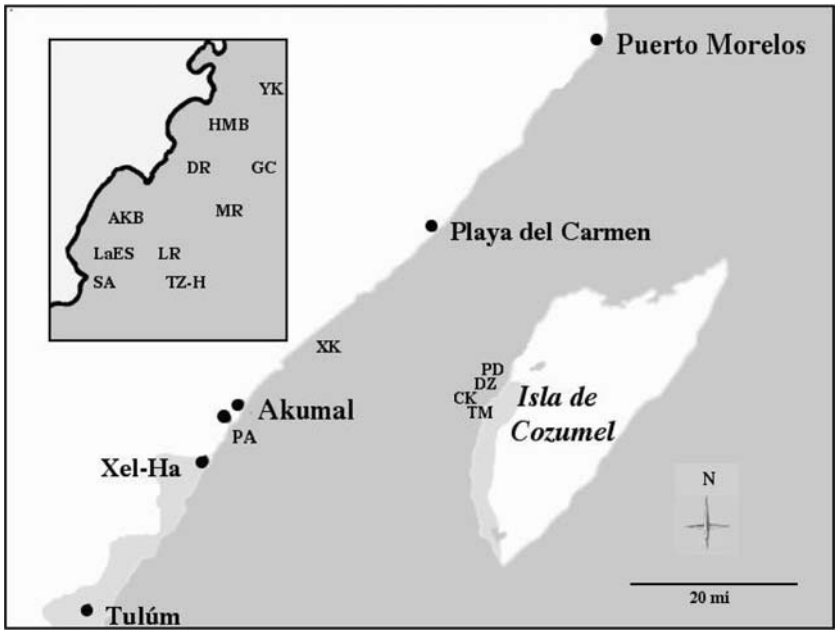


**Fig. 1** Sample sites with inset of Akumal sites. Map of the Yucatan Coast showing approximate survey locations (full site names in Table 1). The three back reef sites in proximity to the Puerto Morelos Laboratory are not shown

**Table 1** Survey sites

| Region | Reef | GPS coordinates | Survey date | Species present |
|---|---|---|---|---|
| | Dick's Reef (DR) | N20°24.018′ W087°18.181′<br>N20°24.149′ W087°18.254′ | A, C | Gv, Gm |
| | Half Moon Bay (HMB) | N20°24.048′ W087°18.269′<br>N20°24.039′ W087°18.306′ | A, B, C | Gv, Gm, Gf |
| | Yalku Bay (YK) | N20°23.412 W087°18.695′<br>N20°24.485′ W087°18.027′ | A, B, C | Gv, Gm |
| Akumal | Tzimin-Ha (TZ-H) | | A | Gv, Gm |
| | South Akumal (SA) | N20°22.548′ W087°19.191′<br>N20°22.668′ W087°19.169′ | A, C | Gv |
| | | N20°23.202′ W087°18.654′ | | Gv, Gm |
| | Las Redes (LR) | N20°27.504′ W087°15.639<br>N20°23.359′ W087°18.621′ | A, B[c], C | |
| | Morgan's Reef (MR) | N20°23.753′ W087°18.378′ | A, C | Gv, Gm |
| | Grouper Canyon (GC) | N20°23.667′ W087°17.890′ | A, C | Gv |
| | Akumal Bay (AKB) | | B[b] | Gv, Gf |
| | La Escuela (LaEs) | | B | Gv, Gf (n = 1) |
| | X'aak (XK) | N20°25.633′ W087°17.300′<br>N20°25.579′ W087°17.291′ | C | Gv, Gm |
| | Puerto Adventuras (PA) | N20°22.810′ W087°19.015′ | C | Gv, Gm |
| Cozumel | Tormentos Tip (TM) | | B | Gv, Gf |
| | Chankanaab (CK) | | B | Gv, Gf |
| | Paradise (PD) | | B | Gv, Gf |
| | Dzul-Ha (DZ) | | B[b] | Gv, Gf |
| Puerto Morelos | Punta Carrata (PU) | | B | Gv, Gf |
| | Picudas Reef (PC) | | B[a] | Gv, Gf |
| | Caricomp Reef (CC) | | B | Gv, Gm |

Summary of all reefs visited: GPS coordinates, when available, are listed in order of increasing depth; species present: *Gorgonia ventalina* (*Gv*), *G. cf. mariae* (*Gm*), and *G. flabellum* (*Gf*); and survey dates: March 2000 (A), June 2000 (B), and March 2001 (C). All data were based upon the mean of three 50 $m^2$ transects per site (reef × depth) except where indicated: [a]1 transect, [b]2 transects, [c]4 transects

recorded, but not included in prevalence and severity analyses, and mortality rates were not included due to the bias of missing skeletons (e.g., some skeletons may have washed out of the survey area previous to the survey). Sea fans with debatable or very slight signs (e.g., $<1\%$ of their total area affected by signs of aspergillosis or fewer than three spots) were designated healthy for conservative prevalence estimates.

Using a mixed model in SAS (SAS System for Windows, Version 8, SAS Institue Inc.), disease dynamics were analyzed for the effects of sea fan species, size (height classes: 0–20, 21–40, 41–60, 61–80, and 81+ cm), and depth (classes: 3–7.5, 9–13.5, and 16.5–21 m), as well as site (local) and regional effects on disease prevalence and severity. We used an unbalanced block design (i.e., all three species and all five size classes were not always represented) with three transects blocked within site. Species and size were fixed effects, and site was random (i.e., non-permanent transects). In June 2000, the following regions were surveyed: Pm, Ak, and Cz, and site was nested within region. Covariance parameters provided estimates of variance for the random effects of the model (Singer 1998), both between and within sites. A difference of least squared means (DLSM) was calculated between regions, species, and size classes within species. A within size class DLSM compared species to determine if *G. cf. mariae* are less vulnerable to infection by *A. sydowii* than similarly sized *G. ventalina* and *G. flabellum*. All multiple comparison values were alpha-adjusted and unequal sample sizes were accounted for with the Tukey–Kramer procedure. The *t* tests within depth class were run in DataDesk®6.0 to compare species to determine if *G. cf. mariae* are less vulnerable to infection by *A. sydowii* than *G. ventalina* and *G. flabellum* at the same depth.

### *Sample collection*

Samples of each sea fan species were haphazardly collected from Yal Ku Bay (*G. cf. mariae*), Half Moon Bay (*G. ventalina*), and Las Redes (both species). Samples (approximately 4 $cm^2$) were excised from the edges of healthy, similarly sized colonies, stored in WhirlPaks with seawater, and immediately transported to the laboratory.

## Laboratory component

### *Extraction and antifungal growth assay*

To examine whether chemical resistance could account for the observed difference in prevalence between species, the growth of *A. sydowii* exposed to sea fan antifungal extracts of *G. ventalina* and *G. cf. mariae* was compared. The 4 $cm^2$ pieces of healthy sea fans were extracted twice in 20 ml dichloromethane (DCM) for 24 h at room temperature. DCM was used as the extracting solvent because of its ability to dissolve non-polar compounds, its volatility, and previous work showing that antifungal activity is highest for samples extracted in DCM (Kim et al. 2000a). Extracts were transferred to pre-weighed

scintillation vials, weighed, and stored at −20°C until assayed. Remaining coral pieces were dried overnight at 60°C and weighed to obtain dry coral weights. Relative extract per sample was standardized by calculating extract per dry coral weight (EPCW, mg/g).

Antifungal activity was assayed against *A. sydowii* (Dube et al. 2002). Following the procedure outlined in Dube et al. (2002), a growth medium was prepared and covered with sea fan extract before inoculation with a standardized suspension of *A. sydowii* spores from San Salvador, Bahamas (Smith et al. 1996) at a concentration of 2,150,000 spores/ml. Plates were sealed and incubated at 25°C. After 4 days, circular *A. sydowii* colonies were visible. NIH Imaging software (v. 1.62, U.S. National Institutes of Health) was used to measure the diameter of each colony (averaged from two haphazardly chosen perpendicular measurements). Colony diameter ($d$) was recorded 5, 7, and 7 days post-inoculation, and area was calculated as $\pi(d/2)^2$. Because growth of *A. sydowii* colonies when exposed to sea fan extract is approximately linear after a short lag (Alker et al. 2001), the average change in area from day 6 to day 7 was used to estimate daily growth rate. Lower fungal growth rates indicate higher antifungal activity of the extract. Three controls were included in the assay: acetone (negative control to test for solvent effect), Hygromycin at 20 mg/ml (positive antifungal control), and no solvent or extract. An ANOVA revealed no site effect, so site was removed from the model and levels of antifungal activity between *G. ventalina* and *G. cf. mariae* were compared with a $t$ test in DataDesk®6.0.

## Results

### Disease surveys

Signs of aspergillosis were observed in all three species of sea fan: *G. ventalina, G. cf. mariae*, and *G. flabellum.* Where the ranges of all species overlapped in June 2000, disease prevalence varied significantly by species (Table 2). Prevalence of disease signs was significantly lower in *G. cf. mariae* than *G. ventalina* ($t=2.76$, $df=192$, $P<0.005$) and *G. flabellum* ($t=2.76$, $df=192$, $P<0.01$), and there was no difference between *G. ventalina* and *G. flabellum* when all size classes were pooled. Since *G. cf. mariae* and *G. flabellum* were only represented in the smallest two of five size classes, it was tested whether the difference in disease prevalence was due to size or species. A within-size-class DLSM revealed no difference between species in the smallest size class (0–20 cm), and there was a trend of higher disease prevalence in *G. ventalina* than *G. cf. mariae* in fans of 21–40 cm in height ($t=-2.85$, $df=116$, $P=0.06$; Fig. 2a). Prevalences of aspergillosis in *G. cf. mariae* and *G. flabellum* did not differ. There were no differences among species in severity.

In March 2000 and 2001 surveys, only transects with *G. ventalina* and *G. cf. mariae* were censused in Akumal; shallow-water *G. flabellum* transects were inaccessible in high surge conditions. Prevalence of disease signs varied significantly by species and sea fan size, while disease severity varied only with size (Table 2). Aspergillosis was

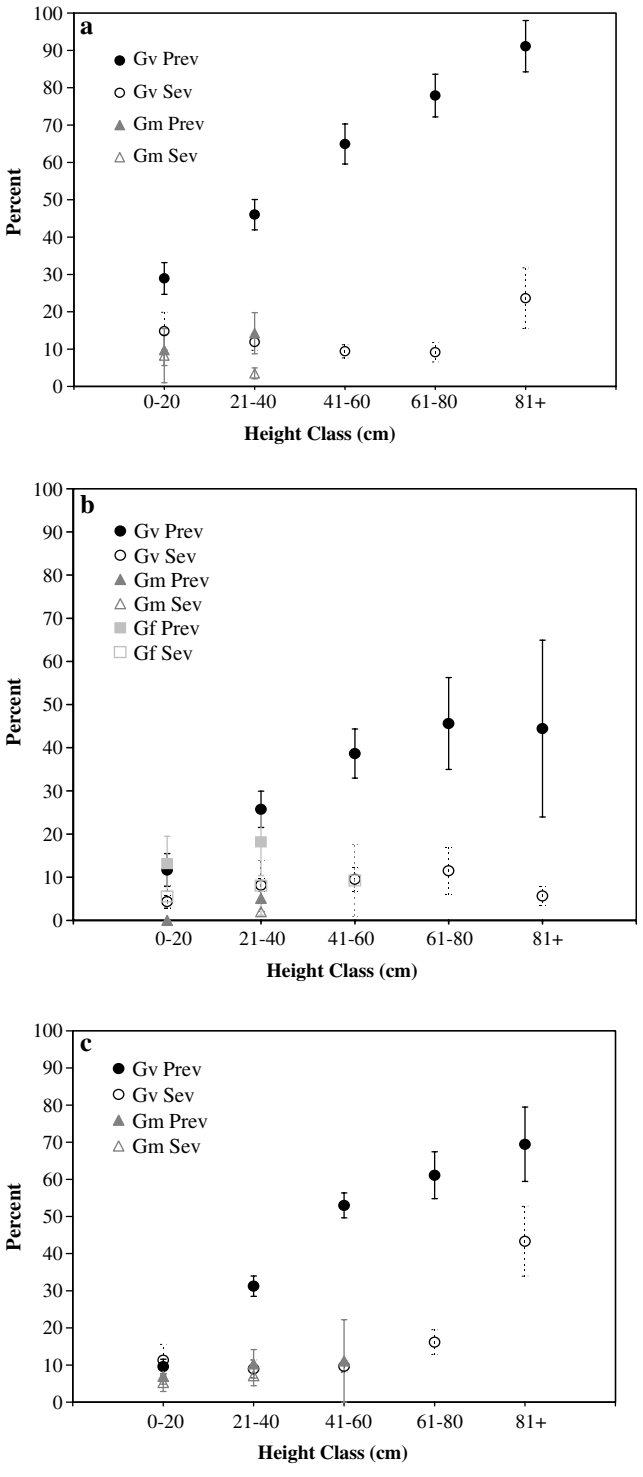


**Fig. 2** Disease surveys. Prevalence (*Prev*), percent of sea fans with disease signs, and severity (*Sev*), percent of a colony infected, of aspergillotic signs in Akumal, Mexico for three species of sea fan: *G. ventalina* (*Gv*), *G. flabellum* (*Gf*), and *G. mariae* (*Gm*): **a** March 2000, **b** June 2000, and **c** March 2001. *Bars* are standard error

**Table 2** Factors determining aspergillosis in the Yucatan

| Dependent variable | Source of variation | *df* | *F* | *P* |
|---|---|---|---|---|
| March 2000 | | | | |
| Prevalence | Species | 1 | 20.84 | <0.0001 |
| | Size | 4 | 29.51 | <0.0001 |
| Severity | Species | 1 | 1.24 | 0.3 |
| | Size | 4 | 4.62 | <0.005 |
| June 2001 | | | | |
| Prevalence | Region | 2 | 12.45 | <0.0001 |
| | Species | 2 | 4.87 | <0.01 |
| | Size | 4 | 7.25 | <0.0001 |
| Severity | Region | 2 | 1.02 | 0.4 |
| | Species | 2 | 0.67 | 0.5 |
| | Size | 4 | 0.78 | 0.1 |
| March 2001 | | | | |
| Prevalence | Species | 1 | 9.61 | <0.005 |
| | Size | 4 | 52.05 | <0.0001 |
| Severity | Species | 1 | 0.72 | 0.4 |
| | Size | 4 | 10.35 | <0.0001 |

Mixed model ANOVA with fixed effects on prevalence and severity of aspergillotic signs on three species of sea fan, *G. ventalina*, *G. cf. mariae*, and *G. flabellum* along coastal regions of the Yucatan Peninsula

significantly more prevalent in *G. ventalina* than in *G. cf. mariae* (Fig. 2; DLSM, 2000: $t=-4.57$, $df=151$, $P<0.0001$; 2001: $t=-3.10$, $df=268$, $P<0.005$) when all size classes were pooled. Within size class DLSM tests revealed no difference between species in the smallest sea fans (0–20 cm) but significantly higher disease prevalence in *G. ventalina* in fans 21–40 cm in height (March 2000: $t=-4.25$, $df=78$, $P<0.001$; March 2001: $t=-6.13$, $df=159$, $P<0.0001$). Disease severity did not differ significantly between species (Table 2). In addition to species differences, size was a significant predictor for disease status (Table 2) within species. Both prevalence and severity increased with colony size (Fig. 2).

## Local and regional variation

In all years, there was significant local variation in prevalence both within ($P<0.0001$) and between ($P<0.05$) sites (Fig. 3). Severity showed little variation between sites ($P>0.05$) but did vary within site ($P<0.0001$). Regionally, prevalence was significantly higher in Ak than Cz (Fig. 4; DLSM, $t=4.95$, $df=192$, $P<0.0001$), but not in Pm ($t=2.08$, $df=192$, $P=0.1$). Prevalence and severity did not vary between Cz and Pm ($t=-1.69$, $df=192$, $P=0.2$).

## Host distribution: density and depth

The prevalence of diseased *G. ventalina* and *G. cf. mariae* colonies correlated with total sea fan density and depth in Akumal, March 2001 (Table 3) when the greatest number of sites was surveyed ($n=17$). Las Redes 7.5 m was identified as an outlier with the highest density and much lower disease prevalence than expected. When this site was included, there was not a strong correlation with density. This site had significantly smaller *G. ventalina* than all other sites (a site average of 19.97 cm vs.

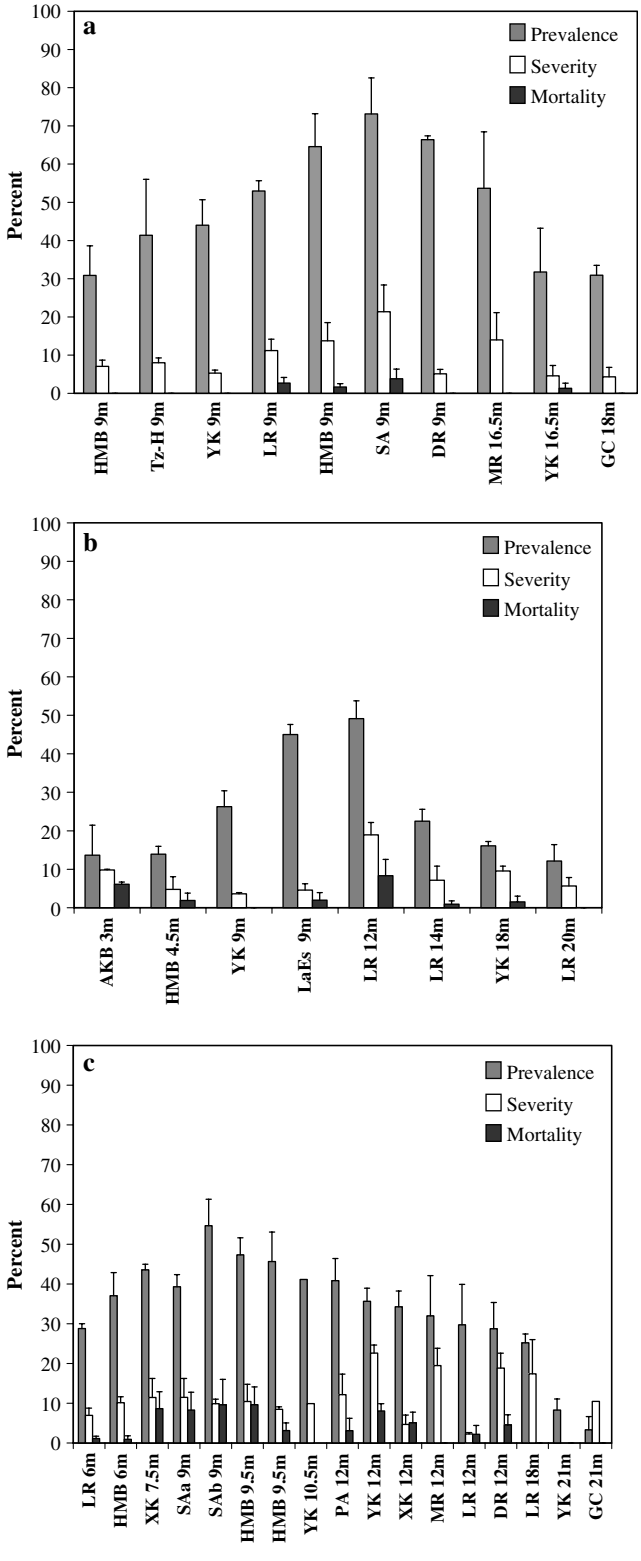


**Fig. 3** Local variation in disease dynamics. *G. ventalina.* Prevalence (percent of sea fans with disease signs), severity (percent of a colony infected) and mortality (skeletons) of sea fans per site in Akumal, Mexico: **a** March 2000, **b** June 2000, and **c** March 2001. Depth data are included in the annotation of the *X* axis. Half Moon Bay (*HMB*) is a large reef, and was surveyed repeatedly on different areas and at various depths (4.5, 6, 9, and 9.5 m), so it appears more than once in (**a**) and (**c**). *Bars* are standard error

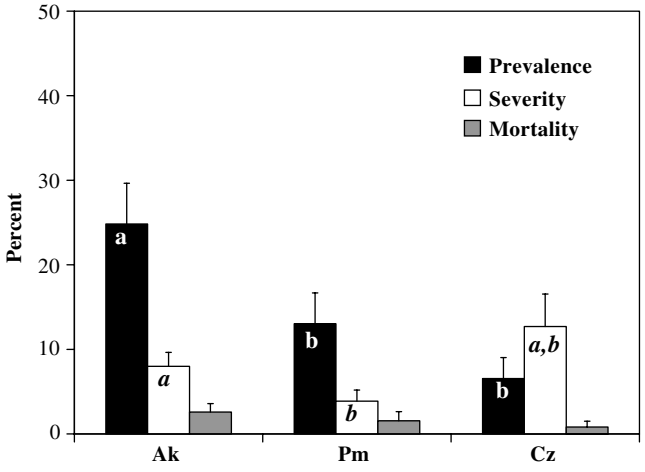


**Fig. 4** Regional variation in disease dynamics. *G. ventalina*, June 2000. Prevalence (percent of sea fans with disease signs), severity (percent of a colony infected) and mortality (skeletons) of sea fans for all regions surveyed in the Yucatan: Akumal (*Ak*), Puerto Morelos (*Pm*), and Cozumel (*Cz*). Differences in prevalence (**a**, **b**) and severity (**a**, **b**) are significant at $P<0.05$. There was no regional effect on mortality rates. *Bars* are standard error

37.08 cm; , $df=16$, $F=18.06$, $P<0.0001$), and since sea fan size is such a strong determinant of infection status, this site was excluded from the analyses. In general, diseaANOVAse prevalence was the highest in *G. ventalina* at intermediate depths: 10–30% at 3–7.5 m, 30–70% at 9–13.5 m, and 5–30% at 16.5–21 m (Fig. 3). Surveys in March and June 2000 showed a similar trend with strong, but non-significant correlations between density and disease, presumably due to fewer survey sites censused. For all years in Akumal, there was significantly higher disease prevalence in *G. ventalina* than *G. cf. mariae* at similar depths (Table 4), showing no effect of depth upon species differences.

## Antifungal assay

Extracts that most effectively inhibited growth of *A. sydowii* were considered chemically most active. There was no measurable fungal growth on positive control plates (Hygromycin B). Growth rates of *A. sydowii* on two treatments of negative control plates (acetone and no solvent) were not different ($t=0.524$, $df=4$, $P=0.3$) and were averaged for statistical analyses. Growth rates of controls (251.55 $mm^2$/day) were significantly higher than plates with sea fan extract for both *G. ventalina* (150.59 $mm^2$/day; $t=-7.58$, $df=14$, $P<0.0001$) and *G. cf. mariae* (129.87 $mm^2$/day; $t=-7.08$, $df=21$, $P<0.0001$), indicating chemical inhibition by the sea fan extracts (Fig. 5). On average, *G. ventalina* and *G. cf. mariae* extracts inhibited growth of *A. sydowii* by 40.13 and 48.37% respectively, with a range of 19–77%. An ANOVA revealed no site effect, so site was removed from the model. A *t* test revealed a trend of higher chemical activity in extracts of *G. cf. mariae* than in those of *G. ventalina* from day 5 to 6 ($t=-1.54$, $df=25$, $P=0.07$). This trend becomes significant from day 6 to 7: *G. ventalina* extracts averaged 177.92 $mm^2$/day, significantly higher than 146.61 $mm^2$/day on *G. cf. mariae* extracts ($t=-2.61$, $df=26$, $P<0.01$). On average, *G. cf. mariae* extracts inhibited the growth of *A. sydowii* by 17.60% more than those of *G. ventalina.*

EPCW was positively related to activity. In addition to more active antifungal compounds at a standardized concentration, *G. cf. mariae* yielded significantly more

**Table 4** Depth effect upon species differences

| Time | Depth | Prevalence, *P* | Severity, *P* |
|---|---|---|---|
| March 2000 | 3–7.5 m | N/A | N/A |
| | 9–13.5 m | <0.0001 | 0.1 |
| | 16.5–21 m | 0.02 | 0.5 |
| June 2000 | 3–7.5 m | 0.04 | 0.5 |
| | 9–13.5 m | <0.001 | 0.4 |
| | 16.5–21 m | <0.01 | 0.2 |
| March 2001 | 3–7.5 m | <0.001 | <0.01 |
| | 9–13.5 m | <0.0001 | 0.7 |
| | 16.5–21 m | 0.05 | 0.2 |

Comparison of prevalence and severity between *G. ventalina* and *G. cf. mariae* in Akumal, Mexico within each of three depth ranges for all survey periods

**Table 3** Correlation between disease prevalence, sea fan density and depth

| Variables | *X* | Density (fans/$m^2$) | | Depth (m) | | Depth (m) | |
|---|---|---|---|---|---|---|---|
| | *Y* | Percent diseased fans per site | | Percent diseased fans per site | | Density (fans/$m^2$) | |
| Month, year | | $r^2$ | *P* value | $r^2$ | *P* value | $r^2$ | *P* value |
| March, 2000 | Gv, $n=8$ | 0.246 | 0.1 | 0.218 | 0.2 | 0.385 | 0.1 |
| | Gm, $n=4$ | 0.323 | 0.1 | 0.044 | 0.6 | | |
| June, 2000 | Gv, $n=10$ | 0.183 | 0.3 | 0.060 | 0.9 | 0.187 | 0.2 |
| | Gm, $n=8$ | 0.139 | 0.6 | 0.039 | 0.8 | | |
| March, 2001 | Gv, $n=16$* | 0.314 | <0.05 | 0.515 | 0.001 | 0.359 | 0.01 |
| | Gm, $n=17$ | 0.258 | <0.05 | 0.249 | <0.05 | | |

A positive correlation between prevalence of aspergillosis in *Gorgonia ventalina* (*Gv*) and *G. cf. mariae* (*Gm*) and total sea fan density, a negative correlation between prevalence and site depth, and a negative correlation for depth versus total sea fan density. Values are the mean of three transects
*Denotes statistics after the removal of a significant outlier, and $n=$sites

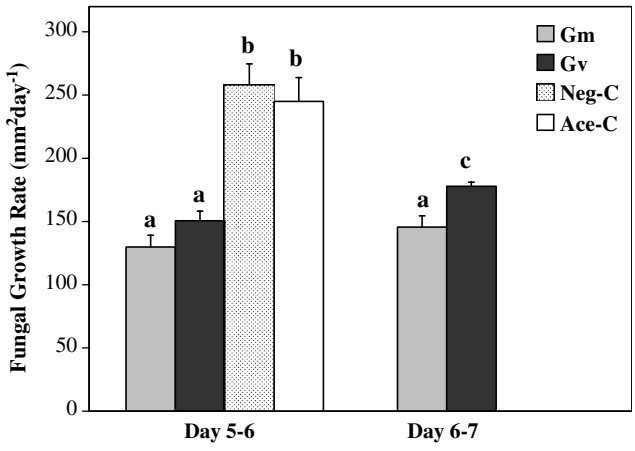


**Fig. 5** Antifungal growth assay. *A. sydowii*. Mean±SE average daily growth rate (per 24 h) of individual *A. sydowii* colonies on sea fan antifungal extracts from *G. ventalina* (*Gv*) and *G. mariae*, (*Gm*) from sites in Akumal, Mexico. Plates were inoculated on day 0, and the increase in area ($mm^2$) was measured from day 5 to 6 and day 6 to 7. Controls for day 6–7 showed extremely high growth rates: Negative (*Neg-C*; nothing added to plate) and Acetone (*Ace-C*; acetone added to plate) control colonies grew into the edges of the plates, and thus were not included. There was no measurable growth in the positive Hygromycin control. Differences in growth rate (**a**–**c**) are significant at $P<0.05$. *Bars* are standard error

extract on average (0.051 g) than *G. ventalina* (0.039 g; $t=1.73$, $df=22$, $P=0.05$).

## Discussion

Host range and other disease dynamics of marine epizootics are poorly understood, thus making the study of coral disease emergence a priority. In order to better understand the patterns of disease distribution and host range of a current marine epizootic, we investigated disease prevalence, severity and variations in chemical resistance in all three species of sea fan present in the Caribbean. This study has confirmed signs of aspergillosis in *G. ventalina*, *G. cf. mariae*, and *G. flabellum*. Aspergillosis has also been detected in several other gorgonian species, including *Plexaurella homomalla* and *P. americana* (Kim et al., unpublished data; Weil et al. 2002). *A. sydowii*, like many other *Aspergillus* species, appears to be a host generalist in gorgonians, and thus a high risk factor for disease emergence (Cleaveland et al. 2001).

Of the three host species surveyed in this study, prevalence of disease is highest in the most abundant species, *G. ventalina*. For *G. ventalina*, there is a significant positive correlation between disease prevalence and sea fan size, with 45–90% of large sea fans showing signs of disease in Akumal, Mexico, while less than 30% of relatively small sea fans (0–20 cm) were affected. Jolles et al. (2002), Kim and Harvell (2004), and Nagelkerken et al. (1997) all found the same trend. *G. cf. mariae*, a small-stature, deep-water species, was present in all regions of the Yucatan surveyed, and consistently showed low disease prevalence and severity. *G. flabellum* and *G. ventalina* had comparable prevalence of disease, but it is difficult to draw conclusions about the relative prevalence of disease in *G. flabellum* since it was only found on 5 of 24 transects in Akumal. While Nagelkerken et al. (1997) detected a slight but non-significant trend of higher prevalence and severity in *G. ventalina* than *G. flabellum* at other Caribbean sites, on the Yucatan, once a sea fan becomes infected, the level of severity is comparable for all three species. It is unclear why severity does not vary by species as does prevalence, but a plausible explanation may be that the extremely low severity values prevent detection of statistical differences.

Since size-specific resistance has been demonstrated in *G. ventalina* from the Florida Keys (i.e., greater antifungal extract activity in small fans relative to large fans) (Dube et al. 2002), three hypotheses were proposed to explain lower disease prevalence in *G. cf. mariae*: (1) *G. cf. mariae* is more resistant due to its smaller stature and reduced contact with water-born propagules;, (2) *G. cf. mariae* produces more potent antifungal extracts, and a higher chemical resistance accounts for the lower disease prevalence than in *G. ventalina*; and (3) there is less wave action at greater depths where *G. cf. mariae* predominates, which may reduce input of infectious propagules for deep-water species as suggested by Nagelkerken et al. (1997). We reject both the small size and the greater depth hypotheses since *G. cf. mariae* has lower prevalence both when paired by size class and when compared over the same depth range as *G. ventalina*. We tested the greater chemical resistance hypothesis as an explanation for the lower prevalence of infections on *G. cf. mariae*. *A. sydowii* colonies grew more slowly on *G. cf. mariae* antifungal extracts than those of comparably sized *G. ventalina*. Extracts of *G. cf. mariae* inhibited the growth of *A. sydowii* 17.60% more than those of *G. ventalina*. In addition, *G. cf. mariae* yielded significantly more EPCW, thus *G. cf. mariae* colonies have more potent extracts and contain a higher biomass of extract than *G. ventalina*. These results support the hypothesis that *G. cf. mariae* is more chemically resistant to aspergillosis than *G. ventalina* (Kim et al. 2000b).

Most infectious diseases show strong density dependence as a result of the transmission process (Anderson and May 1991; Lafferty and Gerber 2002; Jolles et al. 2002). Although density dependence was not detectable in previous studies of aspergillosis in the Florida Keys (Kim and Harvell 2002; Kim et al. 2004), several transects from the Yucatan show higher disease prevalence with higher sea fan density. The detection of density dependence along the Yucatan is consistent with the findings of Jolles et al. (2002), who analyzed spatial distribution of diseased sea fans and detected significant aggregation of severely infected fans. The aggregated distribution of diseased individuals was attributed to a local infection process, and this would be expected to produce density dependence. Density dependence and a clumped distribution of diseased coral colonies have been found in the white plague pathosystem, suggesting that the

disease is contagious (Richardson et al. 1998). The sporadic nature of density dependence and aggregated disease distributions at some sites in the sea fan–aspergillosis pathosystem (Jolles et al. 2002) suggests that outside inputs of the infectious propagules often swamps detectability of a local infection process.

Dube et al. (2002) showed that regional variation in disease pressure in the Florida Keys is not explained by regional variation in host resistance. In the Yucatan, signs of aspergillosis are widely distributed, ranging from Pm in the north to Cz and Ak further south. There was significant regional variation in disease dynamics with prevalence highest near Ak, and severity highest in Ak and Cz. We have no information yet about causes of this regional variation, although lower variance in resistance on reefs with greater disease intensity in the Florida Keys suggests that there is selection for disease resistance, especially at more heavily impacted sites (Dube et al. 2002). Continued monitoring of disease dynamics in the aspergillosis–sea fan pathosystem may reveal causes of local and regional variation in prevalence and severity.

Factors proposed to explain increased rates of disease emergence include pathogen characteristics (e.g., mutation, recombination, and genetic drift), host characteristics (e.g., immunosuppression), host population characteristics (e.g., population size, behavior, and movement), and ecological factors (e.g., changes in land-use, agriculture, and urbanization) (Cleaveland et al. 2001; Morris and Potter 1997; Morse 1995). The original cause of emergence of the aspergillosis epizootic in sea fans could be due to either new exposure, i.e., given the terrestrial origin of aspergilli, sea fans may be naïve and thus highly susceptible hosts, or newly compromised host resistance, e.g., caused by an environmental change such as increasing sea surface temperatures (Kim and Harvell 2004). The multi-species host range and species-level differences in resistance to *A. sydowii* in the Yucatan suggests that host resistance may play an important role in the distribution of aspergillosis among gorgonians. Kim et al. (2000a, b) showed that more than half of 20 gorgonian species tested had equally or less active extracts than *G. ventalina*, suggesting *A. sydowii* is a potential generalist that could be capable of further host shifts and outbreaks. Increased antifungal resistance and decreased prevalence of disease in *G. mariae* provides further evidence that resistance can drive host range.

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