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Variability of aspergillosis in *Gorgonia ventalina* in La Parguera, Puerto Rico

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Abstract.—Temporal and spatial variability of prevalence of the fungal disease aspergillosis (ASP) affecting *Gorgonia ventalina* colonies was assessed at several reef sites in La Parguera, southwest Puerto Rico. During 2005 and 2006, all *G. ventalina* colonies were checked for signs of ASP twice a year, within permanent 20 m² (10x2m) band transects at each of six sites located in three reef zones. Overall, there was high variability in disease prevalence in both space and time (0-28%). The average prevalence of ASP increased significantly from 5.1 ± 9.5% in March 2005 to 14.4 ± 16.0% in September 2006. There was no consistent temporal trend in ASP prevalence affecting all reefs, but shelf-edge reefs had significantly lower prevalence than inner and/or mid-shelf reefs in all sampling periods. Higher disease prevalence found in shallower areas may be related to higher water temperatures in these habitats, and unseasonably high temperatures during the winter and spring of 2006 associated with the extensive bleaching event of 2005.

Keywords.—aspergillosis, *Gorgonia ventalina*, octocoral disease, disease prevalence

Introduction

Diseases affecting coral reef organisms have flourished in recent decades. Aspergillosis (ASP) is a disease which affects a number of shallow-water gorgonian species throughout the Caribbean (Weil et al. 2002; Smith and Weil 2004). The apparent pathogen is the fungus *Aspergillus sydowii* (Smith et al. 1996; Geiser et al. 1998) and the major sign of the disease, apparent tissue necrosis exhibited as dark purple tissue, is most obvious on the three Caribbean sea fans (*Gorgonia ventalina*, *G. flabellum* and *G. mariae*). *Gorgonia ventalina*, the most abundant of the three species, is apparently most susceptible to infection by ASP (Smith and Weil 2004; Mullen et al. 2006).

Aspergillosis prevalence, like that of many coral diseases, is highly variable in space and time. Caribbean-wide surveys undertaken to determine the extent of the 1996 outbreak (Nagelkerken et al. 1997a, b) and subsequent studies have consistently found the disease to be present, often showing high variability in prevalence on a temporal basis for a single locality. Aspergillosis affecting sea fans on reefs of the Florida Keys decreased from 31% in August 1997 to 5.9% in August 2003 (Kim and Harvell 2004), while in Curaçao, disease prevalence was higher than in the Florida Keys with no significant difference between 1995 (52%) and 2005 (43%) (Nagelkerken et al. 1997b; Nugues and Nagelkerken 2006). In Puerto Rico the percentage of diseased sea fans in 1996 was much lower than in Curaçao (10-12%, Nagelkerken et al. 1997b), but prevalence values were similar in 1999 (11%, Weil et al. 2002) and 2002 (6-18%, Toledo-Hernández et al. 2007).

This study assessed the current status of ASP affecting the common sea fan *Gorgonia ventalina* in several reefs off La Parguera, on the southwest coast of Puerto Rico. The goal was the assessment of the temporal and spatial variability in the prevalence of ASP. Disease surveys were performed over a two-year period at varying depths within several reefs along an inshore-offshore gradient. We expected to see variations in disease prevalence with (a) season, due to changes in water temperature, (b) depth, because of differences in water motion and (c) shelf location, because of distance from

the shore and related anthropogenic influences such as nutrient and sediment influx.

Methods

Aspergillosis prevalence (% diseased colonies) affecting *Gorgonia ventalina* in La Parguera, on the southwest coast of Puerto Rico, was assessed at six reef sites in an inshore-offshore gradient. At each reef site, sixteen (16) 20m^2 (10x2m) band transects (total = 96 transects) permanently marked with rebar, were surveyed to assess the spatial and temporal variability of ASP prevalence in the area (Table 1, Fig. 1). At each of two inner-shelf (Enrique and Pelotas) and two mid-shelf (Media Luna and Turrumote) fringing reef sites, eight permanent transects were located in shallow habitats (<6 m) and eight in intermediate depth habitats (6-16 m). At the two shelf-edge reef sites (Weinberg and El Hoyo) all 16 transects were located in deeper habitats (18-23 m). All transects were surveyed during March, when sea temperatures were near their coolest, and during September, when temperatures were near their warmest in both 2005 and 2006. In March 2005, at least 30% of the 16 transects at each site were sampled

Table 1. Characteristics of the six reefs off La Parguera, Puerto Rico where this study was conducted.

Reef	Zone	Location (N)	Location (W)	Distance from shore (km)	Depth range (m)	Slope	Coral cover	Octocoral density
Enrique	Inner-shelf	17°56.658	67°02.213	1.5	1-16	steep	moderate	moderate
Pelotas	Inner-shelf	17°57.442	67°04.176	1	1-16	steep	low	low
Media Luna	Mid-shelf	17°56.093	67°02.931	2	3-20	steep	moderate	high
Turrumote	Mid-shelf	17°56.097	67°01.130	2	2-20	steep	high	low
Weinberg	Shelf edge	17°53.429	66°59.320	6	18-25	gradual	high	moderate
El Hoyo	Shelf edge	17°52.559	67°02.619	8	19-25	gradual	low	low

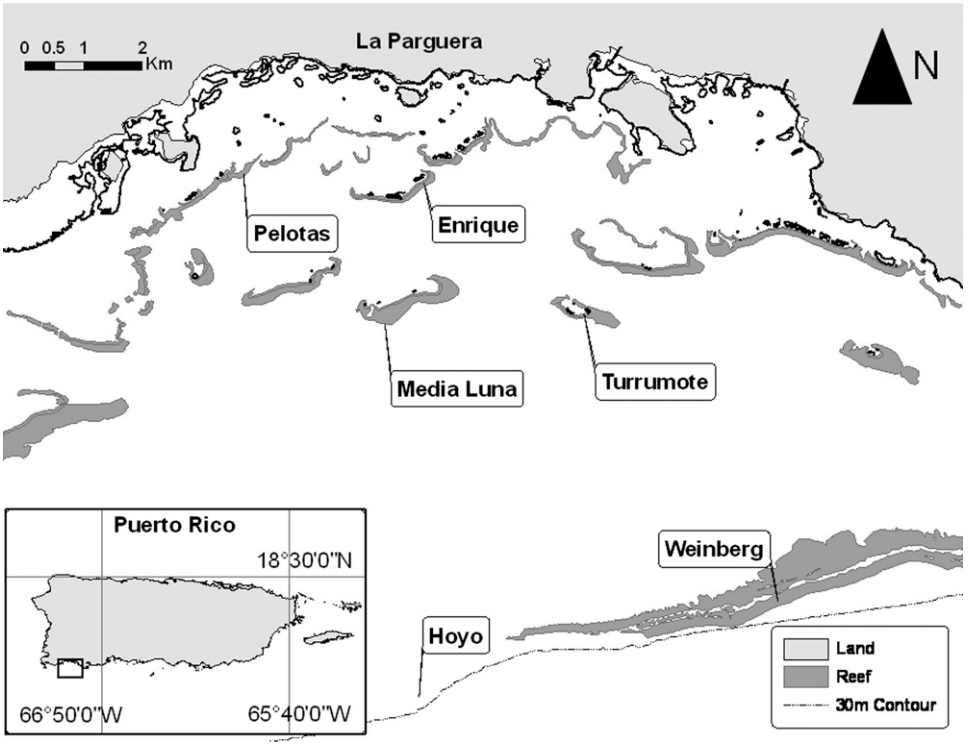

Fig. 1. Map of La Parguera, Puerto Rico with study sites.

TABLE 2. Average aspergillosis (ASP) prevalence values (percentage of sea fans with signs of disease ± standard deviations) for all levels of each factor (zone, reef, depth) for each sampling period. (Zones - March 2005: $n_{(Inner)}$=15, $n_{(Mid\text{-}shelf)}$=20, $n_{(Shelf\text{-}edge)}$=19; September 2005, March 2006, September 2006: n=32. Sites - March 2005: $n_{(Enrique)}$=7, $n_{(Pelotas)}$=8, $n_{(Media\ Luna)}$=9, $n_{(Turrumote)}$=11, $n_{(Weinberg)}$=5, $n_{(El\ Hoyo)}$=14; September 2005, March 2006, September 2006: n=16. Depth - March 2005: $n_{(Shallow)}$=16, $n_{(Intermediate,\ Deep)}$=19; September 2005, March 2006, September 2006: n=32. Total- March 2005: n=54; September 2005, March 2006, September 2006: n=96.)

		Aspergillosis prevalence (%)			
Factor	Level	March 2005	September 2005	March 2006	September 2006
Zone	Inner	1.1 ± 3.1	12.7 ± 12.6	10.8 ± 22.2	9.5 ± 12.3
	Mid-shelf	10.8 ± 11.3	7.5 ± 10.5	16.3 ± 15.9	24.2 ± 18.3
	Shelf-edge	2.8 ± 8.5	6.3 ± 9.0	3.2 ± 6.2	9.2 ± 11.8
Site	Enrique	2.4 ± 4.3	17.0 ± 13.5	20.2 ± 27.4	15.8 ± 14.0
	Pelotas	0 ± 0	7.9 ± 9.7	0 ± 0	2.3 ± 2.9
	Media Luna	6.7 ± 7.7	6.9 ± 7.8	11.1 ± 9.0	20.3 ± 15.0
	Turrumote	14.1 ± 13.0	8.2 ± 12.8	21.4 ± 19.6	28.0 ± 20.9
	Weinberg	0 ± 0	8.3 ± 8.6	6.4 ± 7.6	7.6 ± 7.3
	El Hoyo	3.8 ± 9.8	4.2 ± 9.1	0 ± 0	10.9 ± 15.1
Depth	Shallow	3.8 ± 8.8	12.5 ± 12.7	17.4 ± 21.7	23.1 ± 16.7
	Intermediate	9.0 ± 10.5	7.8 ± 10.4	10.1 ± 16.2	11.4 ± 16.0
	Deep	2.8 ± 8.5	6.3 ± 9.0	3.2 ± 6.2	9.2 ± 11.8
Total		5.1 ± 9.5	8.7 ± 11.0	10.1 ± 16.8	14.4 ± 16.0

(see Table 2 caption). In the three remaining surveys, all 16 transects were surveyed at each site. During each survey, all *G. ventalina* colonies within each band transect were checked for disease signs and counted.

Aspergillosis can be difficult to identify in the field as the presence of hyphae in the tissues can not be determined with the naked eye. For this study, ASP was identified by lesions (dead tissue) surrounded by hard, dark purple tissue, considered indicative of the disease in the field (Nagelkerken et al. 1997a, b; Kim and Harvell 2004). This purpling is a general response to stress which is also displayed by a colony affected by abrasion, predation or overgrowth. Any colony experiencing one of these processes (identified in the field during the surveys) was not considered diseased but was categorized separately and included with healthy colonies for statistical analyses.

Differences in prevalence between sampling periods, overall and for reef zones, reef sites and depths, were statistically analyzed using Friedman Repeated-Measures ANOVAs. Signed rank tests were performed to test for differences between seasons within each year. Differences between reef zones, reef sites and depths within seasons were evaluated with Kruskal-Wallis ANOVAs. ANOVAs with significant results were followed with multiple comparisons tests.

RESULTS AND DISCUSSION

Overall, the prevalence of ASP affecting *Gorgonia ventalina* at the sites studied was significantly higher in September 2006 than in March 2005 (X^2=16.294, df=3, p<0.001) and there was high variability in disease prevalence in both space and time (0-28%) (Table 2, Fig. 2a). This high variability has been found in other surveys of ASP prevalence in Puerto Rico and around the Caribbean. Average ASP prevalence values for *G. ventalina* in individual reefs were similar to values from other surveys in Puerto Rico since the 1995 outbreak [Weil et al. 2002 (La Parguera, Puerto Rico, 4-22%, 1999); Toledo-Hernández et al. 2007 (Puerto Rico, 6-18%, 2002], but were lower than many values from around the Caribbean [Kim and Harvell 2004 (Florida Keys, 8-60%, 1997-2003); Mullen et al. 2006 (Mexico, 5-75%, 2000-2001); Nugues and Nagelkerken 2006 (Curaçao, 10-80%, 1995, 2005); Cróquer and Weil 2009; Weil and Cróquer 2009].

While causality is difficult to prove, there is evidence that high temperatures may

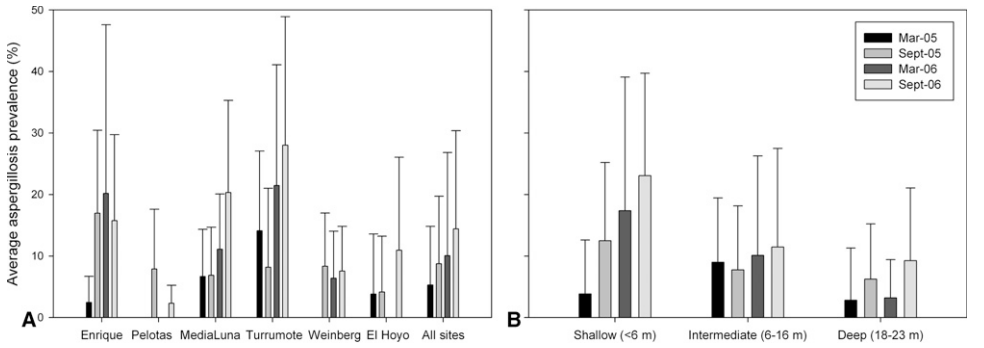

FIG. 2. Grouped bar graphs of average ASP prevalence among *Gorgonia ventalina* colonies (A) at individual reef sites and overall (all reefs) and (B) in different depth ranges from March 2005 to September 2006. See Table 2 caption for n values. Error bars denote standard deviation of transects.

increase prevalence of diseases in general and coral diseases in particular (Harvell et al. 2002; 2007). Prevalence of white syndrome in the Pacific has been linked to high water temperatures (Bruno et al. 2007) and Caribbean yellow band disease prevalence and virulence co-varied significantly with water temperatures in La Parguera over long periods of time (Harvell et al. 2009, Weil unpub. data). Outbreaks of white plague and other syndromes have usually occurred during elevated water temperatures in the Caribbean (Weil 2004). Both the ASP epizootic in 1995 and an earlier epizootic of an unidentified disease affecting sea fans in the early 1980s coincided with rapid temperature increases (Kim and Harvell 2004) and higher temperatures have been reported to benefit the fungus over the host; the growth rate of *Aspergillus sydowii* increases with increasing temperature up to 30°C while the anti-fungal extracts produced by *Gorgonia ventalina* are less effective at such high temperatures (Alker et al. 2001).

Higher disease prevalence was expected during September of each year due to seasonal temperature increases, and lower prevalence was expected in March of 2006. While prevalence values were higher in September of each year (Table 2, Fig. 2a), the difference in prevalence between March and September was only significant in 2006 ($W=1022.000$, $p<0.001$), possibly due to missing values in March 2005. There was no drop in prevalence between September 2005 and March 2006 probably related to the higher-than-normal sea surface water temperatures associated with the 2005 bleaching event. Above average temperatures beginning in the summer of 2005 and lasting through March-April of 2006 likely influenced the higher disease prevalence observed throughout 2006 compared to 2005.

Aspergillosis prevalence varied across reef zones and reefs (Table 2, Fig. 2a). Sea fans at the mid-shelf reefs had significantly higher prevalence than inner and shelf-edge reefs in three of the four surveys ($H_{(March\ 2005)}=13.661$, $H_{(March\ 2006)}=22.298$, $H_{(September\ 2006)}=14.941$, $df=2$, $p<0.001$) while in September 2005, colonies at inner shelf reefs had higher prevalence than those at shelf-edge reefs ($H=7.090$, $df=2$, $p=0.029$). This lower prevalence at the shelf-edge reefs was expected because they are exposed to open Caribbean waters and are farther from point-source coastal anthropogenic influences which may affect susceptibility of local sea fans or limit the input of the spores or hyphae which infect colonies. However, Pelotas, one of the inner-shelf reefs, also had low prevalence values, whereas prevalence at Enrique was more similar to values at the mid-shelf reefs. This may indicate that characteristics of individual reefs had more influence than reef position with respect to ASP prevalence. More reefs (higher replication) in each zone would have to be sampled to characterize definitive trends.

In all sampling periods, disease prevalence at Turrumote was among the highest

of all reefs, peaking at 28.0 ± 20.9% in the summer of 2006 (Fig. 2a). This reef has suffered greatly from white plague and yellow band disease in *Montastraea* spp., and from bleaching events (Weil et al. 2009). Distance from shore may have accounted for some of the differences in ASP prevalence between reefs, but there was not a consistent temporal pattern at all reef sites (Fig. 2a) indicating that there are variable factors affecting prevalence. This spatial and temporal patchiness has been found in other ASP surveys (Kim and Harvell 2004; Nugues and Nagelkerken 2006; Mullen et al. 2006).

Significant differences in disease prevalence over time were found only in colonies located in shallow (<6 m) habitats (X^2=12.162, df=3, p=0.002) (Fig. 2b). During both March (df=2, p<0.01) and September (df=2, p=0.01) of 2006, ASP prevalence was significantly higher in shallow transects (<6 m) than in intermediate transects (6-16 m) of inner and mid-shelf reefs and deeper (18-23 m) transects at the shelf edge (Fig. 2b), contradicting earlier surveys which found that ASP prevalence increased with depth up to 12 meters (Nagelkerken et al. 1997b) or peaked at intermediate depths between 9 - 13.5 m (Mullen et al. 2006). Part of this discrepancy could be due to factors influencing disease prevalence that vary with depth, including host densities, temperature, light quantity and quality, water motion, nutrient influx and turbidity. These factors may affect the dynamics of ASP, but may not be consistent over time and in different areas. Increased water motion may decrease the chance of ASP infection in shallow reef sites (Nagelkerken et al. 1997b); however, these areas may also be warmer and influenced by increased nutrients and more turbid water, which may be more important with respect to disease prevalence.

The overall increasing trend in ASP prevalence throughout this study, particularly in shallow areas of inner and mid-shelf reef sites, suggests that temperature may have been an important large-scale (regional) factor affecting ASP prevalence. Warm water temperatures could also act synergistically with smaller-scale factors, such as nutrient levels and water motion, which vary with depth and distance from shore and may affect disease prevalence.

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