Spatial variability of disease incidence and mortality in the sea fan

Gorgonia ventalina in Puerto Rico (Alcyonacea: Goorgoniidae)

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Abstract: Populations of the common sea fan (Gorgonia ventalina) were decimated by an aspergillosis outbreak throughout the Caribbean two decades ago. Since then, aspergillosis has been considered as the principal cause of mortality in sea fans. However, prevalence and presumably incidence of this disease have been declining in the Caribbean since the mid 1990s. Incidence indicates new cases of disease in previously healthy colonies, while prevalence indicates percent of diseased colonies at a given sample. Most coral disease studies use prevalence rather than incidence to assess the temporal dynamics of diseases. Nevertheless, conclusions based only on prevalence should be handled carefully to avoid misinterpretation. This study was carried out at six reefs in Eastern Puerto Rico. We monitored a total of 448 colonies to (1) obtain estimates of incidence and prevalence of disease and other types of lesions, and (2) to determine causes of sea fan mortality plus their spatial and temporal variation. Three transects (10x1m) were haphazardly placed at each study site. At each transect, every colony was numbered and photographed and its height measured to the nearest cm. Transects were monitored at six months intervals and health status of the colonies was recorded. Also, the colonies were divided into height classes (small, medium and large) for incidence, prevalence and mortality analyses. Incidence and prevalence of disease were low in all reefs, suggesting that disease currently plays a minor role in the regulation of sea fans populations. Detachment was the main cause of mortality, and size structure data suggest that recruitment may compensate for mortality rates in two of the reefs. Spatial differences in size structure and density may be related to environmental and physical characteristics at the reef scale that allow sea fans to reach a safe colony size. Rev. Biol. Trop. 60 (2): 517-526. Epub 2012 June 01.

Key words: Gorgonia ventalina, disease, prevalence, mortality, Caribbean, sea fans.
contribution varying spatially and temporally with habitat. For example, areas with high wave energy such shallow areas in the fore reef should experience high mortality due to detachment (Birkeland 1974).

The demography of gorgonians is strongly size-dependent. Sites subjected to horizontal bedload movement can experience high mortality of small colonies due to smothering by sediments (Yoshioka & Yoshioka 1989, Yoshioka 1994). High mortality of small sea fan colonies can also be expected in sites with high disease incidence, since disease mainly kills small colonies (Toledo-Hernández et al. 2009). However, if detachment is the main cause of mortality, then large colonies of sea fans should exhibit more mortality than small ones, because they are more likely to detach due to an increase in drag force with surface area.

In this study, we followed 448 colonies for one year to evaluate differences in disease incidence and prevalence, and in factors causing mortality across these reefs, particularly disease vs. detachment. We also analyzed small, medium and large colonies separately to identify potential size-based differences in mortality patterns. We hypothesized that if disease incidence and prevalence are low (follow the declining tendency reported in the last five years), detachment and smothering should be the main causes of mortality in large and small colonies respectively. Even so, given a high disease incidence, mortality should be observed mostly in small colonies.

MATERIALS AND METHODS

Study sites: The study was carried out at six reefs in Puerto Rico: Culebrita (CB), Vieques (VI), Humacao (HU), Icacos (IK), Piñeros (IP) and Jobos (JO) (Fig. 1) between September 2006 and September 2007. The study sites can be classified as low-relief, hard-ground reefs dominated by gorgonians following the NOAA’s National Ocean Service benthic classification (Kendall et al. 2001). Most reefs had

![Fig. 1. Study reefs in Puerto Rico. The boxes show amplified views of the geographic locations of each sampling reef. (1) CB Culebrita, VI Vieques Island. (2) IK Icacos, IP Piñeros Island, HU Humacao (3) JO Jobos Bay.](Image)
moderate water transparency, with the exception of JO which showed poor water quality with high sedimentation and low transparency.

Sea fan monitoring: Three transects (10x1m) were haphazardly placed at each study site between 3-5m depth. At each transect, every colony of *G. ventalina* was numbered with an aluminum tag nailed near its base, photographed and its height measured to the nearest cm. Transects were monitored at six months intervals. The health status of each colony was recorded by examination of the photographs. We defined healthy colonies as those with no lesions, purple spots or overgrowth of fouling organisms. Diseased colonies were defined as those with an active disease lesion as described for aspergillosis: a necrotic area surrounded by a purple halo (Petes *et al*. 2003). Colonies with purple spots were those with no necrotic tissue, and having few or numerous small purple spots. Fouled colonies were those showing fouling organisms such as algae or fire corals colonizing the blade. Predated colonies were those showing predation marks by *Cyphoma gibbosum*. Colonies were also divided into three height classes: small (<20cm), medium (20-50cm), and large (50-90cm). Differences in colony density and size (height) among reefs were tested with a Kruskal-Wallis non-parametric analysis (Sokal & Rohlf 1981). Differences in the frequency of health states of sea fans among reefs and among size classes were tested using chi-square analyses.

Incidence and prevalence of disease: Incidence indicates new cases of disease in previously healthy colonies, while prevalence indicates percent of diseased colonies at a given sample. Incidence was calculated as the number of new cases of disease after the first survey for each site (Stedman 2000). Prevalence was calculated as percent of colonies with disease during each survey. Chi square analyses were used to test differences in prevalence of disease among sites and size classes. Changes in the prevalence over time were tested using repeated measures ANOVA with the reefs as independent factors. Prior to analysis, data were pooled by transects and arcsine transformed. Given that purple spots were classified in early literature as a symptom of aspergillosis (Nagelkerken *et al*. 1997, Jolles *et al*. 2002, Mullen *et al*. 2006), we also analyzed prevalence of diseased colonies adding colonies with purple spots in order to compare prevalence levels with the previous literature.

Mortality of sea fans: Mortality was categorized as caused by: (1) disease when 100% of the colony area appeared necrotic, (2) detachment, when the colony disappeared from its known location, or (3) overgrowth when at least 90% of the colony area was covered by algae, fire coral (*Millepora* spp.), or other fouling organisms. Chi square analyses were used to test for differences in the causes of mortality among sites and size classes.

RESULTS

A total of 448 colonies of *G. ventalina* were tagged in the studied reefs. Colony density differed among reefs (Kruskal-Wallis test, H=23.47, p=0.0003, Fig. 2), with Icacos (IK) showing the highest density (6 colonies m\(^{-2}\)±16.25 SE), while, Humacao (1.53 colonies m\(^{-2}\)±0.66 SE), and Jobos (0.97 colonies m\(^{-2}\)±2.60 SE) the lowest. Median colony height differed significantly among reefs, (Kruskal-Wallis test, H=233.079, p<0.001), with Icacos being dominated by small colonies (<20cm), and Humacao and Culebrita by large ones (Fig. 3). A posteriori test for colony height showed Icacos as significantly different from the other reefs, except Vieques; it also showed that Humacao and Culebrita differed significantly from Icacos and Vieques.

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Prevalence and Incidence of disease: Healthy sea fans were the most common state at all reefs (Table 1). Colonies with aspergillosis and purple spots were observed at similar proportions (Table 2). Injuries caused by predation and overgrowth by *Millepora* were rarely observed. No significant differences
were found between diseased and the other lesion categories among sites (Table 2). Disease prevalence (strict definition) varied between 0%-32%. However, this variability was not significantly different (repeated measure ANOVA, prevalence by reef: df=5, F=0.90, p=0.51; by time: df=2, F=1.39, p=0.26; interaction time-reef: df=10, F=1.26, p=0.30). The same tendency was observed among size classes ($\chi^2=3.72$, df=2, p=0.15). If colonies with purple spots are pooled with diseased colonies, prevalence increases from 3.45%-32.7% (Table 3). Although, this difference in prevalence was not significant among sites, it was significant

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**Fig. 2.** Bar diagram showing density of *Gorgonia ventalina* colonies at each study reef. Density is shown as mean number of colonies per 10m$^2$.

**Fig. 3.** Size structure of sea fan colonies per study reef.
The incidence of lesions characterized as aspergillosis was very low, with only eight new cases between the first and second census, and five between the second and third census (Table 4). The mortality varied among reefs ($\chi^2=39.8$, df=5, $p<0.001$) and among size classes ($\chi^2=22.1$, df=2, $p<0.001$). Icacos experienced the highest mortality (29%) and Piñeros the least (0%). Small fans experienced proportionally higher mortality than the medium and large colonies. The main cause of mortality at all reefs was detachment, followed by disease lesions.

### TABLE 1

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
<th>Healthy</th>
<th>Diseased</th>
<th>PS</th>
<th>Fouling</th>
<th>Predation</th>
<th>Millepora</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jobos</td>
<td>29</td>
<td>20</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Vieques</td>
<td>59</td>
<td>37</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Humacao</td>
<td>46</td>
<td>32</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Culebrita</td>
<td>57</td>
<td>25</td>
<td>10</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Piñeros</td>
<td>77</td>
<td>53</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Icacos</td>
<td>180</td>
<td>97</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>45</td>
</tr>
</tbody>
</table>

N=total number of colonies at start of study. Fouling represents overgrowth of algae or bryozoans, predation by Cyphoma and overgrowth Millepora spp, PS=purple spot.

### TABLE 2

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>$\chi^2$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Healthy vs diseased by site</td>
<td>5</td>
<td>9.93</td>
<td>0.077</td>
</tr>
<tr>
<td>2. Healthy vs diseased (including ps) by site</td>
<td>5</td>
<td>9.54</td>
<td>0.089</td>
</tr>
<tr>
<td>3. Healthy vs diseased by size</td>
<td>2</td>
<td>3.72</td>
<td>0.156</td>
</tr>
<tr>
<td>4. Healthy vs diseased (including ps) by size</td>
<td>2</td>
<td>10.2</td>
<td>0.006*</td>
</tr>
<tr>
<td>5. Diseased vs PS vs Fouling vs others by site</td>
<td>15</td>
<td>14.6</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Significant P value is represented with asterisk. Purple spots = ps.

### TABLE 3

<table>
<thead>
<tr>
<th>Initial prevalence</th>
<th>Prevalence at 8 mo</th>
<th>Prevalence at one year</th>
</tr>
</thead>
<tbody>
<tr>
<td>only ASP</td>
<td>ASP + PS</td>
<td>only ASP</td>
</tr>
<tr>
<td>Jobos</td>
<td>0</td>
<td>3.45</td>
</tr>
<tr>
<td>Vieques</td>
<td>4.48</td>
<td>23.72</td>
</tr>
<tr>
<td>Humacao</td>
<td>4.34</td>
<td>21.73</td>
</tr>
<tr>
<td>Culebrita</td>
<td>17.5</td>
<td>31.57</td>
</tr>
<tr>
<td>Piñeros</td>
<td>9.09</td>
<td>20.77</td>
</tr>
<tr>
<td>Icacos</td>
<td>10</td>
<td>19.44</td>
</tr>
</tbody>
</table>

Prevalence was calculated in two ways: (1) only using colonies with aspergillosis (ASP), and (2) including colonies with only purple spot presence (PS) as part of diseased colonies (ASP).
and overgrowth (Table 5). The proportion of detachment varied significantly among reefs ($\chi^2=35.4$, df=5, p<0.001). Icacos experienced twice the expected number of detached colonies, while Jobos and Humacao showed the least. Significant differences were also found in the proportion of detached colonies among sizes ($\chi^2=12.5$, df=2, p=0.002). Small colonies detached proportionally more. Probability of detachment did not vary with the health state of the colony ($\chi^2=3.56$, df=3, p=0.31).

**DISCUSSION**

**Incidence and prevalence of aspergillosis**: Measuring incidence and prevalence are important to understand disease dynamics. Nevertheless, prevalence is more widely used than incidence to assess the temporal dynamics in coral diseases. Incidence requires tagging individual colonies and following them through time to notice changes in health state. Therefore, incidence helps to establish infection rate and pathogen spatial dispersion through a host population. Prevalence data is also used to infer pathogen-host dynamics. However, the conclusions based only on prevalence should be carefully construed to avoid misinterpretation, especially for diseases with low virulence, because diseased individuals are likely to remain in a population for a long time. Thus, prevalence may increase with time even with a low constant incidence.

In our case, incidence of aspergillosis was very low among sites and through time. Thereby, we can infer that infection rate of aspergillosis was very low and no epizootic was occurring in these reefs. The low prevalence observed supports this inference. Prevalence was similar over time at each reef, with no spatial or temporal variation. In fact, there is a general perception that prevalence has decreased since 1994 (Kim & Harvell 2004, Bruno et al. 2011). In the Caribbean, reports

<table>
<thead>
<tr>
<th>Sites</th>
<th>N</th>
<th>Total dead colonies</th>
<th>Death by detachment</th>
<th>Death by disease</th>
<th>Death by overgrowth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jobos</td>
<td>29</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vieques</td>
<td>59</td>
<td>8</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Humacao</td>
<td>46</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Culebrita</td>
<td>57</td>
<td>8</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pineros</td>
<td>77</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Icacos</td>
<td>180</td>
<td>52</td>
<td>48</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

N=initial number of colonies monitored.
suggest geographical variability in the prevalence of aspergillosis. Prevalence of 8.4-22% has been reported for the Florida Keys (Kim & Harvell 2004), and in Venezuela and Bermuda of 10% 12.5% (Weil 2004). However, higher prevalences have been reported in Curacao with 52% (Nugues & Nagelkerken 2006), the Florida Keys with 42-58% (Jolles et al. 2002, Kim et al. 2006), and Yucatan with 30-50% (Mullen et al. 2006). It is possible that part of the variation among these studies is explained by differences in the definition of disease. Tumors, purple spots and colonized tissue can be included within the definition (Petes et al. 2003, Nagelkerken et al. 1997) and sometimes what is considered as diseased colony is not defined. When we pooled colonies with purple spots with diseased ones, prevalence increased dramatically for most of our sites and values were similar to those reported in the studies with high prevalence levels. Thus, a precise definition of the type of lesions is very important to avoid misinterpretation of the results (Work et al. 2008).

Anthropogenic stressors such as elevated temperatures, high sedimentation rates, or low water quality can compromise the immune response of sea fan colonies and make them more susceptible to pathogens or invasive organisms (Bruno et al. 2003, Ward et al. 2007). Given that these factors can exhibit local differences, they may affect infection rate and virulence which may result in spatial variation in disease incidence or prevalence (Kim & Harvell 2004, Mullen et al. 2006, Nugues & Nagelkerken 2007, Ward et al. 2007). In Eastern Puerto Rico, low prevalence and incidence of disease was expected to follow the regional reduction of disease. The lack of temporal and spatial variability found in this study is in agreement with the previous studies. Although the causality of this tendency is more difficult to test, others attribute it to an increase in the immune response of sea fans toward pathogens, or virulence reduction of pathogen(s) as a result of natural selection favoring resistant genotypes after the epizootics that have impacted the Caribbean (Bruno et al. 2011). However, temporal and spatial variability was found in Western Puerto Rico (La Parguera, Flynn & Weil 2009). They explained this variability as resulting from changes in temperature or susceptibility of sea fans to anthropogenic influences due to reduction of water quality. In our case, Culebrita (a site with good water quality) showed the highest prevalence. Jobos and Piñeros, located near-shore and with high sedimentation and poor water quality, did not show high prevalence contrary to expectation. These results suggest that local factors such as composition of the fungal community (Zuluaga et al. 2010) maybe more important than anthropogenic stressors in determining disease prevalence.

**Effect of density and size structure of sea fans on disease:** There are several studies that suggest disease prevalence is density dependent, because neighboring colonies can be more susceptible to infection (Nagelkerken et al. 1997, Jolles et al. 2002). However, our results do not support the conclusion that horizontal transmission of sea fan disease is density dependent. For example, reefs with high density such as Icacos and Piñeros showed disease prevalence that was below sites with low density such as Culebrita. Diseased colonies in transects were haphazardly distributed and no pattern was evident.

The observed spatial variability in sea fan abundance and size structure can be explained by the refuge-size hypothesis (Yoshioka 1994). In our study, dominance of a particular size category was evident at each site. According to the hypothesis, there is a direct relationship between colony size and survivorship. Thus, relatively abundant size classes may reflect local size refuges. For instance, we can explain the lack of small colonies in Culebrita, Piñeros and Humacao reefs as resulting from a recruitment bottleneck and the lack of large ones at Vieques and Icacos reefs suggests either high mortality of large colonies or blade fragmentation. On the other hand, at Humacao the relatively high abundance of large colonies and lack of small ones suggests high survivorship.
of large ones, but low recruitment. In this case, it is possible that the higher depth of this reef (8m) explains this result, because smothering is more likely to occur in deeper sites, because there is less energy (Yoshioka 1994).

Our results also suggest that spatial differences in size structure and density may be related to abiotic factors at the reef scale. Such as, although good water quality was observed in most of our reefs, Jobos, the site with the lowest sea fan density, showed high turbidity and sedimentation. This can be explained, because sedimentation reduces density of sea fans by smothering, especially in small colonies (Yoshioka 1996). On the other hand, high energy wave action may maintain hard substrates free of sediments and promote recruitment by detaching other sessile organisms that compete with sea fans for space (Kojis & Quinn 2001).

Mortality in sea fans: Detachment was the main cause of mortality in sea fans in the studied reefs. Death of colonies by disease or injuries was rare. This is consistent with the low prevalence of disease found in the study sites. Detachment is considered one of the main causes of mortality in most species of gorgonians (Birkeland 1974, Yoshioka & Yoshioka 1991, Toledo-Hernández et al. 2009), and wave action is considered as the principal cause of detachment (Yoshioka & Yoshioka 1989). It also tends to be restricted to shallow water and exposed zones (Whale 1985). Three of our reefs showed low mortality by detachment (Piñeros 0%, Jobos 6.9%, and Humacao 8.7%). Piñeros is a hard ground protected from wave action, and therefore, colonies will suffer less detachment. Jobos was an interesting reef because some of the sea fans were attached to loose stones that may roll over when exposed to high currents or wave action - we observed living colonies moved from their initial location with signs of abrasion. Colonies located in deeper waters (>6m) will be less impacted by wave action, reducing the chance of detachment as observed in Humacao reef.

Health status did not affect the probability of detachment, but colony size did: small colonies were more susceptible. This result is consistent with Toledo-Hernández et al. (2009), who found high mortality in small sea fans especially healthy ones by detachment. Large colonies were less affected by detachment contrary to expectation. They should be more likely to detach due to increase in the drag-force resistance that should be directly proportional to size (Lasker et al. 2003). In our study, the lower probability of detachment observed in large colonies could be explained by size refuge hypothesis. Sites with less energy (currents, waves), may promote growth to a large size; while sites with high energy will promote growth to a relatively smaller size. In general, sea fans are adapted to withstand high energy due to wave action or currents and they are mainly observed in the crest of the reefs. Another explanation, might be small scale variability in reef quality related to protection from high energy. In a reef with “good and bad” spots, in terms of the protection they offer from wave action, the good spots (with high protection) are more likely to be occupied by large colonies than by smaller ones, because of the higher survivorship. We would also expect more turn-over in the bad spots, and thus they would tend to be occupied by small ones as is the case in the Icacos reef.

In conclusion, sea fans populations from Eastern Puerto Rico are not currently affected by an epizootic. It is not known why incidence and prevalence of aspergillosis has declined, but this seems to be a regional tendency. Detachment was the main source of mortality at all reefs, in particular small colonies, contrary to expectation. Wave energy was likely the main cause of detachment given that most of our sites were shallow areas exposed to strong wave energy. Size structure and density could be a response to local physical conditions that allow sea fans to reach a safe colony size. A clear and specific description of disease features will avoid confusion and facilitate comparisons between studies.
ACKNOWLEDGMENTS

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