Epizootiology of Caribbean Yellow Band Disease in La Parguera, Puerto Rico

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Abstract

Caribbean Yellow Band Disease (CYBD) is one of the most important coral diseases affecting *Montastraea* species in the Caribbean. This work includes two studies of the current status of CYBD affecting *Montastraea faveolata* and *M. franksi* in reefs off La Parguera, Puerto Rico from 2008 to 2009. The first study included the study of the spatial and temporal variability in prevalence of CYBD in the important frame-builders *Montastraea faveolata* and *M. franksi*. Diseased and healthy colonies were counted along each of four band transects (10x2m = 20m^2) in each of 3 depth intervals (3-8; 8-15 and > 15m) in two inner- (Pelotas and Enrique) and two mid-shelf (Media Luna and Turrumote) fringing reefs, and two deep bank reefs (Weinberg and Buoy) at the shelf-edge (>18m). Temperature varied normally from 26 to 29 ^{o}C. Contrary to my hypotheses, prevalence of CYBD decreased, although not significantly, from winter 2008 to summer 2009 and it was low when compared to past reports for the wider Caribbean. Intermediate depth (8-15m) habitats showed the highest densities of *M. faveolata* and mean CYBD prevalence decreased (26.9 ± 24.9% to 24.1 ± 23.4%) from 2008-2009 with reef mean values ranged from 0-33%. CYBD prevalence in *M. franksi* decreased (5.4±10.7% to 5.0± 12%) from 2008-2009 at deep (>15m) habitats, where the highest densities of these colonies were found, and mean prevalence ranged from 0-24.4% among reefs. *M. faveolata* populations in the mid-shelf zone showed significantly higher mean CYBD prevalence (28±27%) than inner shelf reefs (9.9±20.6%) and shelf edge reefs (5.3±5.6%) (K-W=31.62; df=2; p=0.00) throughout the study. Mean CYBD prevalence in *M. franksi* was significantly higher (U=1756; p=0.00) at the mid-shelf zone (16.3±31%) compared to shelf-edge zone (1±1.4%). No infected colonies were found in the inner reefs. There was a significant and

positive correlation between densities of *M. faveolata* and CYBD prevalence (Spearman's r=0.7; p=0.00). However, for *M. franksi*, a significantly negative correlation (Spearman's r=-0.5; p=0.04) was found. Results indicate that *M. faveolata* seems to be more susceptible to CYBD compared to *M. franksi* and that deep (>15m) habitats may serve as a refuge from CYBD for both populations. The drop in the proportion of infected colonies in both, *M. faveolata* and *M. franksi* populations could imply either that the disease virulence has dropped, or that there are no more susceptible hosts and therefore, no new infections, or a combination of these. Some of the infected colonies lost more than 90% of their live tissues. Future work should include the study of potential variability in putative pathogens, environmental factors affecting the onset and etiology of this disease and the overall impact on reef communities off La Parguera. The second study included the study of incidence and spatial distribution patterns of CYBD in *M. faveolata* in reefs off La Parguera. The incidence and spatial distribution patterns of CYBD were assessed by counting, tagging, mapping and following through a year all diseased and infected colonies in three 100 m^2 quadrants in two inner, two mid-shelf and two shelf-edge reefs off La Parguera, Puerto Rico. Healthy colonies were checked and photographed every two months from January to December of 2009 to monitor any pattern of disease spread in the population and new diseased colonies. HOBO temperature loggers were deployed to record temperature variability. Temperature increased from 26 in winter to 29 ^{o}C in summer of 2009, which is considered a normal range. Incidence of CYBD was low for all reefs in both seasons, but increased significantly (Sign Test; Z=2.40; p=0.01) from winter to summer with an average of 1.33 newly infected colonies/month in the winter and 2.50 newly infected colonies/month in the summer. Mid-shelf reefs had the highest host abundance and showed significantly higher CYBD incidence (an average of 0.58 infected colonies/month) compared to inner- and shelf-edge zones (KW=9.74; df=2; $p \leq 0.05$). The increased incidence levels in the summer indicate that warmer months seem to favor development of CYBD on *M. faveolata*. Analysis of spatial distribution patterns showed a significant aggregated pattern of CYBD infected colonies and for the whole population (i.e. healthy + diseased) in all reefs at the spatial scales sampled. Aggregated colonies would facilitate spread of infectious agents within populations, but, CYBD has not been showed to be infectious. Similar stressful conditions, then, might trigger the disease in susceptible, aggregated colonies harboring the potential pathogens.

Resumen

La enfermedad coralina banda amarilla en la región del Caribe (CYBD, por sus siglas en inglés) es una de las más dañinas que afectan a los corales del complejo de especies *Montastraea*. Un grupo de bacterias del género *Vibrio* causa esta enfermedad. La enfermedad banda amarilla forma en el coral un anillo de aproximadamente 1-15cm, de aspecto pálido o amarillento, que va desde el centro de la colonia hasta sus bordes matando el tejido coralino. Este trabajo de tesis incluye dos estudios de la CYBD en *Montastraea faveolata* y *Montastraea franksi* en arrecifes de La Parguera, Lajas, Puerto Rico durante el período de 2008 a 2009.

El primer capítulo incluye el estudio de la variabilidad espacial y temporal en la prevalencia (porciento de colonias enfermas en una población) de CYBD en los constructores arrecifales, *M. faveolata* y *M. franksi*. Se contabilizó las colonias enfermas y sanas a lo largo de cada uno de cuatro transeptos de banda (10x2m = 20 m^2), en cada uno de los tres intervalos de profundidad (3-8, llano; 8-15, intermedio y > 15 m, profundo) en dos arrecifes de la zona interior (Pelotas y Enrique), dos arrecifes de la zona intermedia (Media Luna y Turrumote) y dos arrecifes de la zona del borde (Weinberg y Boya) de la plataforma insular de La Parguera. Se muestreó cada año en dos temporadas diferentes para observar diferencias, invierno-primavera y verano-otoño. La temperatura varió de 26 a 29 ^{o}C de una temporada a otra. Hubo una disminución de la prevalencia de CYBD, aunque no significativa, en los meses de invierno-primavera (enero-abril) de 2008 a los meses de verano-otoño (junio-octubre) de 2009. Este valor contrasta con los últimos informes que describen a CYBD en la plataforma insular de La Parguera y el Caribe. Las mayores densidades poblacionales de *M. faveolata* se observaron a

profundidades intermedias (8-15 m). El promedio de prevalencia de CYBD disminuyó del año 2008 al 2009 (26.9 ± 24.9% a 24.1 ± 23.4%, respectivamente) con prevalencias en arrecifes individuales que oscilaron entre 0-33%. La prevalencia de CYBD en *M. franksi* disminuyó del año 2008 al 2009 (5.4±10.7% a 5.0± 12%, respectivamente) en profundidades altas (>15 m), donde encontramos las mayores densidades de estas colonias. Las poblaciones de *M. faveolata* en los arrecifes de la zona intermedia mostraron significativamente mayor prevalencia (28±27%) que arrecifes en la zona interior (9.9±20.6%) y arrecifes en el borde (5.3±5.6%) de la plataforma insular de La Parguera a lo largo del estudio. La prevalencia de CYBD en *M. franksi* fue mayor en la zona intermedia (16.3±31%) en comparación con la zona del borde (1±1.4%) de la plataforma insular. No se encontraron *M. franksi* con señales de CYBD en arrecifes de la zona interior. Hubo una correlación positiva y significativa entre densidades poblacionales de *M. faveolata* y la prevalencia CYBD (Spearman's $r = 0.7$; $p < 0.05$). Sin embargo, para *M. franksi*, la correlación entre densidades poblacionales y prevalencia fue significativamente negativa (Spearman's $r = -0.5$; $p < 0.05$). Los resultados indican que las colonias de *M. faveolata* parecen ser más susceptibles a CYBD en comparación con *M. franksi* y que altas profundidades (> 15m) pueden servir como refugio de CYBD para ambas poblaciones. La disminución de la proporción de colonias infectadas por CYBD en ambas poblaciones podría implicar que la enfermedad ha disminuido, que no hay huéspedes susceptibles, o una combinación de éstas y por lo tanto, no hay nuevas infecciones.

El segundo capítulo incluye el estudio de la incidencia (número de nuevas colonias infectadas por mes) y patrones de distribución espacial de CYBD en *M.*

faveolata, en arrecifes de La Parguera. Se evaluó la incidencia y patrones de distribución espacial de CYBD, contando y elaborando mapas de colonias y monitoreando dichos mapas a través del año 2009. Las nuevas colonias infectadas en 3 cuadrantes de 100 m^2 en cada uno de los dos arrecifes de la zona interior, dos en la zona intermedia y dos arrecifes en el borde de la plataforma insular en La Parguera, fueron fotografiadas cada mes desde enero a diciembre de 2009 para revisar cualquier patrón de enfermedad en la población. La incidencia aumentó significativamente del invierno al verano con un promedio de 1.33 nuevas colonias infectadas/mes en invierno y 2.50 nuevas colonias infectadas colonias/mes en el verano. Los arrecifes de la zona intermedia tuvieron la mayor abundancia de huéspedes y mostraron significativamente mayor incidencia de CYBD (un promedio de 0.58 nuevas colonias infectadas/mes) en comparación con los arrecifes de las zonas interior y del borde de la plataforma insular. Los niveles de mayor incidencia en el verano indican que los meses más cálidos parecen favorecer el desarrollo de CYBD en *M. faveolata*.

Los análisis de los patrones de distribución espacial mostraron un patrón agregado de colonias infectadas con CYBD al igual que toda la población (colonias saludables + enfermas) en todos los arrecifes en las escalas espaciales muestreadas. Las colonias agregadas facilitarían la propagación de agentes infecciosos en las poblaciones, pero CYBD no ha demostrado ser infecciosa en estudios anteriores. Condiciones de alto stress podrían desencadenar la enfermedad en colonias agregadas, susceptibles de albergar patógenos potenciales.

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I want to dedicate this thesis to my family and friends, especially my two children, Kai Bajari and Alicia, my wife Lucila and my mother Carmen, for teaching me how to deal with the obstacles in life and how to fight against all adversity. Also, I want to dedicate this work to the most beautiful place in the world, the archipelago of Puerto Rico, a place which is still waiting for human conscience, peace and freedom. Finally, this work is dedicated to the students of the University of Puerto Rico and the public education system.

The education is a right, not a privilege.

¡¡¡QUÉ VIVAN LOS ESTUDIANTES!!!

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Chapter 1. General Introduction

Coral reefs are one of the most productive ecosystems on Earth (Grigg et al. 1984). Thirty-two of the 34 animal phyla are found in these ecosystems (Porter and Tougas, 2001). This high diversity represents an unexploited source of genetic material and chemically active products useful in medical, pharmaceutical and aquacultural services (Reaka-Kudla, 1997). Coral reefs support valuable fisheries for local consumption and for the aquarium trade (Porter and Tougas, 2001). These reefs provide more than $375 billion annually to the global economy (Pandolfi et al. 2005).

Corals are highly susceptible to stress in the marine environment. Coastal development, sedimentation, nutrient and chemical pollution have been some of the most devastating anthropogenic stressors affecting coral reefs over the last decades (Hughes et al, 2003). These factors along with global warming trends may facilitate the emergence and spread of bacterial, viral, protozoan and fungal pathogens causing coral diseases around the world. It is important to understand that stressors affecting corals could also be affecting coral reef's biodiversity. Nearly 30% of coral reefs worldwide are seriously damaged and close to 60% could be lost by the year 2030 (Hughes et al. 2003; Wilkinson, 2004, 2008).

Even though some of the anthropogenic stressors (e.g., overfishing, pollution and coastal development) can be managed successfully at a local scale, global changes such as water acidification and water temperature increase can accelerate partial/total mortality of susceptible corals (Hughes et al. 2003; Pandolfi et al. 2005; Harvell et al. 2007). Global climate change and anthropogenic stressors have resulted in devastating bleaching and disease outbreaks event in coral reefs around the World over the last three decades

(Hughes et al. 2003; Wilkinson, 2004, 2008). The 1998 massive coral bleaching episode associated with increased sea surface temperatures severely damaged 16% of the worldwide coral reefs (Wilkinson, 2004). Bleaching has been more devastating in Indo-Pacific reefs than in Caribbean reefs (Weil, 2004). In contrast, a higher percent (76%) of coral diseases have emerged within the Caribbean, while only few diseases have been reported for the Indo Pacific (Weil, 2004).

A disease, by definition, is "any impairment to health resulting in physiological dysfunction", involving an interaction between a host, an agent (pathogen, environment, genetics) and the environment" (Martin et al. 1987; Wobeser, 1994). The host will be the one affected, the agent or pathogen will cause the disease and the environment will help in the vulnerability of the host into getting sick.

The increment in infectious diseases in marine environments has been associated with different stressors such as, elevated sea surface temperatures, marine pollution, sedimentation and predation and nutrient enrichment (Harvell et al. 1999, 2007; Bruno et al. 2003; Voss and Richardson, 2006; Weil et al. 2006). However, it has been suggested that there is a non-linear relationship between local environmental quality and diseases in some Caribbean coral reefs. Different flushing rates (e.g., windward vs. leeward reefs) and the high level of potential pathogen connectivity within the Caribbean as a result of its circulation patterns are some of the reasons of this suggested non-linear relationship (Jordán-Dalhgren et al. 2005).

The wider Caribbean is considered a disease hot spot because of the high prevalence and fast emergence of coral diseases (Goreau et al. 1998; Weil et al. 2002; Weil, 2004; Weil et al. 2006; Harvell et al. 2007). Green and Bruckner (2000) reported

29 diseases/syndromes affecting 106 scleractinian species in 54 different nations worldwide. There are about 20 reported diseases affecting 45-zooxanthelated corals and 10 octocorals in the Caribbean (Weil, 2004; Harvell et al. 2007). Disease outbreaks and bleaching events are causing significant mortalities and hence, changing the composition, dynamics and community structure and enhancing the decline of these coral reefs (Weil, 2004; Weil et al. 2006). Two major epizootic events in this region in the early 1980's produced significant mortalities and community changes over large geographic areas. The mass mortalities of the important grazer *Diadema antillarum* (Lessios et al. 1984), and white band disease epizootic affecting reef builders (e.g., *Acropora palmata* and *Acropora cervicornis*) (Gladfelter, 1982), other recent epizootic events include sea fan mortalities produced by aspergillosis (Smith and Weil, 2004), and high diversity of coral mortalities produced by white plague and yellow band diseases have altered reef structures and composition (Weil et al. 2006; Bruckner and Bruckner, 2006; Bruckner and Hill, 2009). New threats (e.g., folliculinid ciliates) that were only reported from the Indo Pacific are now found in the Caribbean (Cróquer et al. 2006).

One of the most recent epizootic events in Caribbean coral reefs is Caribbean Yellow Band Disease (CYBD). It affects important reef-building species such as *Diploria strigosa*, *D. labyrinthiformis*, *Colpophylia natans*, and all three species of the *Montastraea annularis* species complex (Goreau et al. 1998; Santavy et al. 1999; Cervino et al. 2001; Garzón-Ferreira et al. 2001; García et al. 2002; Bruckner and Bruckner, 2006; Weil et al. 2006; Bruckner and Hill, 2009). It has been proposed that a consortium of *Vibrio* initiates Yellow Band Disease in both the Pacific and the Caribbean and mainly affects zooxanthellae (Cervino et al. 2004a,b).

CYBD is characterized by a yellow-discolored irregularly shaped patch on the surface which progresses in diameter (Foley et al. 2005), approximately 1-3cm in width at an increasing rate of a few to several centimeters per month while the inner portion of the lesion dies and then fills with sediment and algae (Santavy et al. 1999; Cervino et al. 2001; Bruno et al. 2003, Gil-Agudelo et al. 2004; Bruckner and Bruckner 2006). Similar to other diseases, CYBD progresses faster during warmer temperatures (Weil, 2004; Cervino et al. 2008; Harvell et al. 2009; Weil et al. 2009a). Moreover, CYBD significantly reduces the reproductive output in infected colonies (Weil et al. 2009b). The disease is distributed from Bermuda to Trinidad, the northern coast of south America and central America and the Gulf of Mexico and prevalence varies significantly across reefs and geographic regions (Santavy et al. 1999; Weil et al. 2002; Jordán-Dahlgren, 2005; Bruckner and Bruckner, 2006; Bruckner and Hill, 2009; Cróquer and Weil, 2009; Weil and Croquer 2009; Weil et al. 2009a). In Mona Island, western coast of Puerto Rico, prevalence levels in the *Montastraea* species complex have increased from less than 1 in 1996 to 50% in 1999 (Bruckner and Bruckner, 2006). Additionally, from 1998 to 2001, outbreaks of CYBD and white plague (WP) affected 30-60% of the *M. annularis* complex colonies in Mona and Desecheo islands (Bruckner and Hill, 2009). In La Parguera, southwest Puerto Rico, prevalence levels increased from 1% in 1999 to 55% in 2007 (Weil et al. 2009a).

The purpose of this study was to document the spatial and temporal variability in incidence, prevalence and spatial distribution patterns of CYBD in *M. faveolata* and *M. franksi* populations in six reefs off La Parguera. Coral reefs in the coastal sector of La Parguera encompass a substantial building block in the natural resources of southwestern

Puerto Rico. These reefs are extremely important for the local economy (i.e. tourism and commercial and recreational fishing activities), and because of the high marine biodiversity they sustain. Moreover, these reefs serve as a natural coastal protection from hurricanes and currents. CYBD affects primarily *Montastraea faveolata and Montastraea franksi* in reefs off La Parguera. These species are two of the most important scleractinian coral and frame builders. This is the first study in Puerto Rico that estimates spatial distribution patterns of a coral disease. Spatial pattern analysis can describe the dynamics of a disease and allow testing of hypotheses regarding mechanisms of infection (Jolles et al. 2002). Studying the spatial distribution patterns of CYBD may help to identify if the disease is infectious among *M. faveolata* and *M. franksi* colonies. The other two components of this study, prevalence and incidence of CYBD at the different zones, habitats and reefs, show the current scenario and dynamics of this disease in southwest Puerto Rico.

1.2 Questions and Hypotheses

A. **Is there significant spatial and temporal variability in YBD incidence (i.e. proportion of new cases of disease per unit time in a population) in *M. faveolata* and *M. franksi* populations in La Parguera?**
    - H_o: There are no significant spatial/seasonal differences in CYBD incidence in *M. faveolata* and *M. franksi* populations in La Parguera.
    - H_a: There are significant spatial and/or seasonal differences in CYBD incidence in *M. faveolata* and *M. franksi* populations in La Parguera.

B. **Is there significant spatial and temporal variation in CYBD prevalence (i.e. proportion of cases of a disease in a population at a specific time) in *M. faveolata* and *M. franksi* populations in La Parguera?**

- H_o: There are no significant spatial/temporal differences in CYBD prevalence in *M. faveolata* and *M. franksi* populations in La Parguera.
- H_a: There are significant spatial/temporal differences in CYBD prevalence in *M. faveolata* and *M. franksi* populations in La Parguera.

C. **Are there significant differences in the spatial distribution of CYBD infected *M. faveolata* and *M. franksi* across the inshore-offshore gradient in La Parguera?**

- H_o: There are no significant differences in the spatial distribution pattern of CYBD infected *M. faveolata* and *M. franksi* populations across the inshore-offshore gradient in La Parguera.
- H_a: There are significant differences in the spatial distribution patterns of CYBD infected *M. faveolata* and *M. franksi* populations across the inshore-offshore gradient in La Parguera.

Chapter 2. Variability in prevalence of Caribbean yellow band disease in La Parguera, Puerto Rico

2.1 Abstract (Chapter 2)

The spatial and temporal variability in prevalence of Caribbean yellow band disease (CYBD) in the important frame-builders *Montastraea faveolata* and *M. franksi* was assessed during summer-fall and winter-spring of 2008 and 2009 in reefs off La Parguera, Puerto Rico. Diseased and healthy colonies were counted along each of four band transects (10x2m = 20m^2) in each of 3 depth intervals (3-8; 8-15 and > 15m) in two inner- (Pelotas and Enrique) and two mid-shelf (Media Luna and Turrumote) fringing reefs, and two deep bank reefs (Weinberg and Buoy) at the shelf-edge (>18m). Temperature varied normally from 26 to 29 ^{o}C. Contrary to our hypotheses, prevalence of CYBD decreased, although not significantly, from winter 2008 to summer 2009 and it was low when compared to past reports for the area and the wider Caribbean. Intermediate depth (8-15m) habitats showed the highest densities of *M. faveolata* and mean CYBD prevalence decreased (26.9 ± 24.9% to 24.1 ± 23.4%) from 2008-2009 with reef mean values ranged from 0-33%. CYBD prevalence in *M. franksi* decreased (5.4±10.7% to 5.0± 12%) from 2008-2009 at deep (>15m) habitats, where the highest densities of these colonies were found, and mean prevalence ranged from 0-24.4% among reefs. *M. faveolata* populations in the mid-shelf zone showed significantly higher mean CYBD prevalence (28±27%) than inner shelf reefs (9.9±20.6%) and shelf edge reefs (5.3±5.6%) (K-W=31.62; df=2; p=0.00) throughout the study. Mean CYBD prevalence in *M. franksi* was significantly higher prevalence (U=1756; p=0.00) at the mid-shelf zone (16.3±31%) compared to shelf-edge zone (1±1.4%). No infected colonies were found in

the inner reefs. There was a significant and positive correlation between densities of *M. faveolata* and CYBD prevalence (Spearman's r=0.7; $p<0.05$). However, for *M. franksi*, a significantly negative the correlation (Spearman's r=-0.5; $p<0.05$) was found. Results indicate that *M. faveolata* seems to be more susceptible to CYBD compared to *M. franksi* and that deep (>15m) habitats may serve as a refuge from CYBD for both populations. The drop in the proportion of infected colonies in both, *M. faveolata* and *M. franksi* populations could imply either that the disease virulence has dropped, or that there are no more susceptible hosts and therefore, no new infections, or a combination of these. Some of the infected colonies lost more than 90% of their live tissues. Surviving ramets are now healthy-looking. Future work should include the study of potential variability in putative pathogens, environmental factors affecting the onset and etiology of this disease and the overall impact on reef communities off La Parguera.

2.2 Introduction

Over the last 30 years diseases in the marine environment have been increasing due to anthropogenic factors and natural stressors (Carpenter et al. 2008; Harvell et al. 2009). Coral diseases are one of the main stressors in Caribbean coral reefs. The Caribbean basin is a "disease hot spot" because of the high number of coral diseases, high prevalence and frequency of outbreaks, high number of susceptible species and faster emergence of new diseases than other regions of the world (Goreau et al. 1998; Green and Bruckner 2000; Weil et al. 2002; Weil, 2004; Weil et al. 2006; Harvell et al. 2007; Weil and Rogers, 2011). Close to 20 diseases affecting 42-zooxanthelated corals and 10 octocorals have been reported for the Caribbean (Weil, 2004; Sutherland et al. 2004), and the emerging and spreading of coral diseases and their prevalence seem to be correlated with elevated water temperatures and bleaching episodes (Harvell et al. 2002; Bruno et al. 2003; Miller et al. 2006; Selig et al. 2006; Bruno et al. 2007; Bruno and Selig, 2007; Muller et al. 2008*;* Harvell et al. 2009; McClanahan et al. 2009; Miller et al. 2009; Weil and Cróquer, 2009; Weil et al 2009a).

Caribbean yellow band disease (CYBD) is a wide-spread and highly prevalent disease affecting coral reefs in the wider Caribbean (Weil et al. 2002; Gil-Agudelo et al. 2004; Weil, 2004; Bruckner and Bruckner 2006; Weil and Cróquer 2009; Weil et al 2009a; Weil and Rogers, 2011). Since its discovery and description over twenty years ago (Santavy et al. 1997; Gil-Agudelo et al. 2004), CYBD distribution have significantly increased to the extent of geographic distribution of susceptible species in the wider Caribbean (Weil et al. 2002; Weil and Croquer 2009; Weil and Rogers, 2011). The putative pathogens have been identified as a consortium of *Vibrio* bacteria in both the Pacific and the Caribbean, which affects the zooxanthellae (Cervino et al. 2004a,b; Cervino et al. 2008), producing the characteristic signs of bleached rings of tissue bordering areas of recent tissue mortality. Although in the Caribbean, the pathogens have been identified from samples of *Montastraea faveolata* only, other important reef species (*M. franksi, M. cavernosa, Diploria strigosa, D. labyrinthyformis, Colpophyllia natans* and *Agaricia agaricites*) have been observed with similar signs (Garzón-Ferreira et al. 2001; Gil-Agudelo et al. 2004). Nevertheless, the *Montastraea annularis* species complex seems to be the most susceptible to this disease (Santavy et al. 1997; Weil, 2004; Gil-Agudelo et al 2004; Bruckner and Bruckner 2006; Bruckner and Hill 2009; Croquer and Weil 2009; Weil and Rogers, 2011).

Prevalence (i.e. number of diseased colonies in a population at a specific time) and virulence (i.e. rate of tissue mortality within infected colonies) have significantly increased in local populations of *Montastraea* spp. and seem to be correlated with increasing sea-water temperatures (Cervino et al. 2008; Bruckner and Hill, 2009; Harvell et al. 2009; Weil et al. 2009a). Prevalence of CYBD has ranged from <2 to 91% (from 1996 to 2009) in many Caribbean localities (i.e. Bermuda, Curacao, Grand Cayman, Grenada, Bonaire, Panamá, Venezuela, Turks and Caicos, Colombia, Puerto Rico and Mexico) (Santavy et al. 1999; Green and Bruckner, 2000; Cervino et al. 2001; Weil et al. 2002; García et al. 2002; Weil, 2004; Jordán-Dahlgren, 2005; Bruckner and Bruckner, 2006; Bruckner and Hill, 2009; Harvell et al. 2009; Weil and Cróquer, 2009; Cróquer and Weil, 2009; Weil et al. 2009a).

In Puerto Rico, CYBD is one of the most prevalent and detrimental diseases affecting scleractinian corals (Ballantine et al. 2008; Weil et al. 2009a). Since 1999, prevalence of CYBD in the *Montastraea faveolata* has increased dramatically with up to 52% of damaged colonies in a single site in Mona Island (Bruckner and Bruckner, 2006) and from 1 to 55% in 2007 in La Parguera (Weil et al. 2009a). After the 2005 bleaching event, CYBD prevalence in *M. faveolata* increased 40% with extensive tissue and colony mortalities associated to this disease, white plague outbreaks and bleaching reaching 60% in some reefs (Weil et al. 2009a).

Differences in prevalence may be due to distribution and virulence of pathogens, local environmental conditions, distribution and abundance of susceptible colonies and/or susceptibility of colonies within the population (Raymundo et al. 2008; Weil and Cróquer, 2009; Weil et al. 2009a). Differences in habitats (depths), sites and zones within a marine area have been important factors when studying prevalence of harmful diseases, such as aspergillosis on *Gorgonia ventalina* (Flynn and Weil, 2009). Weil and Cróquer (2009) found that CYBD on *Montastraea* species was more prevalent at intermediate (5-12m) and deep (>15m) habitats in most Caribbean localities studied. Bruckner and Bruckner (2006) found that this disease was more prevalent at 5-25m habitats in Mona Island. These differences seem to respond to population distribution and densities of susceptible species and environmental conditions in some cases.

The purpose of this study was to assess the spatial and temporal variability of CYBD prevalence in populations of *M. faveolata* and *M. franksi*, two of the most abundant reef-building species, in La Parguera, south-west coast of Puerto Rico. We expected to find differences between habitats (i.e. depths) (due to differential distribution of colonies, changes in light quality and quantity, and water motion); between reef sites and reef zones (due to distance from shore and reef structure), and differences between seasons (due to changes in temperature and day light hours) and years due to changes in virulence and/or colony resistance. The null hypotheses included: $H_0$1= there are not significant differences in CYBD prevalence across depth habitats within reefs; $H_0$2= there are not significant differences in CYBD prevalence between reefs within and across zones; $H_0$3= there are not significant differences in CYBD prevalence between reef zones (inner-shelf, mid-shelf and shelf-edge), and $H_0$4= there are no significant differences in CYBD prevalence between the winter and summer seasons within each year and between years (2008 and 2009); $H_0$5= there is no relationship between CYBD prevalence and densities of susceptible populations of *M. faveolata* and *M. franksi*.

2.3 Materials and Methods

2.3.1 Study area

The south coast of Puerto Rico faces the Caribbean Sea and is characterized by lower wave energy and a wider insular shelf than the north coast (García et al. 2003; Ballantine et al. 2008). Field work for this study was conducted in coral reefs off La Parguera Natural Reserve, on the southwest coast of Puerto Rico. The insular shelf of La Parguera extends 6-8 km offshore. Abundant coral reefs and associated marine communities such as seagrass beds and mangrove forests fringe coral reefs and the coast line. Coral reefs have developed because of oligotrophic waters, broad insular platform, and low river discharges coupled with low human population density in the area in the recent past (Ballantine et al. 2008). Reefs in La Parguera are oriented east to west and reefs could be grouped in three main zones according to distance to the coast line, inner-shelf zone, mid-shelf zone and shelf-edge reef zone (García et al. 1998). Six reefs that have been monitored by the NOAA-CRES project from 2003-2008 were selected for this study. Two reefs each in the inner-shelf zone (Enrique and Pelotas), and mid-shelf zone (Turrumote and Media Luna) and the reefs in the shelf-edge zone (Weinberg and Buoy) were selected (Fig. 2.1; Table 2.1).

2.3.2 Inner-shelf Reefs

Enrique (17°56.658 N and 67°02.213 W) is a fringing reef located 1.5 km from the shore. The reef platform is short (30-50m), depth ranges 1-15m with a steep drop and a moderate coral cover and octocoral density, dominated by *Pseudoterogorgia americana* and *Montastraea* species complex, hydrocorals and zoanthids (Weil, unpubl.data, pers. obs). Pelotas (17°57.442N and 67°04.176W) is a wave-protected reef located 1 km from the shore. An extensive sea grass bed dominates the back reef platform with low coral cover and octocoral density. *Montastraea* species complex and *Pseudopterogorgia americana*, along with other octocorals, hydrocorals and zoanthids dominate the substrate (Weil unpubl.data, pers. obs.). This reef reaches a maximum depth of 15m. These two reefs were selected because of the abundance and distribution of the target species *M. faveolata* and *M. franksi* along the reef depth gradient among other inner-shelf reefs.

2.3.3 Mid-shelf Reefs

Media Luna (17°56.093N and 67°02.931W) is a well developed, wave-exposed; fringing reef located approximately 2.5 km south of the main coast. The reef is approximately 1.7 km long and 686 m wide. It has an extensive shallow platform (1-4m) dominated by octocorals with crustose and submassive corals, zoanthids and CCA. The reef crest and drop off is dominated by massive species such as *M. faveolata, Diploria strigosa, D.labyrinthiformis, Siderastrea siderea, Colpophyllia natans*, and *Porites astreoides*. Reef drops to 20m and the deep areas are dominated by sub-massive and platy species such as *M. franksi, Meandrina meandrites, M. cavernosa, Mycetophyllia aliciae, Undaria humilis* and *Agaricia lamarcki.* Turrumote (17°56.097N and 67°01.130W) is a well developed, wave-exposed fringing reef similar to Media Luna. It is located 2.8 km from the shore and 1.5 km east of Media Luna. The key and reef complex are approximately 780m long and 500m wide. An extensive platform dominated by corals, hydrocorals, CCA, octocorals and zoanthids characterizes the shallow habitat from 0 to 4 m. The reef drops down to 20m where it ends in a sandy bottom. The dropoff is dominated by large colonies of *M. faveolata* and other branching and massive reef-building species (e.g., *Colpophyllia* sp., *Diploria* spp., *Siderastrea* spp. and *Porites* spp., etc.). These reefs were selected because of their high abundance and extended distribution of both *M. faveolata* and *M. franksi* along the reef depth gradient compared to other reefs in this zone.

2.3.4 Shelf-edge reefs

Weinberg (17°53.429N and 66°59.320W) and Old Buoy reef (17°53.11N and 66°59.51W) are both deep spur and groove bank reefs located at the edge of the shelf edge, 8 km offshore. They extend from 18 to 35m deep along the drop-off. . Both reefs are characterized by a high diversity and cover of coral species, and dominated by high densities of medium sized colonies of *M. faveolata*, *M. franksi*, *M. cavernosa*, *D. labyrinthiformis, S. siderea; C. natans* and *Agaricia* spp., crustose coralline algae, sponges and scattered octocorals. These reefs were selected because of their high abundance and extended distribution of both *M. faveolata* and *M. franksi* compared to other reefs in this zone.

2.3.5 Temporal and spatial variability in prevalence of CYBD in *M. faveolata and M. franksi*

Prevalence of CYBD was assessed using the CARICOMP modified, permanent band-transect method (Weil et al. 2002). Number of diseased and healthy colonies of *M .faveolata* and *M. franksi* were counted twice a year along each of four permanent, tagged band transects (10x2m = 20m^2) in each of 3 depth intervals (3-8; 8-15 and > 15m) in the inner and mid-shelf reefs, and 12 band transects between 18 and 25m at the shelf-edge reefs, to assess spatial (along depth profile within reefs, between reefs within zones and across zones) and temporal (winter and summer of 2008 and 2009) variability in CYBD prevalence. Spring and fall months were included in the winter and summer periods, respectively. Disease prevalence for each species was estimated as the number of CYBD diseased colonies over the total number of colonies of *M. faveolata* and *M. franksi* respectively in each band transect. The average prevalence (± SD) was calculated for each depth interval using the normalized prevalence of the four band-transects. The reef average prevalence (and SD) was estimated using data from all 12 band transects. Seasonal variability in sea water temperature was measured using HOBO temperature loggers deployed at shallow (2 m) and deep (15m) habitats in each of the inshore and mid-shelf reefs, and at 20 m in the shelf-edge reefs.

2.3.6 Statistical analyses

The prevalence data did not fulfill the requirements for parametric tests and could not be normalized after arcsine transformations. Significant spatial variability in CYBD prevalence at different scales within each season and each year (across habitats within reefs, reefs within zones and across zones) were evaluated using Kruskal-Wallis ANOVA's. ANOVA's with significant results were followed with multiple comparison tests. Mann-Whitney (U) tests were used to evaluate differences in CYBD prevalence in *M. franksi* across habitats within reefs and between zones because colonies showed signs of the disease only at intermediate (8-15m) and deep (>15m) habitats and at mid-shelf and shelf-edge zones during the period of study (2008-2009). Mann-Whitney (U) tests were also used to evaluate differences in overall prevalence between species. Sign tests were used to test for significant differences between seasons within each year and between years. Differences in temporal variability of average disease prevalence between seasons and years overall and for habitats within reefs, reefs within zones and zones were evaluated using Friedman Repeated Measures ANOVA's. Spearman correlation analyses were used to test the relationship between CYBD prevalence and densities (# colonies/m^2) of *M. faveolata* and *M. franksi*. Statistica 7 software was used to complete the different statistical analyses.

2.4 Results

2.4.1 Temperature variability

Monthly 2008 temperatures increased normally (2.65°C) from winter to summer. In 2009, the temperatures increased slightly higher (3°C) than 2008 during these months but it was also a normal increase (Fig. 2.8). Both individual highest and lowest temperatures were recorded in 2008 with 29.87 °C during August and 25.99 °C during February.

2.4.2 Spatial variability of CYBD prevalence in *M. faveolata* during 2008

During the winter of 2008, prevalence of CYBD in *M. faveolata* was significantly higher at intermediate (8-15m) habitats (pooled data) (31 ± 24.0%) compared to deep habitats (6.0 ± 4.3%) (K-W=23.65; df=2; p=0.00) and shallow (3-8m) habitats (8.31±14.8%) (K-W; p=0.05) (Table 2.2a). No significant differences were found between deep and shallow habitats (K-W; p>0.05).

Mean prevalence of CYBD at intermediate habitats in Pelotas (inner-shelf reef) was 3.6 (±7.1%) (Fig. 2.2a). No infected colonies were found in deeper and shallower habitats. Intermediate habitats at Enrique (inner-shelf reef) showed a higher, but not significant mean prevalence (20.5 ± 14.6%) compared to shallow habitats (3.1 ± 6.2%) (K-W; p>0.05) (Fig. 2.2b). No signs of CYBD were found at deep habitats. Prevalence was also significantly higher at intermediate habitats in Media Luna (41 ± 5.8%) and Turrumote (58.9 ± 24.9%) (mid-shelf reefs) compared to deeper habitats in both reefs

(4.1 ± 8.3% and 12.5 ± 14.4% respectively) and to shallow habitats (5 ±10%) in Media Luna only (K-W; p=0.05 and p=0.01) (Figs. 2.2c and 2.2d). No significant differences were found between deep and shallow habitats in Turrumote (K-W; p>0.05).There was no habitat stratification at the shelf-edge reefs, so no comparisons between habitats were made. Overall average prevalence of CYBD in *M. faveolata* was similar for both the Buoy (3.5±4.5%) and Weinberg (3.8±7.6%) reefs (Figs. 2.2e, 2.2f), and lower than the deep habitats in the mid-shelf reefs.

When data was pooled within reefs, CYBD prevalence was significantly higher in the two mid-shelf reefs, Turrumote (33 ± 26.9%) and Media Luna (15.2 ± 20.2%) compared to the inner reefs, Pelotas (1.2±4.1%) and Enrique (7.9±12.5%), and the shelf edge reefs, Weinberg (3.8±7%) and the Buoy (3.5±4%) (K-W= 25.56; df=5;p=0.00) (Table 2.2a; Fig 2.6a). Within each reef zone, no significant differences were found between Enrique and Pelotas, Turrumote and Media Luna, and Weinberg and the Buoy respectively. Similarly, mean prevalence was higher but not significantly different between Enrique and the two shelf-edge reefs. When pooling data for the reef zones in the inshore-offshore gradient, the mid-shelf zone had significantly higher CYBD prevalence (25.4 ± 24.2%) compared to the inner-reef zone (4.5 ± 9.7%) and the shelf-edge zone (3.7 ± 5.9%) (K-W=20.04; df=2; p=0.00) (Table 2.2a). No significant differences were found between inner and shelf-edge zones.

Contrasting results were found during the summer of 2008. Average prevalence of CYBD was significantly higher at intermediate habitats (22 ± 15.3%) compared to deep (14.8 ± 24.3%) and shallow habitats (7.6 ± 10.4) (K-W= 18.47; df=2; p=0.00) (Table

2.2a). No significant differences were found between deep and shallow habitats (K-W; p>0.05).

In Pelotas, the average prevalence was 3.6 (±7.1%) at intermediate habitats (Fig. 2a). No infected colonies were observed in shallow or deep habitats. Prevalence was significantly higher at intermediate habitats in Enrique (16.1 ± 5.0%) compared to shallow habitats (4.6 ± 9.3%) (K-W; p=0.02) (Fig. 2.2b). No signs of CYBD were found at deep habitats. CYBD prevalence in *M. faveolata* was significantly higher at intermediate habitats (28.8 ± 13%) compared to shallow habitats (5±10%) (K-W; p=0.04) but was not different to deep habitats (15.8 ± 11.1%) in Media Luna (Fig. 2.2c). No significant differences in prevalence were found across depth in Turrumote, although intermediate (39 ± 24%) habitats showed a higher prevalence compared to deep habitats (21 ± 34%) and shallow habitats (15 ± 12%) (K-W; p>0.05) (Fig. 2d). Weinberg had no signs of CYBD and the Buoy had a significantly low CYBD prevalence (1.91 ± 2.2%) (Fig. 2.2e) compared to the deep habitats of the mid-shelf reefs.

Similarly to winter 2008, CYBD prevalence was significantly higher in Turrumote (29.6±34%) compared to all the other reefs but Media Luna (16.5±19%), the other mid-shelf reef. Prevalence was higher but not significant at Enrique (7.9±12.5%) compared to Pelotas (1.2±4.1%) and the Buoy (2 ± 2.28%) (K-W; p>0.05) (Table 2.2a; Fig. 2.6b). When pooling the data within each reef zone, mean CYBD prevalence was significantly higher at the mid-shelf zone (30.5±29.8%) compared to inner (4±7%) and shelf-edge zones (1±1.8%) during the summer of 2008 (K-W=30.93; df=2; p=0.00) (Table 2.2a). No significant differences were found between inner and shelf edge zones.

When all data for 2008 was pooled, prevalence of CYBD was significantly higher at intermediate habitats (26.4 ± 7%) compared to deep (10.4 ± 16.6) and shallow habitats (8.6 ± 12.7%) (K-W= 42.42; df=2; p=0.00) (Table 2.2a). No significant differences were found between deep and shallow habitats. Average prevalence at intermediate habitats in Pelotas was 3.6±7.1%. No colonies were observed with disease signs at deep and shallow habitats. CYBD prevalence was significantly higher at intermediate habitats in Enrique (18.3±5.4%) compared to shallow habitats (3.9±9.3%) (K-W; p=0.02). There were no signs of CYBD prevalence in *M. faveolata* colonies at the deep habitats in Enrique during 2008. Intermediate habitats in Media Luna had significantly higher prevalence (35.1 ± 13%) compared to shallow habitats (5±10%) and deep habitats (15±21.1%) (K-W; p=0.01). Same pattern was observed in Turrumote, where intermediate habitats had significantly higher mean prevalence (50 ± 32%) compared to deep (36 ± 40%) and shallow habitats (25 ± 11%) (K-W; p=0.00) for 2008. Both shelf-edge reefs had low CYBD average prevalence in 2008 (2.7±2.2% and 1.92±0%) for the Buoy and Weinberg respectively) compared to deep habitats in the mid-shelf reefs.

M. faveolata in Turrumote showed a significantly higher mean CYBD prevalence (15.7 ± 33.5%) compared to the other reef sites during 2008 (K-W= 62.23; df=5;p=0.00) (Table 2.2a). At the inner-shelf zone, CYBD prevalence in Enrique (7.4±10.7%) was significantly higher than Pelotas (1.19±4.0%) (K-W; p=0.05). At the mid-shelf zone, CYBD prevalence in Turrumote (31.3±30.5%) was significantly higher compared to Media Luna (15.8±19.4%) (K-W; p=0.02). At the shelf-edge, CYBD prevalence at Buoy (2.3±3.5%) was similar to Weinberg (1.9±5.4%). CYBD prevalence in *M. faveolata* was significantly higher at the mid-shelf zone (28±27%) compared to the inner (4.3±8.6%)

and shelf-edge zones (2.3±4.4%) (K-W=49.74; df=2; p=0.00) (Table 2.2a) for 2008. No significant differences were found between inner and shelf-edge zones.

2.4.3 Spatial variability of CYBD prevalence in *M. faveolata* during 2009

During the winter of 2009, mean prevalence of CYBD in *M. faveolata* was significantly higher at intermediate habitats (23±16.7%) compared to shallow (10.7±6.4%) (K-W= 19.63; df=2; p=0.02) and deep habitats (20± 20%) (K-W; p=0.00) (Table 2.2b). Intermediate habitats in Pelotas (inner-shelf reef) showed a mean prevalence of 3.5±7.1% (Fig. 2.3a) and no disease signs were observed I other depth intervals. Intermediate habitats in Enrique showed a higher (16.1±5.4%) but not significant mean CYBD prevalence (K-W; p>0.05) compared to the shallow (6.2±8.8%) and deep (5±10%) habitats (Fig. 2.3b). Prevalence was significantly higher at intermediate habitats in Media Luna (28.8±14.1%) compared to deep habitats (19.5±14.1%) (K-W; p=0.02) (Fig. 2.3c). Contrary to the summer of 2008, no signs of CYBD in *M. faveolata* were observed in shallow habitats. Intermediate habitats in Turrumote had a higher but not significant mean CYBD prevalence (54.1±53.3%) compared to shallow (15.3±8.5%) (K-W; p>0.05) and deep habitats (42.4±38.7%) (K-W; p>0.05) (Fig. 2.3d). CYBD prevalence was similar at the two shelf edge reefs of 2.5±3.2% and 2.1±4.1% for the Buoy and Weinberg respectively (Figs. 2.3e and 2.3f).

When data was pooled within each reef, Enrique (15 ±28.2%) had a significantly higher prevalence during the winter of 2009 compared to Pelotas (1.2±4.1%) (K-W; p=0.02). At the mid-shelf zone, CYBD prevalence in *M. faveolata* colonies at Turrumote (37.3±38%) was higher compared to colonies in Media Luna (16±16%) but not significant (K-W; p>0.05). At the shelf-edge zone, CYBD prevalence in *M. faveolata* in Weinberg (2.1±4.1%) was similar to Buoy (2.5 ± 3.2%). Turrumote showed a significantly higher CYBD prevalence compared to Pelotas, Buoy and Weinberg (K-W; p=0.00) and was similar to Media Luna (the other mid-shelf reef) and Enrique (inner reef) during winter of 2009 (Table 2.2b; Fig. 2.6c). When data was pooled for each reef zone, mean CYBD prevalence was significantly higher at the mid-shelf zone (26.7 ± 15%) compared to the inner (8.5 ± 20%) and shelf-edge zones (2.28 ± 3.4%) (K-W=18.24; df=2; p=0.00) (Table 2.2b). No significant differences were found between inner and shelf-edge zones.

Similarly to winter 2009, significantly higher prevalence of CYBD in *M. faveolata* was found at intermediate habitats (25.5 ± 15.6%) compared to shallow (12.1 ± 5.1%) and deep habitats (20.4 ± 20%) (K-W= 22.86; df=2; p=0.01) (Table 2.2b). Colonies at intermediate habitats in Pelotas showed a prevalence of 8.9±10.7% (Fig. 2.3a). The deep habitats in Enrique showed a significantly higher CYBD prevalence in *M. faveolata* with 5.1±10% compared to the prevalence at the shallow (6.2±8.8%) and intermediate habitats (16.1±5.4%) (K-W; p=0.04) (Fig. 2.3b). *M. faveolata* colonies at intermediate habitats in Media Luna showed 34.7±12.2% of CYBD prevalence which was significantly higher compared to deep habitats (6.2±12.5%) (K-W; p=0.02) but not significant compared to colonies at shallow habitats (15±19.1%) (K-W; p>0.05) (Fig. 2.3c). *M. faveolata* colonies at deep habitats in Turrumote showed a CYBD prevalence of 54.1±53.3% which was higher but not significant than shallow habitats (15.3±8.5%) (K-W; p>0.05) and intermediate habitats (42.4±38.7%) (K-W; p>0.05) (Fig. 2.3d). *M.*

faveolata colonies at Buoy showed a CYBD prevalence of 9.3±7.0% (Fig. 2.3e) and colonies at Weinberg a prevalence of 7.6±5.2% (Fig. 2.3f).

Average prevalence of CYBD in *M. faveolata* was significantly higher in Turrumote compared to Enrique, Pelotas and Weinberg during summer of 2009 (K-W= 20.42; df=5; p=0.00) (Table 2.2b; Fig. 2.6d). Prevalence was significantly higher in Enrique (14±28%) compared to Pelotas (3±7.1%) (K-W; p=0.04). In the mid-shelf zone, CYBD prevalence was higher but not significant at Turrumote (37.2 ± 38%) compared to Media Luna (19 ± 18%). At the shelf-edge zone, CYBD prevalence increased in *M. faveolata* in both reefs (Weinberg =7.6 ± 5.2% and Buoy = 9.2 ± 7%) compared to 2008 and was not significantly different between them. When pooling data for reef zones for the summer of 2009, mean CYBD prevalence was significantly higher at the mid-shelf zone (28.1 ± 12%) compared to inner (10 ± 20%) and shelf-edge zones (8.4 ± 5.8%) (K-W=13.74; df=2; p=0.05; Table 2.2b) similar to previous seasons in 2008. No significant differences were found between inner and shelf-edge zones.

When all data for 2009 was pooled, significantly higher prevalence of CYBD in *M. faveolata* was found at intermediate habitats (24.3±2%) compared to shallow (11.4±1%) (K-W= 42.37; df=2; p=0.00), but not with deep habitats (20.3±0.3%) (Table 2.2b). *M. faveolata* had an average prevalence of 6.3±10.7% at intermediate habitats in Pelotas. No infected colonies were observed at shallow and deep habitats. Prevalence at the intermediate habitats (16.1±5.4%) in Enrique was significantly higher compared to deep (5±4%) and shallow habitats (6.3±8.8%) (K-W; p=0.01). In Media Luna, prevalence which was significantly higher at intermediate habitats (31.8±12.2%) compared to deep (12.9±12.5%) (K-W; p=0.00) and shallow habitats (7.5±14.9%) (K-W; p=0.00). *M.*

faveolata at deep habitats in Turrumote had significantly higher prevalence (54.1±53.3%) compared shallow (15.3±8.5%) and intermediate habitats (42.4±38.7%) (K-W; p=0.04). At Buoy, CYBD prevalence in *M. faveolata* (5.9±7.0 %) was similar to Weinberg (4.9±5.2%).

CYBD prevalence in *M. faveolata* was significantly higher in Turrumote (37.2±38%) compared to Pelotas (2 ± 5.7%), Weinberg (5.0 ± 4.4%) and Buoy (5.8 ± 5.7%) (K-W= 47.91; df=5;p=0.00; Table 2.2b) but not different from Media Luna (17.4±17%), and Enrique (14±27.4%). Prevalence at Enrique was significantly higher compared to Pelotas (2 ±5.7%) during 2009 (K-W; p=0.00). Reefs at the shelf-edge had lower prevalence of CYBD in *M. faveolata* compared to the other reefs. Buoy (5.8±5.7%) had similar prevalence compared to Weinberg (5.0±4.4%). When pooling data within zones, the mid-shelf zone showed significantly higher prevalence the (27.3±27%) compared to inner (9.9±20.6%) and shelf-edge zones (5.3±5.6%) (K-W=31.62; df=2; p=0.00) (Table 2.2b). No significant differences were found between inner and shelf edge zones.

When all the data was pooled together for La Parguera, CYBD prevalence in *M. faveolata* was significantly higher at intermediate habitats (25.5±24%) compared to shallow (7.9±11.5%) (K-W= 15.17; df=2; p=0.04) and deep habitats (13.4±27%) (K-W; p=0.00). Similarly in other reefs, prevalence was significantly higher overall at intermediate habitats compared to shallow and deep habitats in Enrique (17.3±8% ; 5.1±7% and 13.5±34% respectively) (K-W; p=0.02) Media Luna (33±11%; 6±12% and 13.9±16% respectively) (K-W; p=0.00), Turrumote (46.4±33%; 20.4±10% and

45.5±44% respectively) (K-W; p=0.00). Weinberg and Buoy had only one type of habitat (>15m) so no comparisons across habitats were done.

Overall average CYBD prevalence was significantly higher in Turrumote (29.3±32%) compared to the other reef sites (K-W= 108.8; df=5;p=0.00). Media Luna (15.2±18.4%) had the second higher prevalence followed by Enrique (11.6±20%), the Buoy (4.3±5.1%), Weinberg (3.4±5.4%) and Pelotas (1.7±5%). The mid-shelf zone showed significantly higher prevalence (27.6±28.7%) compared to the inner (7±15.9%) and shelf-edge zones (3.8±5.2%) (K-W=80.49; df=2; p=0.00). No significant differences were found between inner and shelf-edge zones.

2.4.4 Spatial variability of CYBD prevalence in *M. franksi* during 2008

No signs of CYBD were observed in *M. franksi* colonies at shallow habitats during the study period (2008-2009). CYBD mean prevalence was significantly higher at intermediate habitats (21.5±39.7%) compared to deep habitats (6±9.6%) (U=297.0; z=2.42; p=0.00) (Table 2.3a). *M. franksi* populations in Pelotas and Enrique (inner-shelf reefs) showed no signs of CYBD during the study period (2008-2009). Mean prevalence was higher at intermediate habitats in Media Luna (57.5±50.5%) compared to deep habitats (8.5±10.1%), but not significant (U=3.00; p>0.05) (Fig. 2.4a). Similar results were obtained for Turrumote where *M. franksi* had higher prevalence but not significant at intermediate habitats (28.5±48%) compared to deep habitats (10±11%) (U=3.0; p>0.05). At shelf-edge reefs there was only a deep habitat and prevalence was similar in both the Buoy (2±1.6%) and Weinberg (1.8±1.6%) (Fig. 2.4d).

No significant differences in CYBD prevalence in *M. franksi* were found between reefs during the winter of 2008 (K-W= 2.0; df=3; p>0.05) (Table 2.3a; Fig. 2.7a). *M.*

franksi colonies showed no signs of CYBD at the Buoy and prevalence at Weinberg was low (2±1.6%). during the winter of 2008. At the mid-shelf zone, CYBD prevalence at Media Luna (22±37%) was higher but not significant (K-W; p>0.05) compared to Turrumote (15.9 ± 29%). Overall, the mid-shelf zone had significantly higher CYBD prevalence (19 ± 33%) compared to the shelf-edge zone (1 ± 1.4%) (U=104.0; z=2.86; p=0.00) (Table 2.3a).

Similar results were found during the summer of 2008 with higher but not significant prevalence of CYBD at intermediate habitats (18.7±40.3%) compared to deep habitats (6.0±11.9%) (U=306; p>0.05) (Table 2.3a). Within each reef, *M. franksi* had higher prevalence but was not significant at intermediate (25±50%) compared to deep habitats (13.1±11%) (U=5.50; p>0.05) in Media Luna (Fig. 4a) similar to observation in Turrumote, with higher but not significant prevalence at intermediate (50±57%) compared to deep (16±22%) habitats (U=2.0; p>0.05) during this season (Fig. 2.4b). Contrary to the winter, *M. franksi* colonies showed signs of CYBD during the summer (1.1±1.3%), but those in Weinberg disappeared (Fig. 2.4c).

No significant differences in CYBD prevalence in *M. franksi* were found between reefs during summer of 2008 (K-W=5.28; df=2; p>0.05) (Table 2.3a; Fig. 2.7b) with prevalence ranging from a high mean of 15.6±30% in Turrumote and 12.7±28% in Media Luna, to 1.1±1.3% at Buoy. Average CYBD prevalence in *M. franksi* was significantly higher at the mid-shelf zone (14.1±29%) compared to the shelf-edge zone (1±1.0%) (U=91.0; z=3.44; p=0.00) (Table 2.3a).

When data was pooled for 2008, intermediate (20.0±39%) habitats showed a higher but not significant prevalence of CYBD in *M. franksi* compared to deep habitats (6

± 10.7%) (U= 1260; p>0.05; Table 2.3a). Average prevalence was higher but not significantly at intermediate habitats in Media Luna (41±49%) and significantly higher at Turrumote (39±50%) compared to deep habitats (7.4 ± 10.6% and 14 ± 19% respectively) (U=100.00; z=2.90; p=0.02). Both shelf-edge reefs had low and similar CYBD prevalence in *M. franksi* (1.0 ± 1.0% at the Buoy and 1 ± 1.4% at Weinberg).

Data pooled within reefs for the year showed similar patterns with mean CYBD prevalence significantly higher at Turrumote (15.7±33.5%) and Media Luna (17.4±33%) compared to Weinberg and the Buoy during 2008 (K-W= 20.78; df=3;p=0.00) (Table 3). No significant differences in prevalence were found between Media luna and Turrumote and between Weinberg (1±1.4%) and the Buoy (1±1.0%) Turrumote. Average CYBD prevalence in *M. franksi* was significantly higher at the mid-shelf zone (18.6±33%) compared to the shelf-edge zone (1±1.2%) (U=393.0; z=3.1; p=0.00) (Table 2.3a).

2.4.5 Spatial variability of CYBD prevalence in *M. franksi* during 2009

During the winter of 2009 higher prevalence of CYBD in *M. franksi* was measured at intermediate habitats (12±34%) compared to deep habitats (5.2± 12.3%) (U=285; p>0.05) Table 2.3b). No signs of CYBD were observed in *M. franksi* at deep habitats in Media Luna but at intermediate habitats prevalence was 7±9.4% (Fig. 5a). CYBD prevalence was higher but not significant at intermediate habitats in Turrumote (50±57%) compared to deep habitats (17.8±25.3%) (U=2.00; p>0.05) (Fig. 2.5b). Populations of *M. franksi* had similar CYBD prevalence at both Weimberg (1±0.5%) and the Buoy (1±1.2%) (Figs. 2.5c, 2.5d) deep shelf-edge reefs.

No significant differences were found in CYBD prevalence in *M. franksi* between reefs during winter of 2009 (K-W= 12.09; df=3; p>0.05) (Table 2.3b; Fig. 2.7c).

Prevalence ranged from a high 24±38% in Turrumote to lower values in Media Luna (2.3±5.9%), Buoy (0.6±1.2%) and Weinberg (0.5±0.5%). CYBD prevalence was significantly higher at at the mid-shelf zone (13.3±29%) compared to the shelf-edge zone (1±0.8%) (U=120; z=2.78; p=0.01) (Table 2.3b).

During the summer of 2009, higher prevalence of CYBD in *M. franksi* was measured at intermediate habitats (15±33%) compared to deep habitats (5±12%) but were not significant (U= 316; p>0.05) (Table 2.3b). Prevalence was higher at intermediate habitats in Media Luna (10.4±12.5%) compared to deep habitats (2.7±5.5%) but not significant (U=5.50; p>0.05) (Fig. 2.5a). Similarly, prevalence was higher but not significant at intermediate habitats in Turrumote (50±57%) compared to deep habitats (23±22%) (U=2.0; p>0.05) (Fig. 2.5b). Prevalence was low but similar at both Weinberg (1±0.3%) and the Buoy (1.5±3%) at the shelf-edge (Figs. 2.5c and 2.5d).

No significant differences in CYBD prevalence in *M. franksi* between reefs were found in summer of 2009 (K-W= 9.36; df=3; p>0.05) (Table 2.3b; Fig. 2.7d). At the mid-shelf zone, CYBD prevalence was significantly higher at Turrumote (24.4±18%) compared Media Luna (1±3.2%) (K-W; p<0.05). At the shelf edge zone, prevalence was low overall, with higher values at the Buoy (1.8±2.9%) compared to Weinberg (0.2±0.3%) but not significant (K-W; p>0.05), and CYBD prevalence was significantly higher at the mid-shelf zone (12.6±29%) compared to shelf-edge zone (1±2.1%) (U=123.5; z=2.70; p=0.02) for summer of 2009 (Table 2.3b).

When data was pooled for 2009, higher but not significant prevalence in *M. franksi* was observed at intermediate habitats (13±33%) compared to deep habitats (5±12%) (U=1202; p>0.05) (Table 3). In Media Luna, prevalence was similar in both intermediate (5±9.8%) and deep habitats (5±7.5%). *M. franksi* had significantly higher CYBD prevalence at intermediate habitats in Turrumote (50±53%) compared to deep habitats (23±20%) (U=8.0; p=0.00), and it was similar between the Buoy (1.0±2.2%) and Weinberg (1 ± 0.3%) at the shelf-edge.

When data was pooled within reefs, *M. franksi* CYBD prevalence was significantly higher in Turrumote (24.2±30%) compared to Weinberg (1 ± 0.3%) and Buoy (1.0±2.2%) in 2009 (K-W= 19.82; df=3;p=0.00) (Table 2.3b). CYBD prevalence was significantly higher (K-W; $p<0.05$) in Turrumote (24.2±30%) compared to Media Luna (1.7±7%), and there were no significant differences between Buoy (1.2±2.1%) and Weinberg (0.3±0.4%). The mid-shelf zone showed significantly higher CYBD prevalence (13±29%) compared to the shelf-edge zone (1±1.6%) (U=487.5; z=3.20; p=0.00) (Table 2.3b).

When data for both years was pooled together, no significant differences were found between intermediate (17±36%) and deep habitats (5.2±11%) habitats.

Intermediate habitats at Media Luna showed higher but not significant prevalence (23.2±39%) compared to deep habitats (8±9.2%; U=126; $p>0.05$). In Turrumote however, CYBD prevalence was significantly higher at intermediate (45±50%) habitats compared to deep habitats (21±18%; U=36.0; p=0.00).

Overall variability of CYBD prevalence in *M. franksi* was significantly higher in Turrumote (22±35%) compared to Media Luna (7.6±20%), Weinberg (1±1.1%) and Buoy (2.4±1.6%) (K-W= 37.23; df=3;p=0.00). No significant differences were found between reefs at the shelf-edge zone. CYBD prevalence was significantly higher at the mid-shelf zone (16.3±31%) compared to shelf-edge zone (1±1.4%) ($U=1756$; $z= 3.35$; $p=0.00$).

2.4.6 Temporal variability of CYBD prevalence in *M. faveolata*

When data was pooled within habitat for all reefs within each season, no significant differences in mean CYBD were found across seasons in populations of *M. faveolata* at shallow (3-8m) habitats. Mean prevalence varied from 8.31±14.8% in winter to 7.6±10.4% in summer of 2008, down to 5.4±8% in winter and up to 12.1±5.1% in summer of 2009 (Fig. 2.9). At intermediate habitats no significant differences were found in mean CYBD prevalence in *M. faveolata* across seasons. Contrary to previous observations, CYBD prevalence decreased from 31±24% in winter to 15.9±14.1% in summer 2008 and then increased to 23±16.7% in the winter of 2009 and to 25.5±15.6% in the summer (Fig. 2.9)

In the deep habitats CYBD prevalence did not vary significantly across seasons, but, mean prevalence in *M. faveolata* increased from winter 2008 (6.0 ± 4.3 %) to summer (29.6 ± 26.5 %) of 2008 (Sign Test; $z=1.3$; $p>0.05$) (Fig. 2.9) then a slight decrease in winter (20±20%) and summer (20.4±20%) of 2009 (Fig. 2.9).

Pooling all data within each reef and seasons showed no significant differences in CYBD prevalence in *M. faveolata* across seasons in none of the six reefs studied in 2008 and 2009. Prevalence in Pelotas varied from 1.2 ± 4.1% in winter 2008 to 3 ± 7.1% in summer 2009; and from 7.9±12.5% in winter and summer 2008 to 15±28.2% in winter and 14±28% in summer 2009 in Enrique, the two inner reefs. Prevalence varied from 15.2±20.2 and 16.5±19% in winter and summer of 2008 to 16±16% in winter and 19±18% in the summer of 2009 at Media Luna; and from 33±26.9% in winter to

29.6±34%in summer 2008 to 37.3±38% in winter and 37±23% in summer of 2009 (Fig. 10).

CYBD prevalence in *M. faveolata* populations at the two shelf-edge reefs was generally low and no significant differences across seasons was found for both reefs (X^2=0.10; df=3; p>0.05). At the Buoy, prevalence varied from 3.5±4% in winter to 1.1±0.2 in summer of 2008, increasing to 2.5±3.2% in winter of 2008 and to 9.2±7% in summer of 2009. (Fig. 2.10). At Weinberg, prevalence decreased from 3.8±7% in winter to no obvious signs of the disease in the summer of 2008. Then, it increased 2.1±4.1% in the winter and to 7.6±5.2% in the summer of 2009.

CYBD prevalence in *M. faveolata* showed a no significant increase from winter 2008 (4.5±9.7%) to summer 2009 (10±20%) at the inner-shelf zone. There were no significant differences in prevalence among seasons (X^2=5.60; df=3; p>0.05) (Table 2; Fig. 2.11). Prevalence was similar in winter (4.5±9.7%) and summer (4±7%) of 2008, then it increased in winter (8.5±1.6%) and in summer (10±20%) of 2009 (Fig. 2.11). The mid-shelf zone showed a low but not significant increase in CYBD prevalence in *M. faveolata* from winter 2008 (25.4±24.2%) to summer 2009 (28±12%) (X^2=7.50; df=3; p>0.05) (Fig. 2.11). Prevalence increased from winter (25.4±24.2%) to summer (30.5±29.8%) of 2008 but was not significant (Sign Test; z=0.00; p>0.05), then it dropped in winter (26.7±15) of 2009, slightly increasing in the summer (28±12%). At the shelf-edge, prevalence in *M. faveolata* increased not significantly from winter 2008 (3.7±0.5%) to summer 2009 (8.4±5.8%) and no significant differences were found between the four seasons (X^2=4.12; df=3; p>0.05). In this zone, prevalence in *M. faveolata* during 2008 showed a decrease from winter (3.7±0.5%) to summer (1±1.8%)

Then increased slightly in winter (2.28±3.4%) and again in summer (8.4±5.8%). (Fig. 2.11).

When data was pooled within years no significant differences in CYBD prevalence were found for none of the three deep habitats between 2008 and 2009. In shallow habitats mean prevalence was higher (11.4 ± 1%) in 2009 compared to 2008 (8.6 ± 12.7%); at intermediate habitats, mean prevalence was higher (26.4 ± 7%) in 2008 compared to 2009 (24.3 ± 2%); and in deep habitats, CYBD in 2009 (13.8 ± 3%) was higher compared to 2008 (10.4 ± 16.6%) (Table 2.2ab; Fig. 2.12).

When data was pooled for each reef in each year, average disease prevalence in *M. faveolata* at Pelotas was similar for both 2008 (1.19 ± 4.0%) and 2009 (2 ± 5.7%) (Table 2.2ab; Fig. 2.13). Prevalence increased from 2008 to 2009 in Enrique (7±10 to 14±27), Media Luna (15.8 ± 19.4% to 17.4 ± 17%), Turrumote (31.3±30.5% to 37.2±38%) Buoy (2.3±3.5% to 2.4±5.7%) and Weinberg (1.9±2.4% to 2.6±4.4%) (Table 2.2ab; Fig. 2.13).

When data was pooled for each zone each year, mean CYBD prevalence in *M. faveolata* was not significantly different between 2008 and 2009 in neither of the reef zones. Mean prevalence increased from 2008 (4.3±8.6%) to 2009 (9.9±20.6%) at the inner-shelf zone; decreased from 28±27% to 27.3±27% in 2009 at the mid-shelf zone; and increased from 2008 (2.3±4.4%) to 2009 (5.3±5.6%) at the shelf-edge zone (Table 2.2ab; Fig. 2.14).

2.4.7 Temporal variability of CYBD prevalence in *M. franksi*

No signs of CYBD were observed in *M. franksi* in shallow (3-8m) habitats during the period of study. There was a non-significant decrease in CYBD prevalence from winter 2008 (21.5±39.7%) to summer 2009 (15±33%) at intermediate (8-15m) habitats (X^2=6.23; df=3; p>0.05) (Table 2.3ab; Fig. 2.15). Prevalence decreased from winter (21.5±39.7%) to summer (18.7±40.3%) of 2008; and then in winter (12±34%) of 2009, with a slight increase (15±33%) in the summer (Fig. 15). No significant differences in CYBD prevalence were found across seasons at deep (>15m) habitats (X^2=1.39; df=3; p>0.05) (Fig. 2.15). Prevalence varied from 6 ± 9.6% on winter 2008 to 5 ± 12% in summer 2009.

There were no signs of CYBD in *M. franksi* at Pelotas and Enrique during the study. When data was pooled for each reef, no significant differences between the four seasons In Media Luna(X^2=1.92; df=3; p>0.05) (Table 2.3ab; Fig. 2.16 Prevalence in *M. franksi* varied a little from winter (15.9±29%) to summer (12.7±28%) of 2008 (Table 2.3ab; Fig. 2.16), with a sharp but significant decrease to 2.3±5.9% in winter of 2009, and to 1±3.2% in the summer (Sign Test; z=0.5; p>0.05) (Table 2.3ab; Fig. 2.16). No significant differences in CYBD were found across season in Turrumote. Prevalence varied from 22±37% in winter of 2008 to 24.4±18% in summer of 2009, Prevalence decreased from winter (22±37%) to summer (15.6±30%) of 2008, increasing again in winter of 2009 (24.7±36%) and remaining high (24.4±18%) in the summer (Table 2.3ab; Fig. 2.16). No significant differences in CYBD prevalence in *M. franksi* was found across seasons for the Buoy and Weinberg at the shelf-edge. No signs of the disease were observed during winter 2008 (Table 2.3ab; Fig. 2.16). Prevalence was low and varied from 0.6±1.2% in winter to 1.8±2.9 % in summer 2009 at the Buoy, and 0.5± 0.5% in winter to 0.2±0.3% in summer of 2009 at Weinberg (Table 2.3ab; Fig. 2.16).

The mid-shelf zone showed a not significant decrease in CYBD prevalence in *M. franksi* from winter 2008 (19±33%) to summer 2009 (12.6±29%) (X^2=4.20; df=3; p>0.05) (Table 2.3ab; Fig. 2.17). Mean prevalence decreased from winter (19±33%) to summer (14.1±29%) of 2008, and then to winter (13.3±29%) and summer (12.6±29%) of 2009 to (Table 3; Fig. 17). At the shelf-edge zone, CYBD prevalence in *M. franksi* was similar during the four seasons (Table 2.3ab; Fig. 2.17).

When data was pooled for each year, averages CYBD prevalence in *M. franksi* decreased from 2008 (20 ± 39%) to 2009 (13 ± 33%) in intermediate habitats (Table 2.3ab; Fig. 2.18). Average disease prevalence in *M. franksi* decreased from 2008 (6 ± 10.7%) to 2009 (5±12%) in deep habitats (Table 2.3ab; Fig. 2.18).

When data was pooled within each reef, average disease prevalence in *M. franksi* decreased from 17.4 ± 33% in 2008 to 1.7 ± 7% in 2009 at Media Luna (Table 2.3ab; Fig. 2.19). A contrasting pattern was observed for Turrumote where prevalence increased from 15.7 ± 33.5% to 24.2 ± 30%, respectively (Table 2.3ab; Fig. 2.19) (Sign Test; z=1.33; p>0.05). Average prevalence was similar from 2008 (1 ± 1.0%) to 2009 (1.2 ± 1%) at the Buoy and Weinberg (1 ± 1.4% and 0.3 ± 0.4% respectively) (Table 2.3ab; Fig. 2.19). When data from each reef zone was pooled, no significant differences in CYBD prevalence were observed between 2008 and 2009 for the mid-shelf (18.6 ± 33% in 2008 and 13 ± 29% in 2009)and shelf-edge (1 ± 1.2% in 2008 and 1 ± 1.6% in 2009) zones respectively(Table 2.3ab; Fig. 2.20).

2.4.8 Differences in CYBD prevalence between *M. faveolata* and *M. franksi*

CYBD prevalence was significantly higher in *M. faveolata* (25.5±24%) compared to *M. franksi* (17±36%) (U=601.0; z=7.35; p=0.00) at intermediate habitats (Table 2.4).

Similarly, prevalence was higher but not significant in *M. faveolata* (13.4±27%) compared to *M. franksi* (5.2±11%) at deep (>15m) habitats (U=12692; z=0.17; p>0.05) (Table 2.4). No comparisons were done at shallow (3-8m) habitats because no signs of CYBD were observed in *M. franksi* during the period of study. No comparisons between species were done at Pelotas and Enrique because no signs of CYBD were observed in *M. franksi* in these reefs. *M. faveolata* showed a significantly higher CYBD prevalence (15.2±18.4%) compared to *M. franksi* (7.6±20%) (Table 4) (U=746.0; z=3.29; p=0.00) in Media Luna and in Turrumote (29.3±32% compared to 22±35% respectively (Table 2.4) (U=499.0; z=4.91; p=0.00). Prevalence in *M. faveolata* was higher (4.3±5.1%) but not significant compared to *M. franksi* (2.4±1.6%) at the Buoy (Table 2.4) (U=1045.0; z=1.12; p>0.05) and Weinberg (3.4±5.4% and 1.0±1.1% respectively) (U=1104.0; z=0.3; p>0.05). No comparisons were done at the inner-shelf zone because no signs of CYBD prevalence were found in *M. franksi* at this zone during the period of study. CYBD prevalence was significantly higher in *M. faveolata* (27.6±28.7%) compared to *M. franksi* (16.3±31%) at the mid-shelf zone (Table 2.4) (U=2488.0; z=5.78; p=0.00). Similarly to the mid-shelf zone, CYBD prevalence was significantly higher in *M. faveolata* (3.8±5.2%) compared to *M. franksi* (1.0±1.4%) (Table 2.4) at the shelf-edge zone (U=15340.0; z=4.50; p=0.00). When all data was pooled together, prevalence of CYBD for La Parguera averaged 11± 23% for the two years of study.

2.4.9 Relationship between CYBD prevalence and densities of *M.faveolata* and *M. franksi*

Highest average densities of *M. faveolata* overall were found at the mid-shelf zone and intermediate (8-15m) habitats (Table 2.5). Spearman correlation analysis showed a significant positive correlation (between densities of *M. faveolata* and CYBD prevalence Spearman's r=0.7; p=0.00) when all prevalence and density data were pooled within seasons for each reef site (Fig. 2.21). In contrast, there was a significant, but negative correlation (Spearman's r=-0.5; p=0.04) between densities of *M. franksi* and CYBD prevalence when all prevalence and densities data were pooled within seasons for each site (Fig. 2.22). Highest average densities of *M. franksi* overall were found at deep (>15m) habitats and at the shelf-edge zone (Table 2.5).

2.5 Discussion

Overall in this study, CYBD prevalence values in *Montastraea* species complex (e.g. *M. faveolata* and *M. franksi*) were lower than other reports in different localities of the Caribbean where CYBD have affected the *Montastraea* species complex with 40, 56 and 91% in 1997 in the coasts of St. John, Turks and Caicos and Bonaire, respectively (Cervino et al. 2001); 34 and 22% in 2001 at the Gulf of Mexico and the Mexican Caribbean, respectively (Jordán-Dahlgren et al. 2005) and 22% in 1997 in the coasts of Curaçao (Bruckner and Bruckner, 2000). However, in this current study, *Montastraea* species showed higher CYBD prevalence than *Montastraea* species in a study in Panamá in 1996 (0-5%) (Santavy et al. 1999) which CYBD was starting to spread throughout the Caribbean and Venezuela in 2000 (<2%) (García et al. 2002).

Montastraea species complex have been found to be the most affected by CYBD in reefs off La Parguera, Puerto Rico in the last decade increasing from 3.2% in 1998 to 42.7% in 2007, specifically in *M. faveolata* and *M. franksi* at the mid-shelf reefs where densities of these two species are higher than reefs in the inner-shelf and shelf-edge zones (Weil et al. 2009). Prevalence of CYBD has significantly increased since 1999 in reefs off Mona Island (Bruckner and Bruckner, 2006; Bruckner and Hill 2009). Outbreaks of CYBD and white plague from 1998-2001 have affected up to 60 % of *M. annularis* complex in Mona reefs (Bruckner and Hill, 2009). CYBD prevalence in *M. faveolata* increased up to 55% in 2007 in Turrumote where this species lost 50-60% of live tissue at intermediate and deep habitats over 4 years (Weil et al. 2009). Turrumote also showed the highest prevalence of aspergillosis in *Gorgonia ventalina* in a recent study (in La Parguera (Flynn and Weil, 2009), and furthermore, one of the highest white plague disease (WPD) prevalence compared to eight other reefs studied in La Parguera (Weil et al. 2009). This pattern is curious since it is unlikely that populations of the affected colonies would have the highest proportion of susceptible colonies to different diseases in the same reef. A more likely explanation is that some environmental stressor might facilitate the infections to develop in the corals and octocorals in this reef. Perhaps, fresh water seepage or higher sedimentation rates transport associate pathogens in the reef. In the present study, the highest CYBD prevalence observed in an individual site was found in Turrumote. Furthermore, this reef site showed the highest CYBD prevalence in both species through the different seasons. It also had the highest densities of *M. faveolata* overall. This high abundance of potential hosts may explain the high prevalence of CYBD in this species. The positive and significant correlation between overall densities of *M. faveolata* and CYBD prevalence suggests that CYBD is spreading through susceptible colonies of *M. faveolata* mainly at the intermediate (8-15m) habitats and mid-shelf reefs (Turrumote and Media Luna) where CYBD prevalence was higher and the highest densities of these colonies were found. This is damaging for these reefs since *M. faveolata* is one of the most important frame builders in La Parguera and the wider

Caribbean. Several coral reefs in Guam have shown a similar pattern where the coral genera currently most injured by diseases are the ones providing the most structure (Myers and Raymundo, 2009). Contrastingly, the highest densities of *M. franksi* were found in shelf-edge reefs (Buoy and Weinberg) and deep (>15m) habitats, but showed a low CYBD prevalence through the study period. Furthermore, the correlation between overall densities of *M. franksi* and CYBD prevalence was significantly negative (Fig. 20). Bruckner and Bruckner (2006) found higher CYBD prevalence in *M. annularis* compared to *M. franksi* in reefs off Mona Island. The authors suggested that the difference in prevalence amongst *M. annularis* complex may be due to different levels of susceptibility of each species towards YBD or to other factors such as depth or nutrients. In the current study, the higher CYBD prevalence in *M. faveolata* than *M. franksi* in all habitats (depths) within reefs, reefs within zones and zones suggests that *M. franksi* is less susceptible to CYBD. Defense mechanisms or studies on different genotypes of *M. franksi* that may be causing this resistance should be done to sustain this theory. *Gorgonia ventalina* and *Acropora cervicornis* have differential susceptibility to aspergillosis and white band disease, respectively (Dube et al. 2002; Vollmer and Kline, 2008). This may be happening with *M. franksi* and CYBD. Weil and Cróquer (2009) suggested that YBD tended to be more prevalent at 5-12m and >15m habitats in some countries in the wider Caribbean. In the present study, prevalence of CYBD was highest at 8-15m habitats. Weil and Cróquer (2009) recognized that this CYBD distribution found in the different reefs through the Caribbean is due to the combination of 1) host population abundance and distribution, 2) presence of potential pathogens and 3) different environmental factors.

In both years, temperature increased normally from winter to summer. The monthly average temperatures through 2008-2009 did not increase over the maximum tolerance level (30^{o}C) in which zooxanthellate corals usually suffers from bleaching and become more vulnerable to diseases (see Ballantine et al. 2008). A consortium of *Vibrio* spp. which is the apparent causal agent of YBD (Cervino et al. 2008) may set off the disease without the colonies being stressed, but if the sea water temperature increases, *Vibrio* spp. may induce a faster spreading of YBD (Cervino et al. 2008). The significantly negative correlation between densities and CYBD prevalence in *M. franksi* suggests that in deep (>15m) habitats and shelf-edge reefs, where these colonies were mostly seen and the lowest values in prevalence found (even for *M. faveolata*) may be due to a more resistant clade of zooxanthellae in deeper colonies (specially *M. franksi*) were quantity of light is lower and quality is poorer than shallower depths. This clade may be resistant to CYBD as well. Zooxanthellae from *Symbiodinium* sp., clade C, have been found on healthy *M. annularis* tissues while *Symbiodinium* sp., clade A, have been found in tissues of the same species showing YBD signs in Panama (Sutherland et al. 2004). Studies on depth distributions of zooxanthellae populations in *M. faveolata* and *M. franksi* colonies in La Parguera could lead to interesting findings in the study of CYBD. Another possibility of the lower CYBD prevalence found in the deeper habitats may be depth susceptible pathogens and different light conditions or a combination of these (Weil et al. 2009). Prevalence of CYBD showed a general pattern of decreasing from 2008 to 2009. This could mean that may be CYBD is dropping after several years as a dominating disease in reefs off La Parguera (Weil et al. 2009) due to apparently increasing resistant colonies to this disease or may be a shift towards a more virulent disease such as White

Plague Disease (WPD) (pers. obs.). A recent study in La Parguera supports the idea that WPD sustains a highly dynamic and changing bacterial community in the holobiont (Weil et al. 2009). This means that may be this disease is infecting more hosts than CYBD due to the dynamic behavior of other potential pathogens responsible of causing it, besides *Aurantimonas coralicida*.

Table 2.1: Description of the study sites in La Parguera, Puerto Rico (modified from Flynn and Weil, 2009)

Site	Zone	Location (N)	Location (W)	Distance from shore (km)	Depth (m)	Slope	Coral cover
Enrique	I. shelf	17°56.658	67°02.213	1.5	1-14	steep	moderate
Pelotas	I.shelf	17°57.442	67°04.176	1	1-12	steep	low
M.Luna	M. shelf	17°56.093	67°02.931	2	4-16	steep	moderate
Turrumote	M. shelf	17°56.097	67°01.130	2	2-15	steep	low
Weinberg	S. edge	17°53.429	66°59.320	6	18-23	gradual	high
Buoy	S. edge	17°53.110	66°59.510	6	18-23	gradual	high

Table 2.2a: Summary table of the spatial (different scales) and temporal (seasonal) variability of CYBD prevalence (%) (mean ± SD) in *M. faveolata* in La Parguera during 2008. Sample size (number of colonies surveyed) in parenthesis

CYBD prevalence % in *M.faveolata*				
Factor	**Level**	**winter 2008**	**summer 2008**	**total 2008**
Habitat	3-8m	8.31±14.8(145)	7.6±10.4 (133)	8.6±12.7 (278)
	8-15m	31±24.0(270)	21.8 ± 15.3(209)	26.4±7 (479)
	>15m	6.0 ± 4.3 (195)	14.8 ± 24.3 (195)	10.4 ± 16.6(390)
Reef	Pelotas	1.2 ±4.1 (61)	1.2±4.1 (61)	1.19±4.0(122)
	Enrique	7.9±12.5 (114)	7±9 (109)	7.4±10.7(223)
	M. Luna	15.2±20.2(111)	16.5±19 (99)	15.8±19.4(210)
	Turrumote	33±26.9 (171)	29.6±34 (117)	31.3±30.5(288)
	Buoy	3.5±4 (109)	1.1±0.2 (101)	2.3±3.5(210)
	Weinberg	3.8±7 (44)	0 (50)	1.9±5.4(94)
Zone	Inner-shelf	4.5±9.7 (175)	4±7 (170)	4.3±8.6(345)
	Mid-shelf	25.4±24.2(282)	30.5±29.8 (216)	28±27(498)
	Shelf-edge	3.7±0.5 (153)	1±1.8 (151)	2.3±4.4(304)

Table 2.2b: Summary table of the spatial (different scales) and temporal (seasonal) variability of CYBD prevalence (%) (mean ± SD) in *M. faveolata* in La Parguera during 2009. Sample size (number of colonies surveyed) in parenthesis

CYBD prevalence % in *M.faveolata*				
Factor	**Level**	**winter 2009**	**summer 2009**	**total 2009**
Habitat	3-8m	10.7±6.4 (127)	12.1±5.1 (128)	11.4±1(255)
	8-15m	23±16.7 (199)	25.5±15.6(200)	24.3±2(399)
	>15m	14.3±20 (192)	13.5±18 (159)	13.8±3(351)
Reef	Pelotas	1.2±4.1 (61)	3±7.1 (61)	2 ±5.7(122)
	Enrique	15±28.2 (110)	14±28 (111)	14±27.4(221)
	M. Luna	16±16 (89)	19±18 (88)	17.4±17(177)
	Turrumote	37.3±38 (107)	37 ±23 (107)	37.2±38(214)
	Buoy	2.5±3.2 (99)	2.3 7% (89)	2.4±5.7(188)
	Weinberg	2.1±4.1 (52)	2.8 5.2 (65)	2.6±4.4(117)
Zone	Inner-shelf	8.5±1.6 (171)	10±20 (120)	9.9±20.6(291)
	Mid-shelf	26.7±15(196)	28.1±12 (195)	27.3±27(391)
	Shelf-edge	2.28±3.4 (151)	8.4±5.8 (120)	5.3±5.6(271)

Table 2.3a: Summary table of the spatial (different scales) and temporal (seasonal) variability of CYBD prevalence (%) (mean ± SD) in *M. franksi* in La Parguera during 2008. Sample size (number of colonies surveyed) in parenthesis

CYBD prevalence % in *M.franksi*				
Factor	**Level**	**winter 2008**	**summer 2008**	**total 2008**
Habitat	3-8m	0(8)	0(8)	0 (16)
	8-15m	21.5±39.7(48)	18.7±40.3(44)	20.0±39 (92)
	>15m	6±9.6(934)	6±11.9(930)	6±10.7 (1864)
Reef	Pelotas	0(33)	0(33)	0 (66)
	Enrique	0(14)	0(14)	0 (28)
	M. Luna	22±37(41)	12.7±28(47)	17.4±33 (88)
	Turrumote	15.9±29(66)	15.6±30(59)	15.7±33.5 (125)
	Buoy	0(382)	1.1±1.3(364)	1±1.0 (746)
	Weinberg	2±1.6(454)	0(465)	1±1.4 (919)
Zone	Inner-shelf	0 (47)	0(47)	0(94)
	Mid-shelf	19±33(107)	14.1±29(106)	18.6±33 (213)
	Shelf-edge	1±1.4(836)	1±1.0 (829)	1±1.2 (1665)

Table 2.3b: Summary table of the spatial (different scales) and temporal (seasonal) variability of CYBD prevalence (%) (mean ± SD) in *M. franksi* in La Parguera during 2009. Sample size (number of colonies surveyed) in parenthesis

CYBD prevalence % in *M.franksi*				
Factor	**Level**	**winter 2009**	**summer 2009**	**total 2009**
Habitat	3-8m	0(8)	0(8)	0 (16)
	8-15m	12±34(40)	15±33(54)	13±33 (94)
	>15m	5.2±12.3 (904)	5±12(854)	5±12 (1754)
Reef	Pelotas	0(33)	0(33)	0 (66)
	Enrique	0(13)	0(12)	0 (25)
	M. Luna	2.3±5.9(34)	1±3.2(47)	1.7±7 (81)
	Turrumote	24±38(58)	24.4±18(58)	24.2±30 (116)
	Buoy	0.6±1.2(360)	1.8±2.9 (300)	1.2±2.1 (660)
	Weinberg	0.5± 0.5(454)	0.2±0.3(466)	0.3±0.4 (920)
Zone	Inner-shelf	0(46)	0(45)	0(91)
	Mid-shelf	13.3±29(92)	12.6±29(105)	13±29 (197)
	Shelf-edge	1±0.8 (814)	1±2.1(766)	1±1.6 (1580)

Table 2.4: CYBD prevalence (% mean±SD) in *M. faveolata* and *M. franksi* for both seasons and years pooled together

Factor	Level	*M. faveolata*		*M. franksi*	
		% mean	SD	% mean	SD
Habitats	3-8m	7.9	11.5	0	0
	8-15m	25.5	24	17	36
	>15m	13.4	27	5.2	11
Sites	Pelotas	1.7	5	0	0
	Enrique	11.6	20	0	0
	M. Luna	15.2	18.4	7.6	20
	Turrumote	29.3	32	22	35
	Buoy	4.3	5.1	2.4	1.6
	Weinberg	3.4	5.4	1.0	1.1
Zones	Inner-shelf	7.0	15.9	0	0
	Mid-shelf	27.6	28.7	16.3	31
	Shelf-edge	3.8	5.2	1.0	1.4

Table 2.5: Overall average densities (#col./m^2) of *M. faveolata* and *M. franksi* for each factor

Factor	Level	*M. faveolata*	*M. franksi*
Habitat	3-8m	1.7	0.1
	8-15m	2.8	0.5
	>15m	2.3	4.2
Site	Pelotas	0.3	0.1
	Enrique	0.5	0.1
	M. Luna	0.4	0.2
	Turrumote	0.5	0.3
	Buoy	0.4	1.5
	Weinberg	0.2	1.9
Zone	Inner-shelf	0.4	0.1
	Mid-shelf	0.5	0.2
	Shelf-edge	0.3	1.7

Figure 2.1: Map of the study sites in La Parguera, Puerto Rico

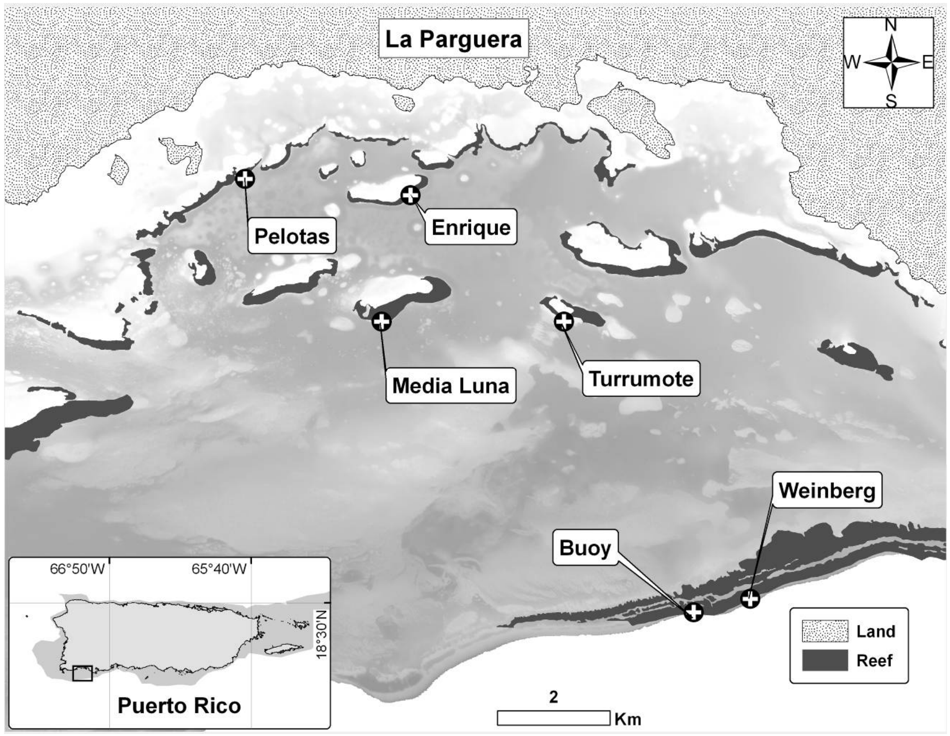


Figure 2.2a-f: Grouped bar charts of % CYBD prevalence (mean ± SE) among *M. faveolata* colonies across habitats within reefs for winter and summer 2008

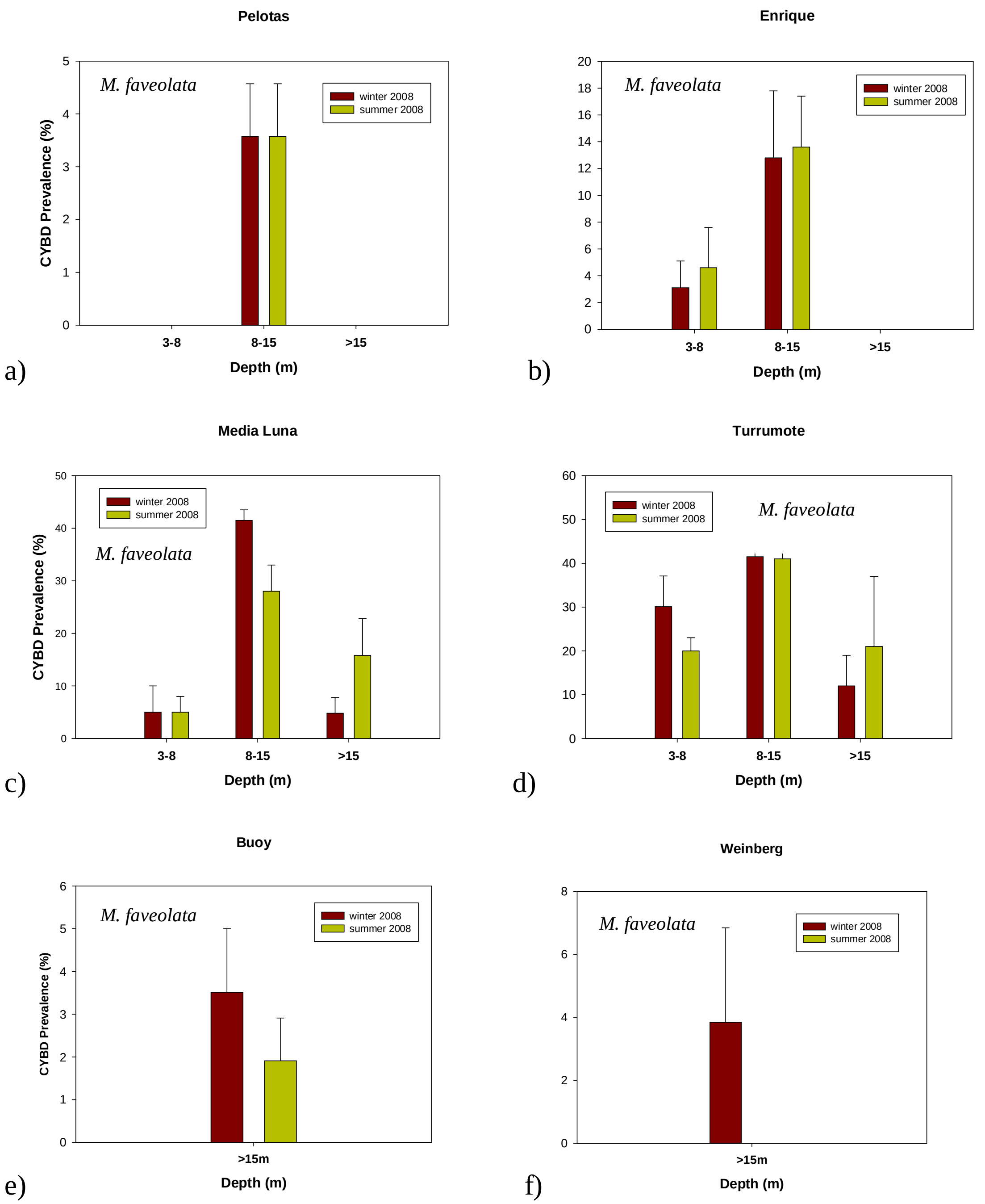
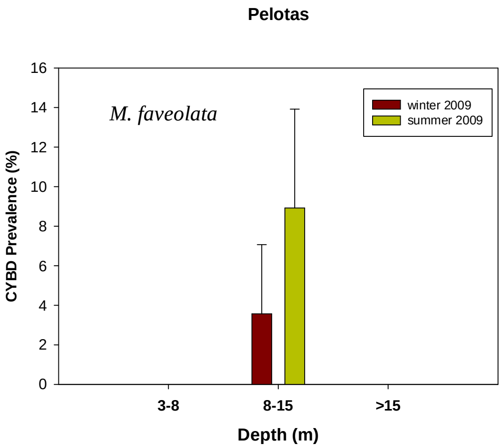
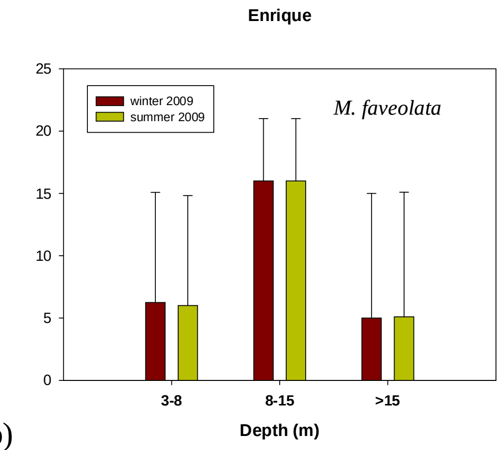


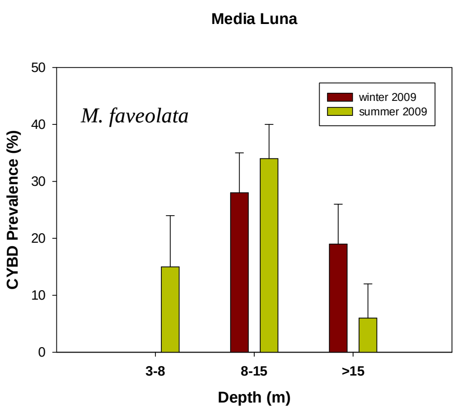
Figure 2.3a-f: Grouped bar charts of % CYBD prevalence (mean ± SE) among *M. faveolata* colonies across habitats within reefs for winter and summer 2009



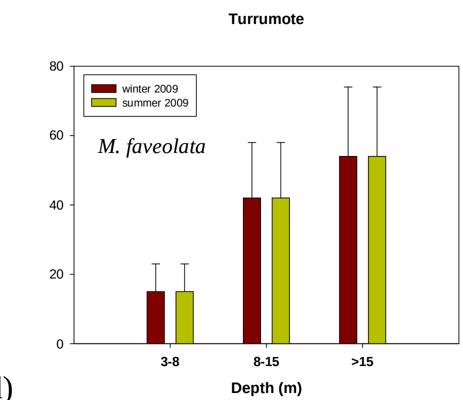
a)



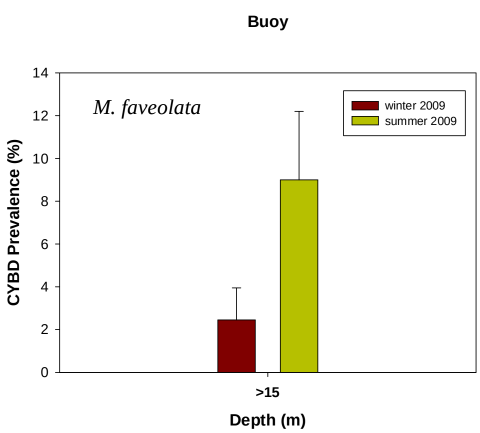
b)



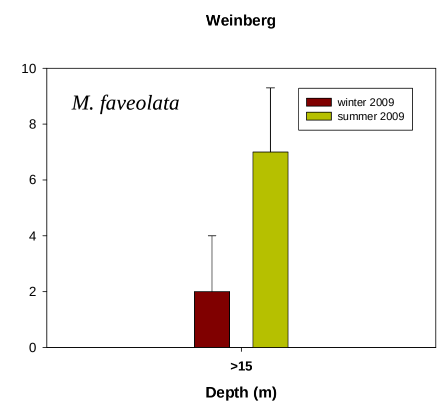
c)



d)



e)



f)

Figure 2.4a-d: Grouped bar charts of % CYBD prevalence (mean ± SE) among *M. franksi* colonies across habitats within reefs for winter and summer 2008

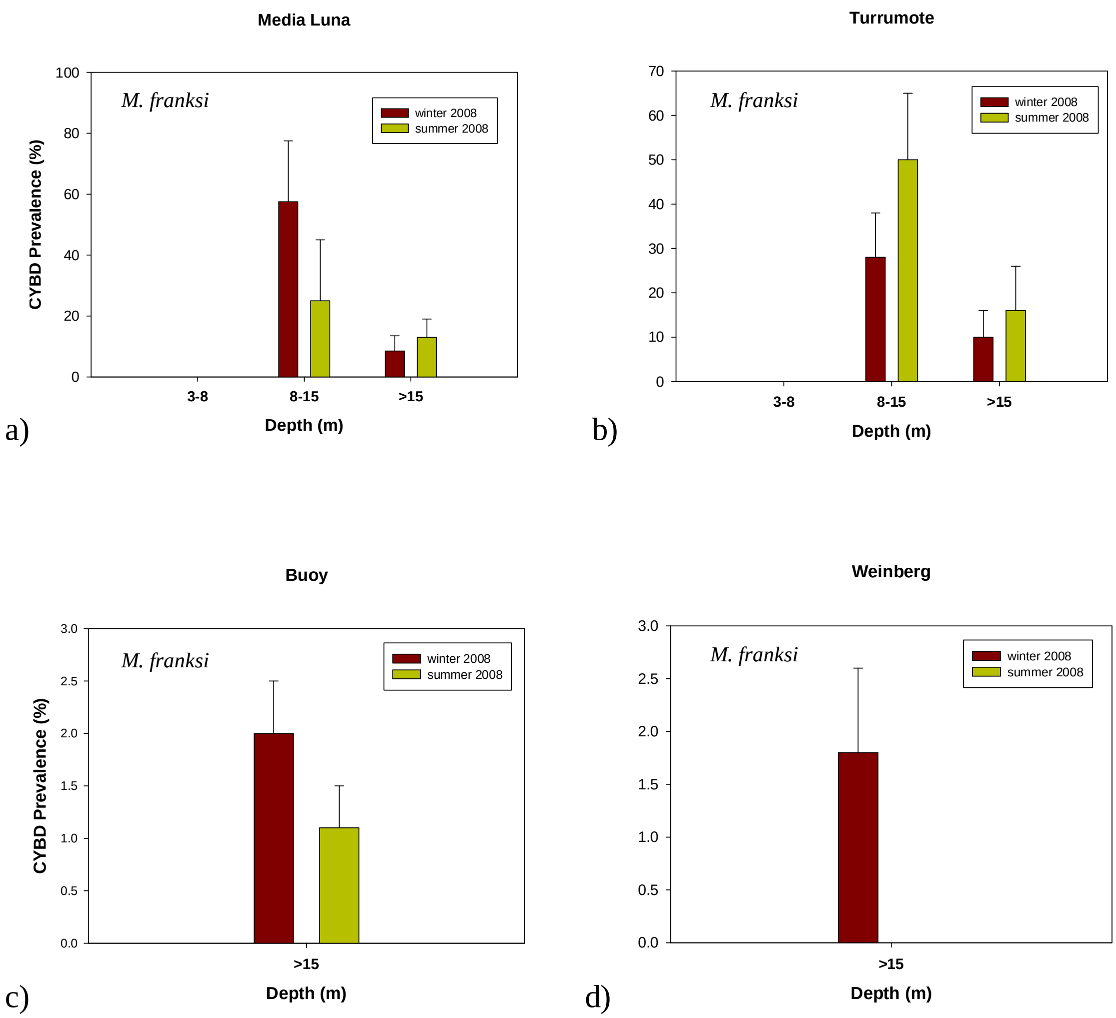
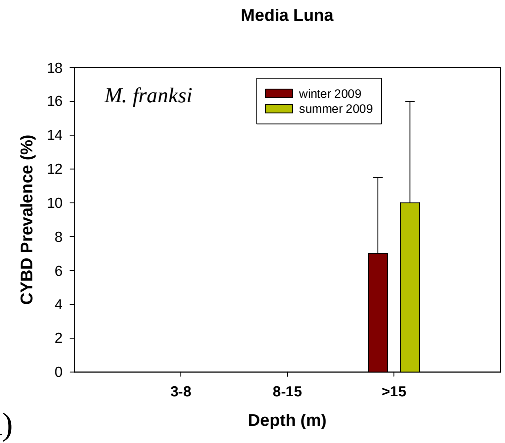
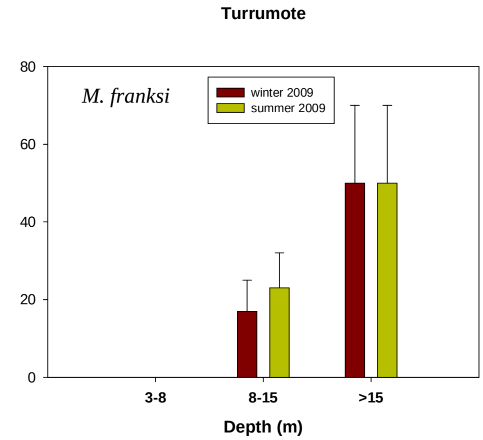


Figure 2.5a-d: Grouped bar charts of % CYBD prevalence (mean ± SE) among *M. franksi* colonies across habitats within reefs for winter and summer 2009



a)



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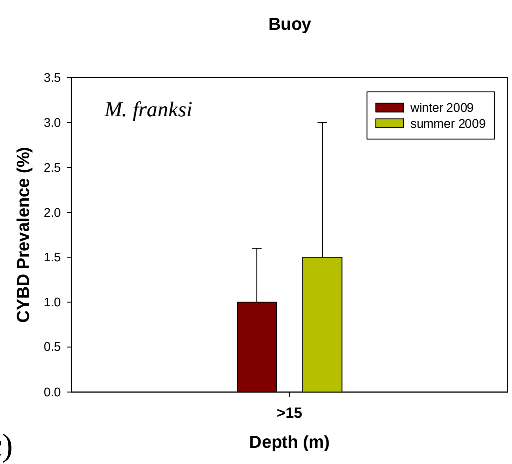
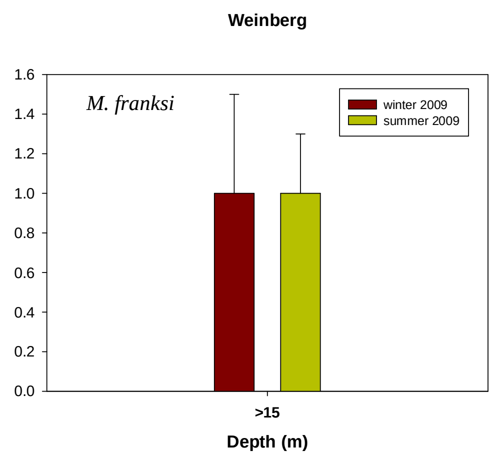


c)



d)

Figure 2.6a-d: Spatial variability of CYBD prevalence (mean ± SE) in *M. faveolata* between reefs within seasons. (Different letters denote significant differences between groups)

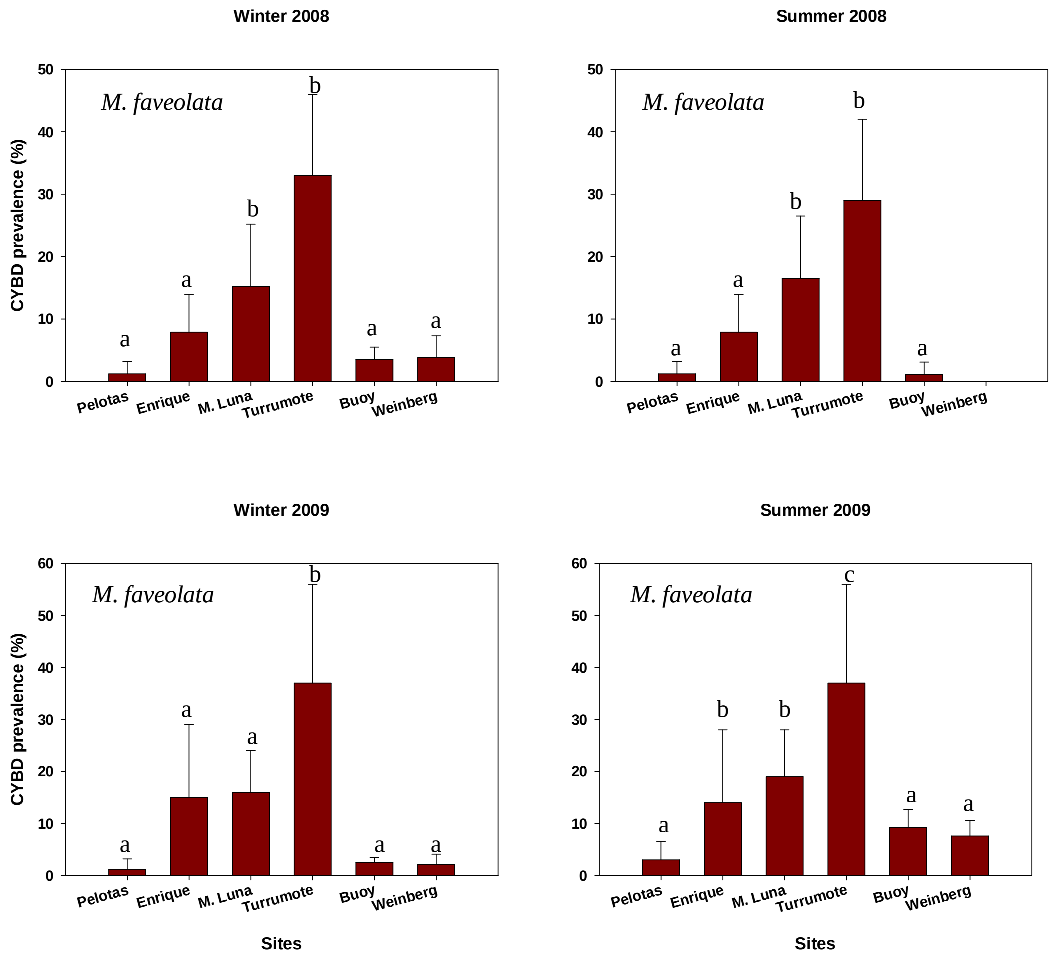


Figure 2.7a-d: Spatial variability of CYBD prevalence (mean ± SE) in *M. franksi* between reefs within seasons. (Different letters denote significant differences between groups)

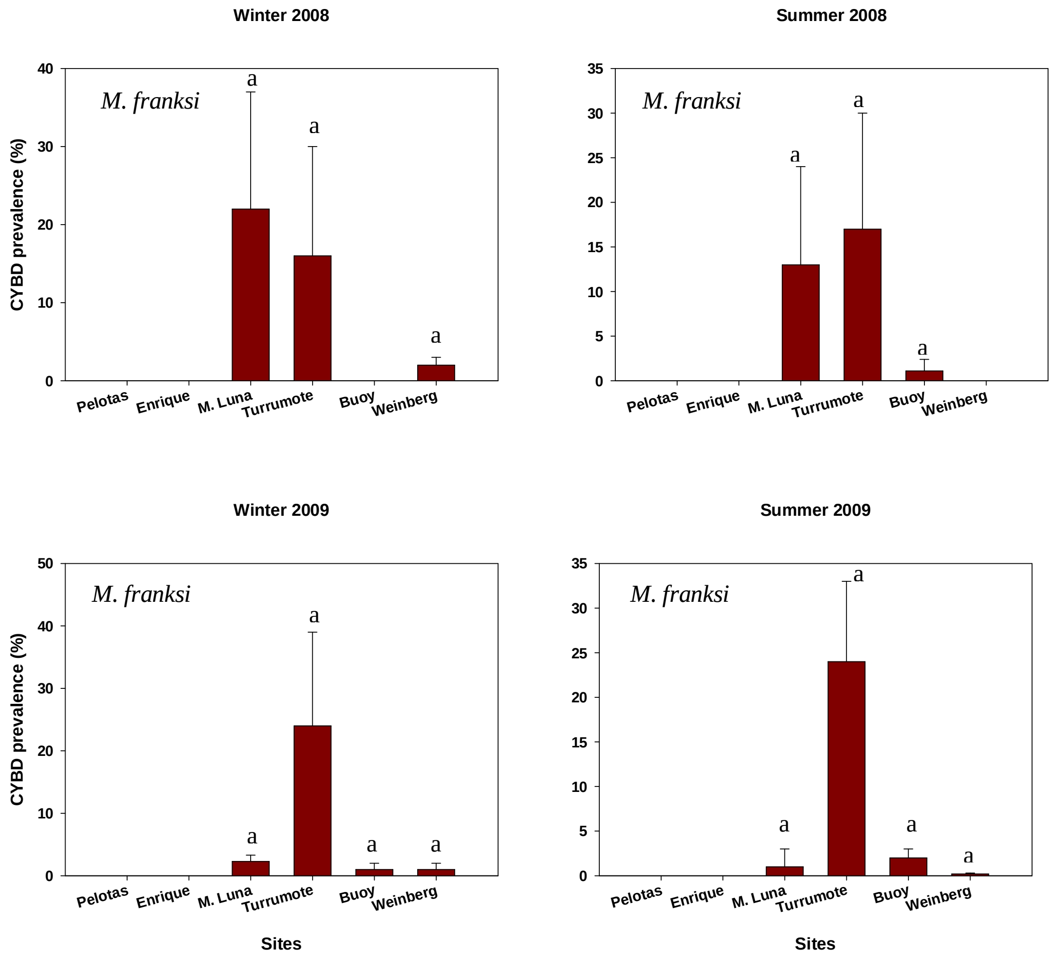


Figure 2.8: Line graph of monthly average seawater temperatures from January 2008-February 2010 in La Parguera, Puerto Rico.

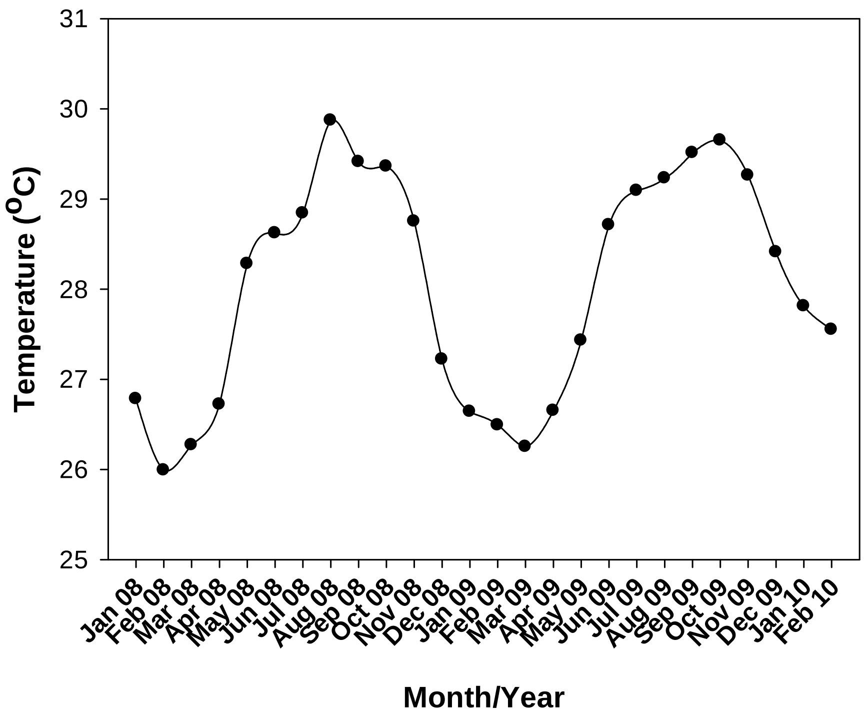


Figure 2.9: Grouped bar charts of % CYBD prevalence (mean ± SE) in *M. faveolata* across habitats within reefs between the four seasons of study (winter 2008-summer 2009)

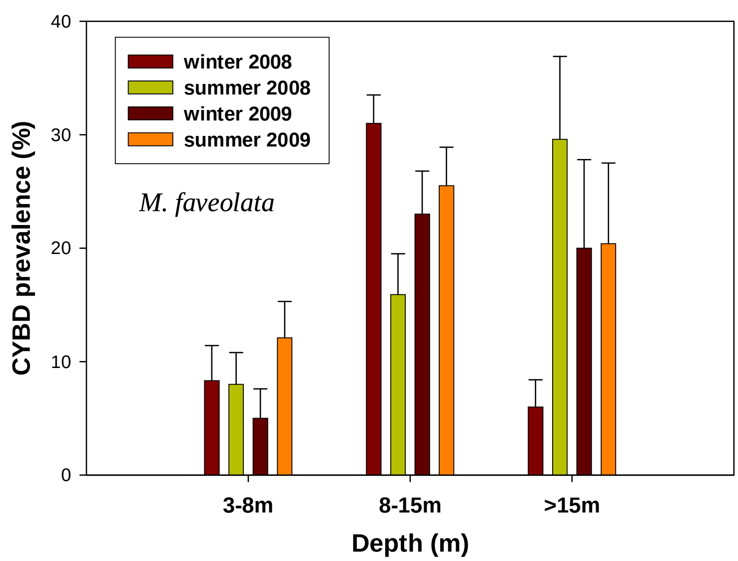


Figure 2.10: Grouped bar charts of % CYBD prevalence (mean ± SE) in *M. faveolata* reefs within zones between the four seasons of study (winter 2008-summer 2009)

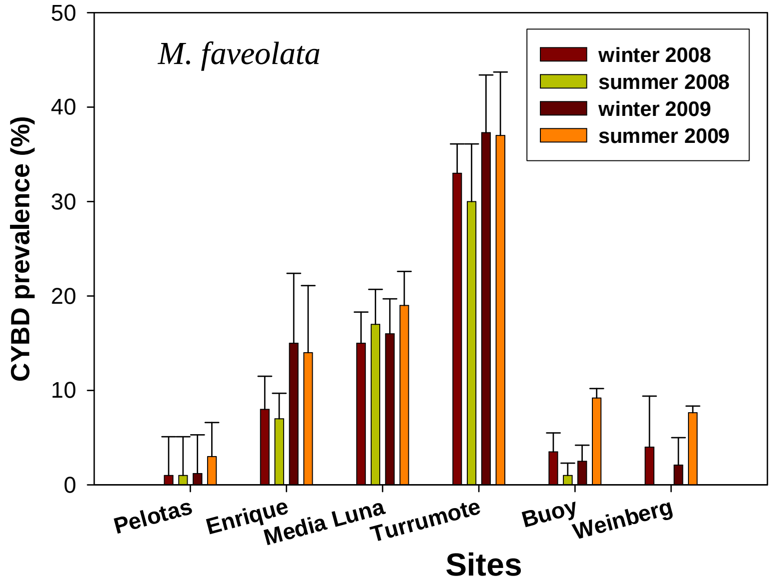


Figure 2.11: Grouped bar charts of % CYBD prevalence (mean ± SE) in *M. faveolata* between zones among the four seasons of study (winter 2008-summer 2009)

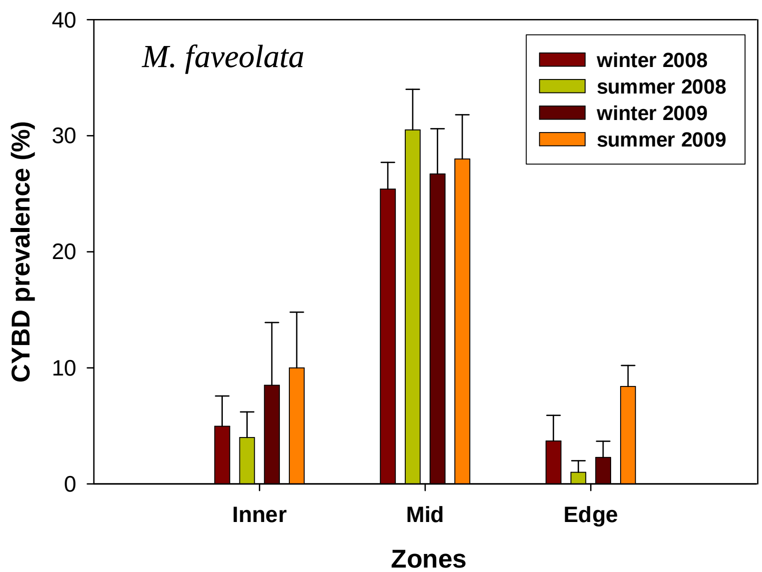


Figure 2.12: Grouped bar charts of % CYBD prevalence (mean ± SE) in *M. faveolata* across habitats within reefs between years 2008 and 2009

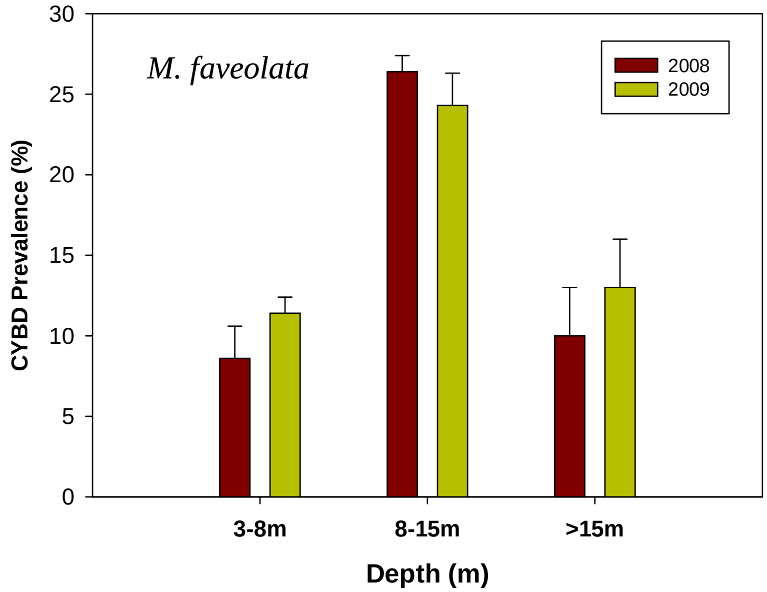


Figure 2.13: Grouped bar chart of % CYBD prevalence (mean ± SE) in *M. faveolata* between sites for 2008 and 2009

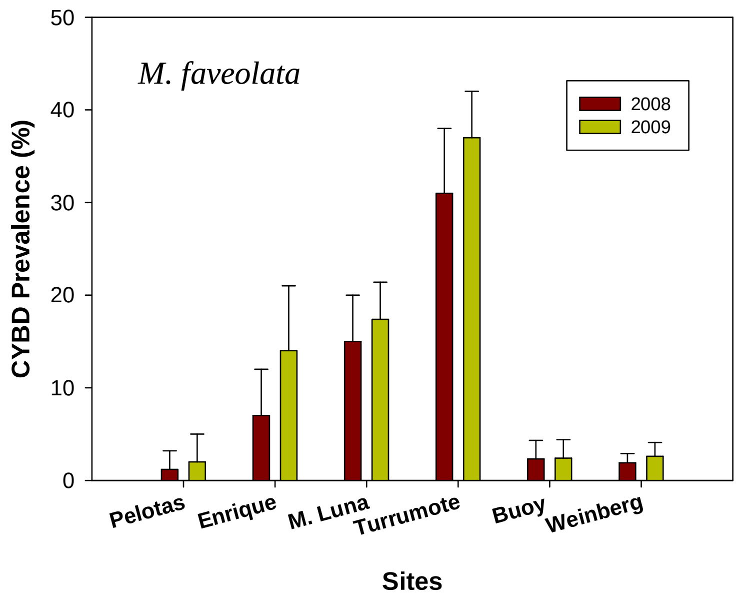


Figure 2.14: Grouped bar chart of % CYBD prevalence (mean ± SE) in *M. faveolata* between zones for 2008 and 2009

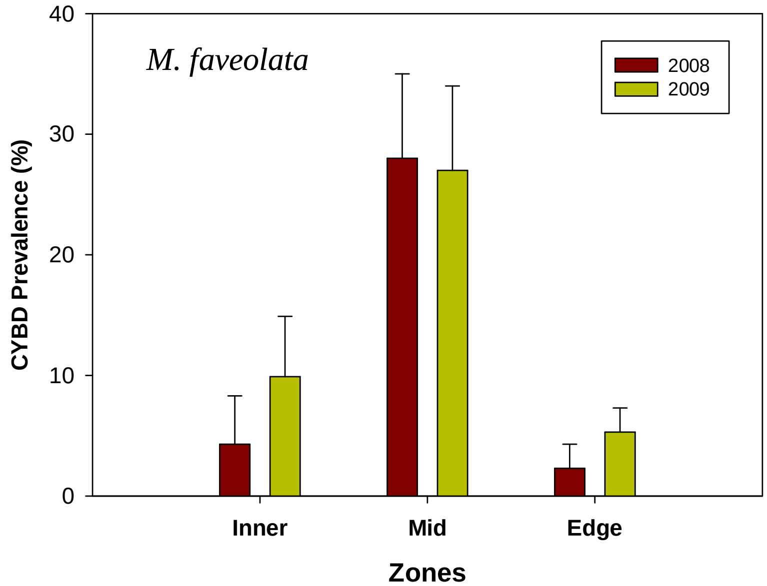


Figure 2.15: Grouped bar chart of % CYBD prevalence (mean ± SE) in *M. franksi* across habitats within reefs between the four seasons of the study (winter 2008-summer 2009)

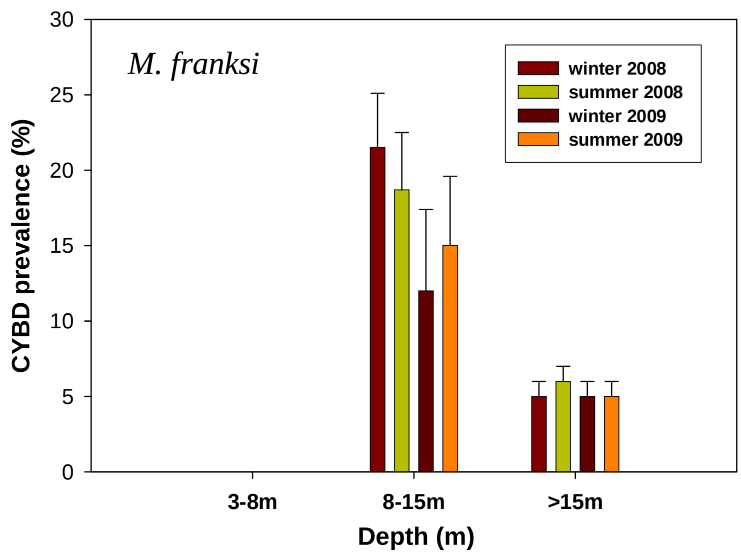


Figure 2.16: Grouped bar chart of % CYBD prevalence (mean ± SE) in *M. franksi* reefs within zones between the four seasons of the study (winter 2008-summer 2009)

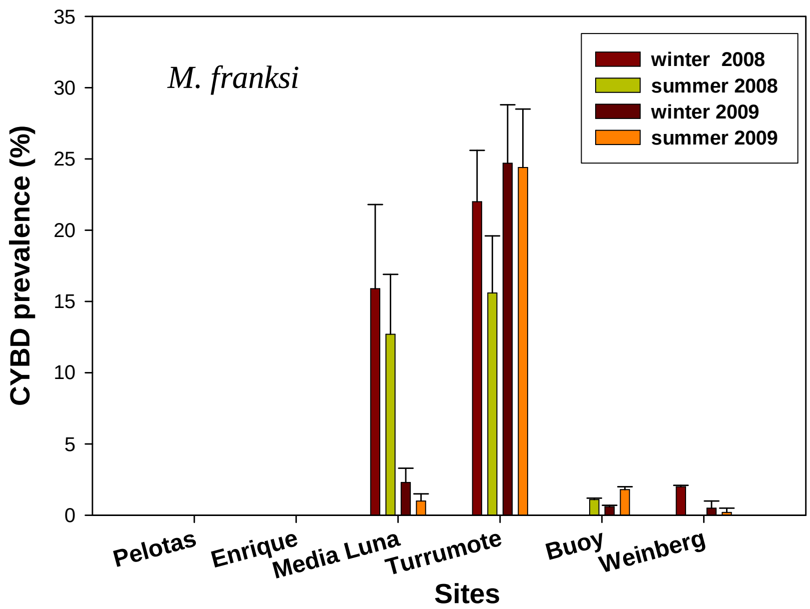


Figure 2.17: Grouped bar chart of % CYBD prevalence (mean ± SE) in *M. franksi* across habitats within reefs between the four seasons of the study (winter 2008-summer 2009)

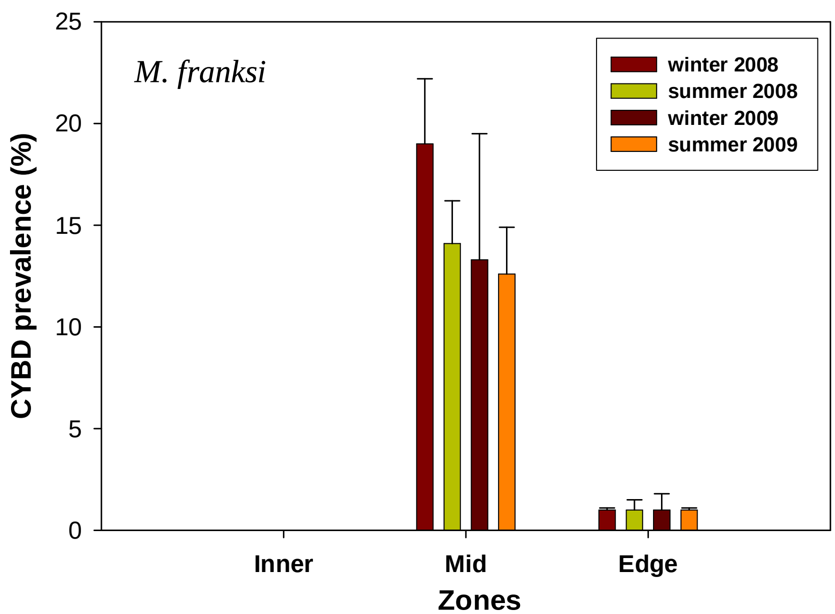


Figure 2.18: Grouped bar chart of % CYBD prevalence (mean ± SE) in *M. franksi* across habitats within reefs between years (2008-2009)

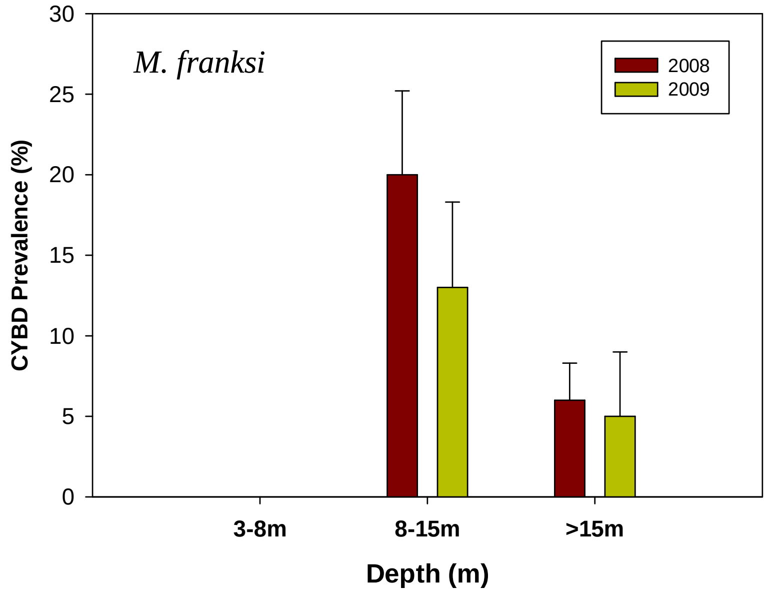


Figure 2.19: Grouped bar chart of % CYBD prevalence (mean ± SE) in *M. franksi* between reefs within zones between years (2008-2009)

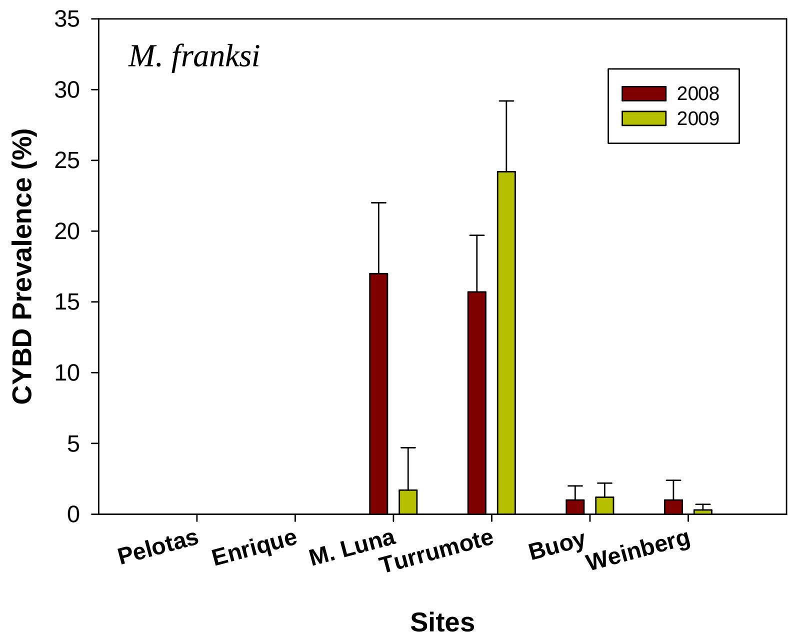


Figure 2.20: Grouped bar chart of % CYBD prevalence (mean ± SE) in *M. franksi* between zones for 2008 and 2009

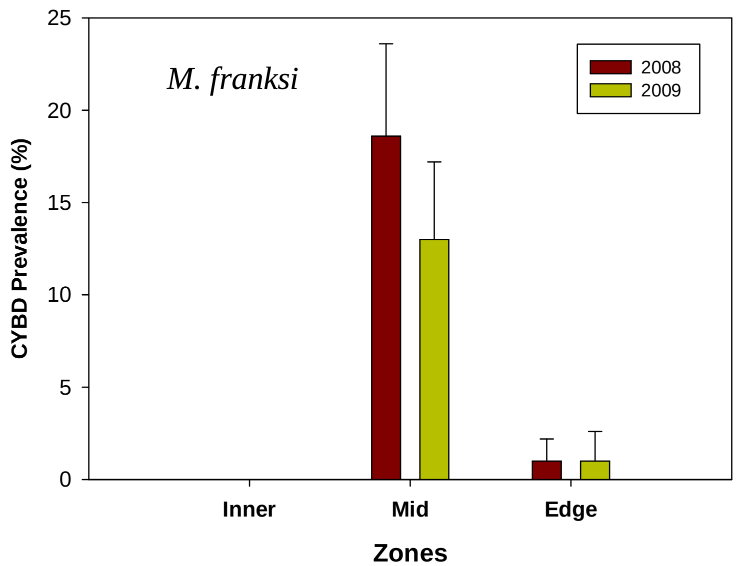


Figure 2.21: Spearman correlation analysis between overall average densities (#col./m^2) of *M. faveolata* and CYBD prevalence

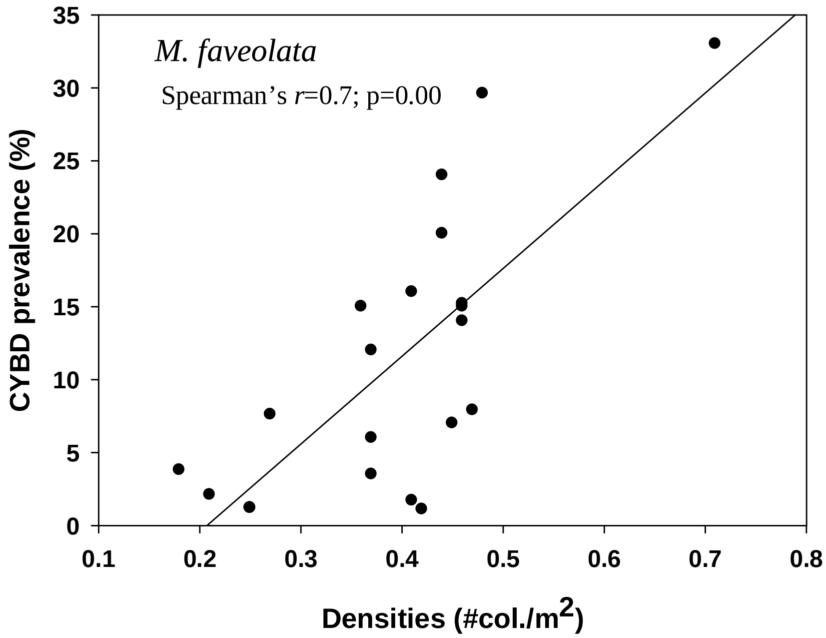
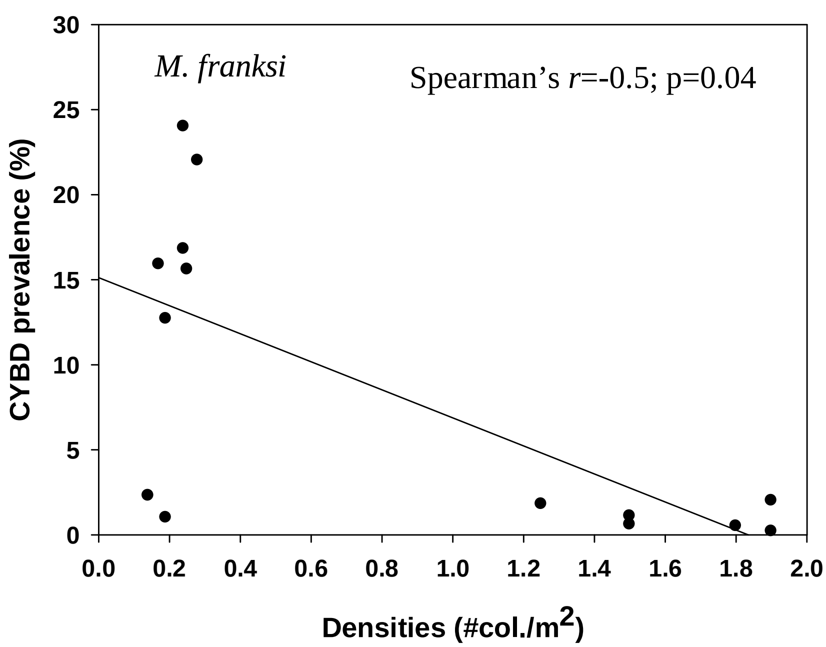


Figure 2.22: Spearman correlation analysis between overall average densities (#col./m^2) of *M. franksi* per site and CYBD prevalence



Chapter 3. Incidence and spatial dispersion of Caribbean Yellow Band Disease in La Parguera, Puerto Rico

3.1 Abstract (Chapter 3)

There is little information on the incidence, the number of new diseased colonies overtime, and on the aggregation patterns of diseased colonies for most coral diseases in the Caribbean. The incidence and spatial distribution patterns of Caribbean Yellow Band Disease (CYBD) in the important frame-builder coral *Montastraea faveolata* were assessed by counting, tagging, mapping and following through a year all diseased and infected colonies in three 100 m^2 quadrats in two inner, two mid-shelf and two shelf-edge reefs off La Parguera, southwest Puerto Rico. Healthy colonies were checked and photographed every two months from January to December of 2009 to monitor any pattern of disease spread in the population and new diseased colonies. HOBO temperature loggers were deployed to record temperature variability. Temperature increased from 26 in winter to 29 ^{o}C in summer of 2009, a normal range. Incidence was low for all reefs in both seasons, but increased significantly (Sign Test; Z=2.40; p=0.01) from winter to summer with an average of 1.33 newly infected colonies/month in the winter and 2.50 newly infected colonies/month in the summer. Mid shelf reefs had the highest host abundance and showed significantly higher CYBD incidence (an average of 0.58 infected colonies/month) compared to inner- and shelf-edge zones (KW=9.74; df=2; $p \leq 0.05$). The increased incidence levels in the summer indicate that warmer months seem to favor development of CYBD on *M. faveolata*. Analysis of spatial distribution patterns showed a significant aggregated pattern of CYBD infected colonies and for the whole population (i.e. healthy + diseased) in all reefs at the spatial scales sampled.

Aggregated colonies would facilitate spread of infectious agents within populations, but, CYBD has not been showed to be infectious. Similar stressful conditions, then, might trigger the disease in susceptible, aggregated colonies harboring the potential pathogens.

3.2 Introduction

Coral diseases are one of the main causes in the decline of Caribbean coral reefs. Over the last two decades the fast emergence and high virulence of coral reef diseases have produced substantial declines in live coral tissue and colonies (Goreau et. al., 1998; Richardson, 1998; Harvell et al. 1999; Green and Bruckner, 2000; Weil et. al, 2002; Weil, 2004; Miller et al. 2006; Weil et al. 2009a). The recent increment in diseases in the marine environment has been associated with different stressors such as, elevated sea surface temperatures, marine pollution, sedimentation (Miller et al. 2006; Harvell et al. 2007; Weil et al. 2009a; Weil and Rogers 2011), and nutrient enrichment (Bruno et al. 2003; Voss and Richardson, 2006). Caribbean Yellow Band Disease (CYBD) is one of the most damaging coral diseases affecting important reef builders in the wider Caribbean. In Puerto Rico, CYBD is one of the most prevalent and detrimental diseases disturbing scleractinian corals (Bruckner and Bruckner 2006; Ballantine et al. 2008; Bruckner and Hill 2009; Weil et al. 2009a). CYBD has showed a high prevalence in “pristine” areas far from anthropogenic disturbance (Weil et. al, 2002; Bruckner and Bruckner, 2006), however very little is known about the incidence and the spatial distribution of this disease.

One of the main gaps in coral disease studies is the lack of data concerning the multi-annual spatial and temporal variability of diseases (Borger and Steiner, 2005). It has been suggested that predictive models should be used to study spatial distribution patterns of coral diseases (Sokolow et al. 2009; Williams et al. 2010). Foley et al. (2005) showed that *M. annularis* colonies infected with CYBD were less clustered (i.e., more regular) than the distribution of healthy colonies between 10-30m in Akumal Bay, Mexico.

Spatial dispersion patterns describe how organisms are arranged in a particular habitat or community. Spatial patterns can be described as regular (i.e. uniform distribution), random (i.e. haphazard distribution) or aggregated (i.e. clumped distribution). Patterns of infection of diseases can be studied by applying spatial distribution patterns (Jolles et al. 2002) (at the proper scales) which could help in characterizing the etiology of the disease and identifying potential mechanisms of infection. Clustered or aggregated disease patterns in a population along a reef could mean that the disease is infectious and water-borne, and the closer the hosts, the faster (incidence) and highest (prevalence) the rate of infection. A more regular pattern could mean that a disease is not necessarily infectious. Spatial distribution patterns of aspergillosis affecting *Gorgonia ventalina* were highly aggregated and prevalence was high in the Florida Keys (Jolles et al. 2002).

Prevalence is the proportion of infected colonies in a population, thus, higher prevalence might suggest high susceptibility in host colonies and/or higher densities of susceptible colonies (Jolles et al. 2002). In Puerto Rico, CYBD is one of the most prevalent and detrimental diseases causing high tissue and colony mortality in the important reef building genus *Montastraea* (Bruckner and Bruckner 2006; Weil et al. 2009a; Bruckner and Hill 2009). Prevalence of CYBD in the *Montastraea annularis* species complex increased dramatically to 52% in reefs off the western end of Mona Island (Bruckner and Bruckner, 2006), and from 1% in 1999 to 55% in 2007 (Weil et al.

2009a) in La Parguera, on the southwest coast of Puerto Rico. After the 2005 bleaching event, CYBD prevalence increased 40% in *M. faveolata* with high mortalities associated to this disease, white plague outbreaks and bleaching reaching 60% in some reefs (Ballantine et al. 2008; Weil et al. 2009a). Even though this disease seems to be caused by a consortium of *Vibrio* bacteria (Cervino et al. 2004; 2008), little is known about the mechanisms of spread of the disease and its infectiousness. Several experimental attempts in the field and lab have failed to demonstrate infectiousness (Weil et al. 2008). Therefore, we expect that the distribution of diseased colonies in natural population not to be aggregated.

The goals of this study were to assess the incidence of CYBD in *M. faveolata*, one of the most abundant and important reef-building species, and to characterize the spatial distribution patterns of CYBD infected colonies of this species reefs off La Parguera, Puerto Rico Furthermore, the relationship between spatial patterns and incidence of CYBD within reefs was estimated. The null hypotheses were: there are no significant spatial/seasonal differences in CYBD incidence in *M. faveolata* populations in La Parguera and there are no significant differences in the spatial dispersion pattern of CYBD infected colonies across an inshore-offshore gradient. Results from this study would help in understanding the etiology and potential mechanisms of infection of CYBD, the rate of infection over time and how the distribution patterns of the population (susceptible colonies) may affect the spread and potential impact of the disease.

3.3 Materials and methods

Field work was conducted in six reefs off La Parguera: two in the inner-shelf (Pelotas and Enrique), two in the mid-shelf (Media Luna and Turrumote) and two in the shelf-edge (Weinberg and Buoy) (Table 1) off La Parguera Natural Reserve, in the southwest coast of Puerto Rico (Fig. 1). Coral reefs in La Parguera are considered the best developed reefs of Puerto Rico (Ballantine et al. 2008). A broad insular shelf, low water energy, favorable environmental conditions and low human impact over long periods of time have allowed extensive development of diverse coral reef communities (Ballantine et al. 2008).

3.3.1 Temporal and spatial variability of CYBD incidence in *M. faveolata*

The average incidence (i.e. number of new cases of a disease per unit time in a population) of CYBD affecting *Montastraea faveolata* in La Parguera was assessed through winter-spring (WI) and summer-fall (SU) of 2009. Incidence of CYBD was assessed using three 100 m^2 areas in each reef ($300m^2$) positioned between 3-9m and separated by at least 20m. Each quadrant corner was marked with a tagged re-bar, and before each survey, the perimeter was marked with a measuring tape, parallel tape lines were extended every two meters to form five 2 x 10m bands, and all diseased and healthy colonies of *M. faveolata* were checked, counted and mapped so the location and status of every single colony within the $100m^2$ was known after each survey. Healthy colonies were checked and photographed every month from January to December of 2009 to monitor new CYBD infections. HOBO temperature loggers were deployed at the beginning of the study to record temperature variability in each locality. Disease incidence data (i.e. proportion of newly infected colonies per month) did not fulfill the requirements for parametric tests and could not be normalized after arcsine transformations. Differences in incidence between the extreme seasons (winter and summer) were evaluated using Sign Tests. Differences between reef zones and sites were evaluated using non parametric Kruskal-Wallis ANOVAs. Statistica 7 software was used to run the different analyses.

3.3.2 Spatial dispersion of CYBD in *M. faveolata*

Spatial dispersion patterns were assessed for healthy; CYBD infected an all colonies of *M. faveolata* within each of the $100m^2$ quadrants using the nearest neighbor methods for spatial maps (Clark and Evans, 1954) in each of the six reefs (Table 1). The nearest neighbor method is based on comparing the distribution of the distances that occur from an individual to its nearest neighbor in space (Krebs, 1999). This distribution can be defined as:

$$R= r_A/r_E$$

where R is the index of aggregation, r_A is the mean distance to the nearest neighbor and r_E is the expected distance to nearest neighbor. If the spatial pattern is random, R=1, when clumping occurs, R approaches zero and in a regular pattern, R approaches an upper limit around 2.15. A simple test of significance for deviation from randomness was used and defined as:

$$z= (r_A-r_E) / S_r$$

where z is the standard normal deviate and S_r is the standard error of the expected distance to the nearest neighbor (see Krebs, 1999 for definitions and calculations).

3.4 Results

Significant differences in CYBD incidence in *M. faveolata* were found among reef sites (K-W=12.13; df=4; $p \leq 0.05$). Pelotas (an inner-shelf reef) showed no signs of CYBD over the study period, so no incidence could be measured. Turrumote and Media Luna (mid-shelf reefs) showed the highest CYBD incidence levels throughout the study both with 0.58 newly infected colonies/month, followed by Enrique (inner-shelf reef) with 0.41 newly infected colonies/month. Buoy and Weinberg (shelf edge reefs) showed the lowest incidence levels in the study (0.16 newly infected colonies/month). Photographic time series of the different tagged colonies at the different sites showed colonies that were healthy in the winter and then developed CYBD during the summer (Figs. 3.2A-C).

CYBD incidence in *M. faveolata* was significantly higher in mid-shelf reefs (0.58 newly infected colonies/month) compared to inner (0.41 newly infected colonies/month) and shelf-edge reefs (0.16 newly infected colonies/month) throughout 2009 (KW=9.74; df=2; $p \leq 0.05$). There was a positive but not significant correlation (Spearman correlation analysis) between densities of *M. faveolata* (Table 3.3) and incidence data (Table 3.2) (Fig 3.4).

The average CYBD incidence on *M. faveolata* ranged from 1.33 newly infected colony/month during the cold season (winter) to 2.50 newly infected colony/month during the warm water season (summer) of 2009 (Sign Test; Z=2.40; p=0.01) (Table 3.2). Monthly average temperatures in 2009 increased from 26.65 in the winter to 29.65 ^{o}C in summer (Fig. 3.3). During February, (26.5 ^{o}C) and June 2009 (28.8 ^{o}C), CYBD incidence was highest (Table 3.2). February finished with 3 new colonies infected in

Media Luna, 1 in Turrumote and 2 in Buoy (n=6). In June, 1 newly infected colony was found in Enrique, 2 in Media Luna and 4 in Turrumote (n=7). The highest average temperature was in October (29.65 ^{o}C) and the lowest in March (26.25 ^{o}C), but no newly CYBD infected colonies were found during these months. Analysis of the spatial distribution patterns of CYBD in *M. faveolata* showed an aggregated pattern for diseased colonies and for all colonies (pooled diseased + healthy) analyzed together. This aggregated pattern was found in all of the 100m^2 areas surveyed (Table 3.4) and was significantly different from randomness after a test of significance (Table 3.5). There were no CYBD infected colonies of *M. faveolata* in the 100m^2 areas surveyed in Pelotas (i.e. inner shelf reef) so these were excluded from the analyses. The highest average densities of *M. faveolata* were found in the mid shelf reefs (1.38 and 0.82 col./m^2 in Media Luna and Turrumote, respectively; Table 3.3) and spatial patterns were highly aggregated as well (Table 3.4).

3.5 Discussion

In La Parguera and the west coast of Puerto Rico, winter average temperatures used to drop below 25.5^{o}C but in the last 10 years winter mean temperatures have not dropped below 26.5^{o}C potentially compromising the immune response of corals or increasing virulence of pathogens (Harvell et al. 2009). During this period, CYBD prevalence increased significantly in the southwest and west coast of Puerto Rico (Weil et al. 2009a; Bruckner and Hill, 2009). Furthermore, CYBD used to be highly seasonal, with higher prevalence during summer and very low prevalence, and sometimes completely disappearing during winter-spring. However, this pattern disappeared as winter temperatures were warming up in the mid 2000’s (Harvell et al. 2009; Weil et al.

2009a). In the present study, incidence levels of CYBD remained low even with the higher than normal temperatures during winter. However, the increased incidence levels during summer-fall showed that warmer months seem to favor development of CYBD in *M. faveolata* colonies.

Weil and Cróquer (2009) recognized that CYBD distribution found in the different reefs through the Caribbean is due to the combination of host population abundance and distribution, presence of potential pathogens and different environmental factors. The positive but not significant correlation between densities of *M. faveolata* and CYBD incidence found in the present study suggests that this disease is affecting abundant and susceptible hosts mainly at mid-shelf reefs (Turrumote and Media Luna) were highest densities and CYBD incidence levels were found compared to inner and shelf-edge reefs. This non significance could be due to the low sample size when making this comparison since not all the three $100m^2$ quadrant in each site showed incidence of CYBD. Moreover, it was impossible to find a relationship between incidence of CYBD and densities of colonies in most of these quadrants individually. A significant positive correlation between prevalence of CYBD and densities of *M. faveolata* has been found in these same reefs indicating the vulnerability of these species towards CYBD (Soto-Santiago and Weil, in preparation). Our results concur with other studies where the number of diseased colonies was related to the abundance of susceptible species (Reigl, 2002; Borger, 2003; Borger, 2005; Borger and Steiner, 2005).

CYBD infected colonies showed an aggregated pattern in nearly all of the $100m^2$ areas surveyed. The same spatial dispersion pattern was found for the whole population (i.e. healthy + infected) as well. Foley et al. (2005) found contrasting results in Akumal, Mexico, when studying spatial dispersion patterns of YBD in *Montastraea annularis*. They found that YBD affected colonies showed a more regular (less clustered) spatial pattern compared to the whole population (healthy + infected) and concluded that proximity to other *M. annularis* may be offering protection from YBD perhaps in the form of barriers to disease agents or genetic resistance of corals which occur in space. In our study, this pattern of less clustering in infected CYBD colonies compared to the whole population was not observed in *M. faveolata* colonies since these showed highly aggregated spatial patterns (similarly to the whole population) in all reefs in La Parguera. This difference in spatial pattern compared to what Foley et al. (2005) found may be due to differences in the distribution of the species within sites in each of both studies and/or differences in the spatial scales used to study the dispersion of the disease. In the present study, spatial scale was $100m^2$ in each of the quadrants studied, but Foley et al. (2005) studied a spatial scale between 10-30m. Spatial patterns can change at different scales of observation and this is highly important when studying spatial dispersion of coral diseases (Foley et al. 2005). Jolles et al. (2002) found that aspergillosis-infected colony distribution was randomly distributed when prevalence was low and highly aggregated when prevalence was high in *Gorgonia ventalina*. It is important to recognize that prevalence (proportion of infected colonies in a population) is not the same epidemiological measure as incidence (new cases of diseased colonies over time). However, both parameters can indicate the level in which a disease is affecting a population. In fact, incidence gives information on the peril of contracting the disease, whereas prevalence indicates how extensive the disease is. In the present study, *M.*

faveolata infected with CYBD were aggregated even with low incidence levels in each of the reefs studied.

Table 3.1: Description of the study sites in La Parguera, Puerto Rico (modified from Flynn and Weil, 2009)

Site	**Zone**	**Location (N) (W)**	**Distance from shore (km)**	**Depth range (m)**	**Slope**	**Coral cover**
Enrique	Inner shelf	17°56.658 67°02.213	1.5	1-14	steep	moderate
Pelotas	Inner shelf	17°57.442 67°04.176	1	1-12	steep	low
M.Luna	Mid shelf	17°56.093 67°02.931	2	4-16	steep	moderate
Turrumote	Mid shelf	17°56.097 67°01.130	2	2-15	steep	low
Weinberg	Shelf edge	17°53.429 66°59.320	6	18-23	gradual	high
Buoy	Shelf edge	17°53.110 66°59.510	6	18-23	gradual	high

Table 3.2: Number of newly infected *M. faveolata* colonies per month in 2009 at the different sites. Surveys were completed every month beginning in January

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Enrique	-	-	-	2	-	1	-	-	2	-	-	-
M. Luna	-	3	-	-	-	2	-	2	-	-	-	-
Turrumote	-	1	-	-	-	4	-	-	2	-	-	-
Buoy	-	2	-	-	-	-	-	-	-	-	-	-
Weinberg	-	-	-	-	-	-	-	-	2	-	-	-

Table 3.3: Average densities of *M. faveolata* (±SD) at the different sites during 2009

Site	# col/m^2	SD
Enrique	0.32	0.16
Media Luna	1.38	0.42
Turrumote	0.82	0.41
Buoy	0.25	0.14
Weinberg	0.34	0.20

Table 3.4: Index of aggregation, R values, with sample size (in parenthesis) for the three $100m^2$ quadrants (Q) in the different sites

		Q 1		Q 2		Q 3	
Factor	**Level**	**Healthy + YBD**	**YBD**	**Healthy + YBD**	**YBD**	**Healthy + YBD**	**YBD**
Reef	Pelotas	-	-	-	-	-	-
	Enrique	1.02 (47)	1.27 (2)	0.63 (26)	0.5 (2)	0.70 (16)	0.48 (2)
	Media Luna	0.55 (80)	0.66 (15)	0.48 (124)	0.7 (16)	0.46 (150)	0.60 (28)
	Turrumote	0.68 (70)	0.86 (40)	0.65 (61)	0.69 (13)	0.58 (27)	0.48 (8)
	Buoy	0.64 (11)	1.39 (5)	0.84 (14)	1.75 (5)	0.75 (36)	0.92 (5)
	Weinberg	0.89 (17)	0.84 (2)	0.66 (20)	1.01 (5)	0.66 (51)	0.48 (6)

Table 3.5: Z and p values (in parenthesis) from the test of significance for the three $100m^2$ quadrants in the different sites

		Q 1		Q 2		Q 3	
Factor	**Level**	**Healthy + YBD**	**YBD**	**Healthy + YBD**	**YBD**	**Healthy + YBD**	**YBD**
Reef	Pelotas	-3.84 (p=.000)	4.72 (p=.011)	-	-	-	-
	Enrique	0.29 (p=.179)	3.57 (p=.176)	-3.47 (p=.000)	-4.03 (p=.000)	-2.23 (p=.000)	- 1.4(p=.000)
	Media Luna	-7.5 (p=.000)	-2.45 (p=.000)	-10.9 (p=.000)	-2.29 (p=.000)	-12.5 (p=.000)	-4 (p=.000)
	Turrumote	-5 (p=.000)	-1.65 (p=.000)	-5.2 (p=.000)	-2.1 (p=.001)	-4.12 (p=.000)	-1.4 (p=.165)
	Buoy	-2.27 (p=.000)	1.69 (p=.141)	-1.13 (p=.003)	3.22 (p=.399)	-2.85 (p=.000)	-0.25 (p=.005)
	Weinberg	-0.7 (p=.038)	-0.4 (p=.000)	-2.83 (p=.000)	0.08 (p=.011)	4.51 (p=.000)	-2.42 (p=.005)

Figure 3.1: Map of the study sites in La Parguera, Puerto Rico.

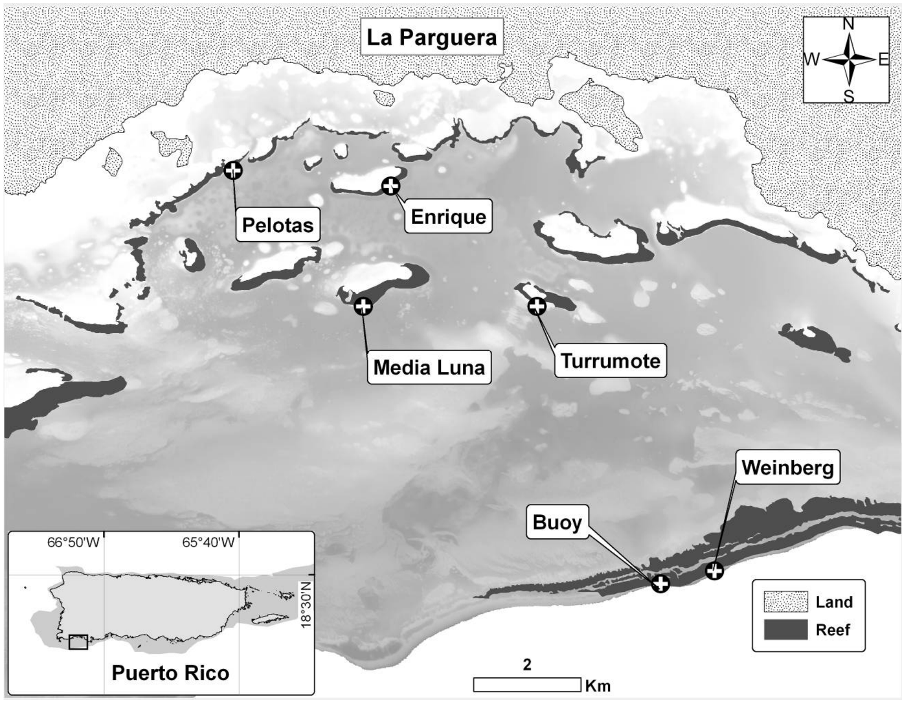


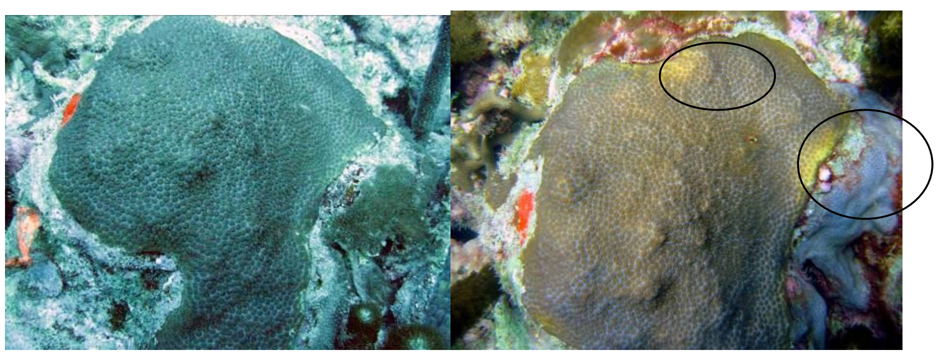
Figure 3.2A: Photographic time series of a colony at Turrumote showing signs of YBD. a) January 2009;b) April 2009; c) October 2009

(a) (b) (c)

Figure 3.2B: Photographic time series of a colony at Enrique showing signs of YBD. a) January 2009; b) October 2009

(a) (b)

Figure 3.2C: Photographic time series of a colony at Media Luna showing signs of YBD. a) January 2009; b) October 2009



(a) (b)

Figure 3.3: Line graph of monthly average seawater temperatures from January-December 2009 in La Parguera, Puerto Rico.

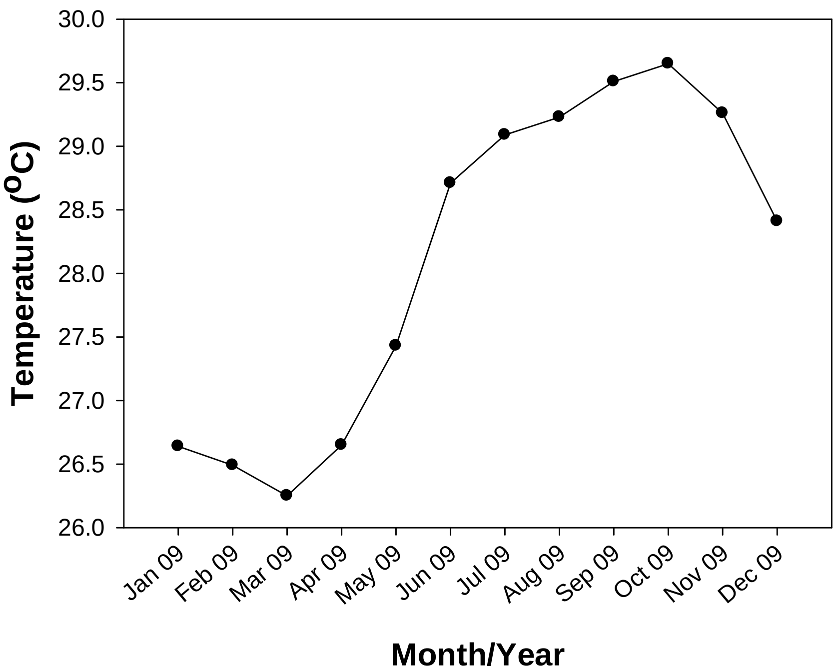
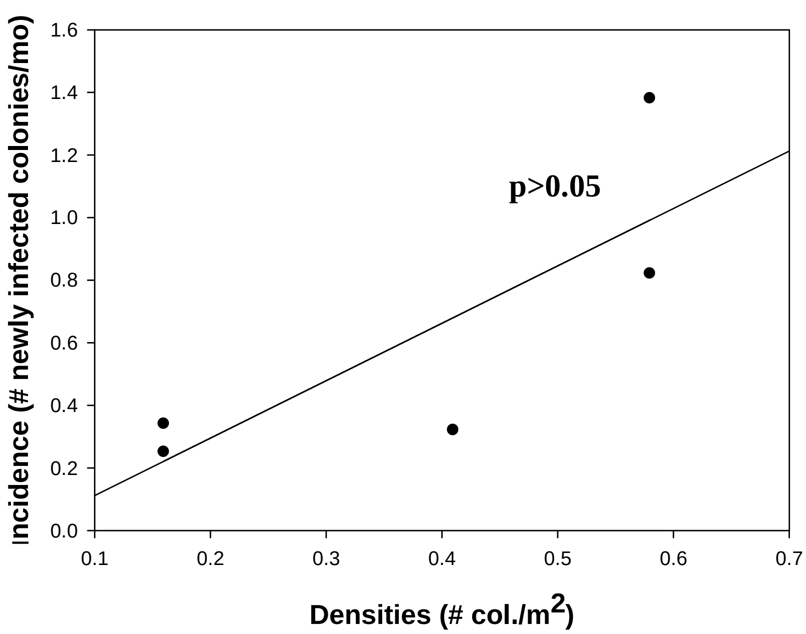


Figure 3.4: Spearman correlation analysis between densities of *M. faveolata* and incidence of CYBD at the different sites



Conclusions and recommendations for future work

- CYBD in La Parguera has dropped over the last two years due to apparently increasing resistant colonies to this disease or may be a shift towards a more virulent disease such as White Plague Disease (WPD).
- The positive and significant correlation between overall densities of *M. faveolata* and CYBD prevalence suggests that CYBD is spreading through susceptible colonies of *M. faveolata* mainly at the intermediate (8-15m) habitats.
- The higher CYBD prevalence in *M. faveolata* than *M. franksi* in all habitats (depths) within reefs, reefs within zones and zones suggests that *M. franksi* is less susceptible to CYBD.
- Defense mechanisms or studies on different genotypes of *M. franksi* that may be causing this resistance should be done to sustain this theory.
- Deeper habitats may serve as a refuge from CYBD for both species. This may be due to to a more resistant clade of zooxanthellae in deeper colonies (specially *M. franksi*) were quantity of light is lower and quality is poorer than shallower depths. Another possibility of the lower CYBD prevalence found in the deeper habitats may be depth susceptible pathogens and different light conditions or a combination of these.
- Studies on depth distributions of zooxanthellae populations in *M. faveolata* and *M. franksi* colonies in La Parguera could lead to interesting findings in the study of CYBD.

- Further research on spatial dispersion patterns at different spatial scales and more replication in more reefs within zones is needed to understand better CYBD dynamics in La Parguera. Incidence studies of CYBD should include more replication and more years of study to observe CYBD incidence patterns over time.
- Coral diseases have shown associations to different environmental variables at different spatial scales (Williams et al. 2010). Predictive models such as boosted regression tree (Williams et al. 2010) and metapopulation models (Sokolow et al. 2009) can be helpful to study spatial distribution patterns of CYBD at different spatial scales. Also, GIS and remote sensing techniques can help substantially to the creation of more accurate maps to study spatial dispersion patterns (Foley et al. 2005) of CYBD over time in reefs off La Parguera.
- Results of the current study shows the importance of prevalence, incidence and spatial dispersion patterns of CYBD and how these can be used to describe the dynamics of this damaging coral disease in reefs off La Parguera, Puerto Rico.

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