A SURVEY OF CORAL DISEASES IN THE FLOWER GARDEN BANKS NATIONAL MARINE SANCTUARY (GULF OF MEXICO)

by

Tomáš J. Oberding

A Thesis Submitted
In Partial Fulfillment of the
Requirements for the Degree of

Master of Science in Mariculture

Texas A&M University-Corpus Christi
Department of Physical and Life Sciences
Corpus Christi, Texas
May, 2004

APPROVED: _____ DATE: _____

Dr. Quenton R. Dokken, Co-Chair
Texas A&M University-Corpus Christi

_____

Dr. Joanna B. Mott, Co-Chair
Texas A&M University-Corpus Christi

_____

Dr. Joe M. Fox, Member
Texas A&M University-Corpus Christi

_____

Dr. Grady M. Price Blount, Chair
Department of Physical and Life Sciences
Texas A&M University-Corpus Christi

_____

Dr. Diana M. Marínez, Dean
College of Science and Technology
Texas A&M University-Corpus Christi

Format: Aquaculture International

ABSTRACT

Awareness and understanding of diseases of sessile organisms, specifically scleractinian corals, are increasingly important factors in the preservation of the world's reefs. A baseline study of the level of disease at the Flower Garden Banks National Marine Sanctuary was undertaken between July and September of 2001. The study was conducted on the East and West banks with three, 50m transects at each bank. A total area of 74.11 m^2 was surveyed using digital video. Analysis of the transects indicated bleaching, black band disease, and unknown disease types as the most frequently encountered disease conditions, accounting for 16.87, 3.25, and 2.49%, respectively, of the total area surveyed. *Millepora* spp experienced the highest percentage of bleaching, with 78.64% of all *Millepora* spp present exhibiting signs of bleaching which accounted for 3% of the total area surveyed. *Montastraea annularis*, the most prevalent coral species present, exhibited the highest levels of bleaching and unknown diseases. The highest percentage of black band disease was seen on *Diploria strigosa*, with 1.51% of the total area surveyed. Forty-four water samples were taken directly from the water column above the coral surface. From the original 44 samples, 36 bacteria were isolated. Bacterial identification included two *Vibrio* species. The highest bacterial cell densities were associated with black band disease. The highest concentration of bacteria, 76 and 46 cells/mL, were found in water above *Porites asteroids* and *P. porites*. Overall, more research remains to be completed at the site, with surveys during the colder-water months to balance the summer sampling. Disease monitoring should also be included as a cornerstone of any coral reef long term monitoring program.

Table of Contents

List of Tables

List of Figures

Acknowledgements

I would like to thank the following individuals, without whose help this experience would have been much more difficult, if not impossible:

-My parents: Milada Oberding-Colton and Dr. Eugene Paul Colton, for their complete and unquestioning support, and insightful assistance in finding my *raison d'etre*.

-Dr. Dokken for the opportunity and funding of this project.

-Dr. Mott for gracious utilization of her laboratory.

-Dr. Fox for essential help with perspective, and advice.

-Dr. McKee for initial belief in my abilities and acceptance into the graduate program.

-Chris Rigaud and Noe Barrera for field sample collection.

-Pam Brown for microbial expertise and lab assistance.

-Anna Jorgenson for data organization and editing.

Introduction

Coral reefs are an important component of many tropical coastal habitats, essential to both the aquatic and terrestrial inhabitants of these areas. Coral reefs reduce erosion by controlling wave action and also serve as habitat for many economically important animals such as ornamental fish and invertebrates (Moberg and Folke 1999).

Many of the world's coral reefs are currently threatened by degradative anthropogenic activities including: unsustainable fishing, poor land use, ozone depletion, global warming (Moberg and Folke 1999), and ballast water discharge (Daszak et al. 2001). Sheppard (2000) cited a more detailed list of influences including: urbanization, aquaculture, exotic species, deforestation, land-fill/reclamation, mining, erosion, industry, sediments from runoff, debris, pesticides, oil, shipping, ballast water, aquatic and terrestrial climate changes, freshwater inflow, and diseases. Kullenberg (1999) in his listing of impacts included radioactive waste. The *in-situ* effects of increasing nutrient loads on reefs have been recognized since 1974 (Kinsey and Domm 1974).

Many of the world's reefs are located along the coasts of developing nations. Coastal populations that depend on the coral ecosystem for their livelihood tend to have a greater negative impact on their marine environments than those whose daily existence is not dependent upon local reefs (Riegl and Luke 1998). As the human population increases, its impact on oceanic habitats will also increase, accelerating rates and expanding the types of degradation associated with human activities (Awosika and Marone 2000).

Despite the study of coral disease being a relatively new scientific endeavor, 29 coral diseases have been described. Though much of the research on coral diseases was

initially conducted in the western Atlantic Ocean and Caribbean Sea, diseases of reef coral are a worldwide problem. Diseases were reported in the Great Barrier Reef and the South Pacific as early as 1992 (Miller 1996). A major worldwide effect of coral disease on reefs is in species composition of the reef (Green and Bruckner 2000). Changes in coral species composition often negatively impact species dependent upon corals for part of their diet or habitat, and this can thus potentially alter the entire ecosystem.

History

Most studies of coral disease during the late 1970's consisted of microscopic examination of diseased coralline tissue and focused on bacteria as the causative agent; however, techniques for speciation and enumeration of the various bacteria were limited (Antonius 1973; Ducklow and Mitchell 1979). Black band disease (BBD) was the first disease of scleractinian corals reported in the Caribbean Sea (Antonius 1973). Mitchell and Chet (1975) demonstrated increasing bacterial growth rates on coral resulting from the impact of pollution. The pollution stimulated mucous formation on corals. These corals then became more susceptible to bacteria (e.g. *Desulfovibrio* sp. and *Beggiatoa* sp.). This research was followed by that of Dustan (1977) and Gladfelter (1977) who first reported and described white band disease (WBD) of branching coral and plague of plate-type corals, respectively. Both diseases appeared with no readily apparent microbial association, with progression rates similar to that of BBD, at several millimeters per day.

During the early 1980's, quantitative studies of coral disease increased. White band disease continued to spread rapidly throughout the Caribbean Sea and was identified as a major threat worldwide to coral populations (Gladfelter 1982). By 1984, four

principal coral diseases had been differentiated: BBD, WBD, plague, and "shut-down," a disease manifested by the cessation of biochemical processes (Antonius 1981). A bacterial pathogen, *Phormidium corallyticum*, had been postulated as the causative agent of BBD (Antonius 1981; Rutzler and Santavy 1983).

By the 1990's, additional diseases had been identified and descriptions of various other coral-related diseases were reported (Carlton and Richardson 1995; Richardson 1998). A persistent problem with respect to these newly described diseases has been the increased potential for misdiagnosis. The primary reason for misdiagnosis of diseases is variability in gross pathology, whereby a single disease may appear in various forms, some of which may be similar outwardly to a potentially unrelated disease. Currently, coral diseases for which disease agents have been identified include: BBD, WBD type II, and plague type I (*P. corallyticum*, *Vibrio carchariae*, *Sphingomonas* sp. respectively; Antonius 1981; Ritchie and Smith 1998; Richardson 1998). WBD type I has been the only disease shown to significantly change the structure of the reefs by altering species composition (Ritchie and Smith 1995a; Ritchie and Smith 1995b), however, the causative agent has not been identified.

Environmental and Anthropogenic Impacts

Coral disease epizootics often follow an environmental disturbance. For example, in the South Pacific, most black band disease appears to occur after heavy rains (Antonius 1981). Agricultural and industrial pollution and nutrients from terrestrial runoff have been shown to have direct negative effects on coral and positive effects on algal growth (Chrost and Faust 1999; McClanahan et al. 2002). Logging activities in both watersheds and coastal areas increase the duration and extent of sediment

entrainment and, hence, periods of high nutrient deposition on the reefs (Koop et al. 2001). High nutrient levels in the water facilitate algal growth which, if not removed by herbivores, can prevent coral larval recruitment (Nyström et al. 2000). Increased turbidity of local waters from terrestrial runoff has been postulated to increase physiological stress in corals (Littler and Littler 1996). Environmental stressors such as those mentioned have been implicated in increased susceptibility of coral to spread of disease (Littler and Littler 1996). Yentsch et al. (2002) determined that turbidity caused by terrestrial runoff increased levels of algal growth and decreased incident light intensity to a point where photosynthesis was substantially reduced. Corals grown in laboratories under low-light conditions simulating those found in reefs which experience terrestrial runoff and nutrient enrichment produced thin, fragile skeletons (Chrost and Faust 1999). These thin skeletons are thought to be more susceptible to damage from physical environmental stress.

Industrial air pollution and greenhouse gases (e.g. carbon dioxide) appear to negatively impact reefs by amplifying the effects of solar radiation. Chrost and Faust (1999) determined that higher levels of solar radiation could positively affect bacterial growth rates. If this increase in bacterial cell densities is coupled with slower growth rates of coral on the Great Barrier Reefs, the potential for cascading and compounding effects exists (Chrost and Faust 1999). The decrease in skeletal growth rates leads to thinner skeletons, which are more susceptible to environmental damage (Chrost and Faust 1999). In turn, environmentally damaged or stressed coral are more susceptible to disease, and hence the potential for epizootics is increased.

Decrease in incident light penetration, caused by increased turbidity, produces a low-nutrient condition within the coral by decreasing photosynthetic rates of zooxanthellae. As a result, corals occupy narrower niches of depth: if they grow closer to the surface in turbid conditions, their skeletons become susceptible to physical damage; if the corals grow deeper to avoid physical damage, zooxanthellae are unable to achieve adequate photosynthetic rates (Yentsch et al. 2002).

In addition to increased nutrient levels in the water column and turbidity caused by runoff from populated coastal areas, recent studies in the Florida Keys have found indicators of human fecal contamination in runoff. Lipp et al. (2002), reported the presence of *Clostridium perfringens* in 66.7% of samples taken from the surface of scleractinian corals, probably living in the mucus surface of microorganisms from the environment. The bacterium did not appear to be causing necrosis in the coral, but its aggregation in the mucus may have an influence upon the organisms which feed upon the coral.

Similar anthropogenic impacts to those observed in the Florida Keys have also been observed in the Gulf of Kutch and the Indian Ocean. Almost 90% of the corals in the Indian Ocean were observed to have some form of necrosis (Ravindran et al. 1999). Included were signs of BBD, WBD, bleaching, fleshy algal overgrowth, and necrotic-lesions. This study also indicated that predation, agricultural, and industrial pollution from the Indian Subcontinent contributed to the poor conditions (Ravindran et al. 1999).

Bruckner and Bruckner (1997a) observed BBD, WBD and white plague in Puerto Rico after Hurricane Hortense in September of 1996, in an area previously considered void of disease. Hurricanes have been implicated in increasing species diversity on coral

reefs (Bythell et al. 2000), as recruits inhabit areas cleared of coral damaged from the hurricane. Similar increases in species diversity have been observed in areas of dynamite fishing (Rubek 1988). Indonesian and Philippine coral reefs possess high species diversity and have been substantially impacted by dynamite fishing and disease (Spalding and Jarvis 2002). Apparently, high species diversity is not necessarily indicative of a healthy climax community, but rather may reflect a successional community highly susceptible to additional impacts (Rubek 1988; Riegl and Luke 1998).

Not only are diseased corals being discovered in new regions, but new diseases are also being identified. Two of these were recently found in the Indo-Pacific region. The first, *Halofolliculina corallasia* (a colonial heterotrich ciliate), was identified as the primary pathogen associated with the disease Skeleton Eroding Band. Apparently, this ciliate colonizes the skeleton of coral, with growth progressing in a manner similar to BBD (Antonius 1999a). The second disease was an algal infection, termed PEY, which destroys coral on reef crests and forms a tightly attached skin under which coral tissue is absent. The red alga, *Metapeyssonnelia corallepida* (Rhodophyta), is the causative agent of PEY and was previously found only in the Mediterranean Sea. Recently, *M. corallepida* has spread to the Caribbean Sea (Antonius 1999b). With the exception of WBD, none of the diseases currently studied appear to have specific host requirements, indicating these diseases could impact many different genera of coral (Green and Bruckner 2000).

Koh (1997) described a form of microbial interaction between cyanobacteria and scleractinian corals, finding that eight species of coral had intracellular antimicrobial compounds that prevented or slowed the colonization of detrimental cyanobacteria.

These compounds may be used in by biotechnological research for the development of scleractinian corals with an increased ability to compete with harmful cyanobacteria, an important factor in the future remediation of reefs.

Coral-type diseases appear not to be limited to coral. Coral-types diseases are diseases that appear similar to diseases of coral and affect organisms which are similar to coral such as coralline algae. Coralline algae have been found to be susceptible to a disease referred to as CLOD (coralline lethal orange disease; Littler and Littler 1995). Over a one-year period in Fiji, a previously pristine reef became 100% infected with CLOD. This type of pathogen could potentially disrupt the reef building process.

Problems with reef health continue to be reported. For example, Daszak et al. (2001) postulated that "pathogen pollution" was responsible for widespread occurrence of pathogens via anthropogenic activities such as ballast water discharge. This process has the potential to cause increased future epizootics, mass mortalities, and extinction events.

Stress and Bleaching

Most researchers agree that bleaching is a symptom of stress (Hagman and Gittings 1992; Ravindran et al. 1999; Dokken et al. 2000). Bleaching is caused by the expulsion of endosymbiotic zooxanthellae and gives a semi-transparent appearance to the coral polyp, manifested as a white calcified skeleton (Richardson 1998). The loss of symbionts from the polyp tissue can ultimately result in death of the polyp. Over 63% of all coral nutrients are derived from photosynthetic activity of the zooxanthellae (Gulko 1998). Stress resulting in bleaching was initially associated with increased water temperatures inhibiting photosynthesis (Goreau and Hayes 1994). Subsequent studies have shown that increases in solar radiation, industrial and agricultural pollution, as well

as reduced salinity, and/or a combination of the previous can cause bleaching (Banin et al. 2000). There may also be a positive response to the stress by potentially pathogenic microorganisms which then proceed to negatively affect coral (Kushmaro et al. 1996). Fisk and Done (1985) reported an incidence of non-temperature related bleaching on the Great Barrier Reef in 1982. Incidences of bleaching with varying degrees of severity have been reported on corals reefs around the world (Hoegh-Guldberg 1999).

Rationale

Despite observations of coral disease, no previous disease studies have been conducted at the Flower Garden Banks National Marine Sanctuary (FGBNMS; Dokken et al. 2000). The purpose of this thesis was to preliminarily identify the level of disease in this unique location. Once a baseline of disease at the site has been established, future research can be conducted to track the levels of individual diseases within the sanctuary. Also, the speciation of bacteria associated with disease could help in more efficient diagnosis of that disease. A distinguishing characteristic of the FGBNMS is that these reefs occur within 50 km of their northern physiological limit. These relatively isolated reefs are subject to fewer terrestrial impacts than other more intensely studied reefs around the world. This relative isolation allows for a unique study site comparatively free of terrestrial and anthropogenic impacts from which to compare the incidence and spread of disease with other reefs around the world.

Objectives

A baseline evaluation of diseased corals was undertaken at the FGBNMS with the following objectives:

1) Evaluate the extent and diversity of coral disease at the FGBNMS;

2) Compare bacterial concentrations in water columns above diseased corals.

Methods

Study site

The Flower Garden Banks National Marine Sanctuary (FGBNMS; NOAA permit # FGBNMS-2001-006) is the northernmost coral reef system located in the Gulf of Mexico on the North American Atlantic Shelf. It consists of two reefs located above diapirismically formed Jurassic salt banks 190 km south-southeast of Galveston, TX (Rezak et al. 1985; Fig. 1). The sanctuary is surrounded by oil and gas platforms, making the banks susceptible to potential contamination by accidental releases of oil or human waste. A possible source of bacterial contamination is ballast water associated with trans-national shipping occurring along the Texas Louisiana coastline (USACE 2002; Daszak et al. 2001). The East Bank is located at 27° 54.5'N, 93° 36.0'W (mooring #2) and the West Bank at 27° 52.5'N, 93° 49.0'W (mooring #5; Gittings et al. 1992; Dokken et al. 2000). The majority of the coral present in the area are found between the 15 and 36 meter isobaths (Gittings et al. 1992; Dokken et al. 2000). Coral above the 36 m isobaths represent nearly 50% of the entire cover, encompassing a total area of approximately 1.3 km^2. Twenty of the 65 western Atlantic hermatypic coral species have been identified at this site (Gittings et al. 1992; Dokken et al. 2000).

Previous studies have shown of twenty species of coral identified, six constitute 90% of the total coverage, with *Montastraea annularis* complex representing 30% of the total (Bright and Pequegnat 1974; Bright et al. 1984; Dokken et al. 2000). No shallow water gorgonian species or branching corals of the genus *Acropora* have been found at the FGBNMS (Gittings et al. 1992; Dokken et al. 2000).

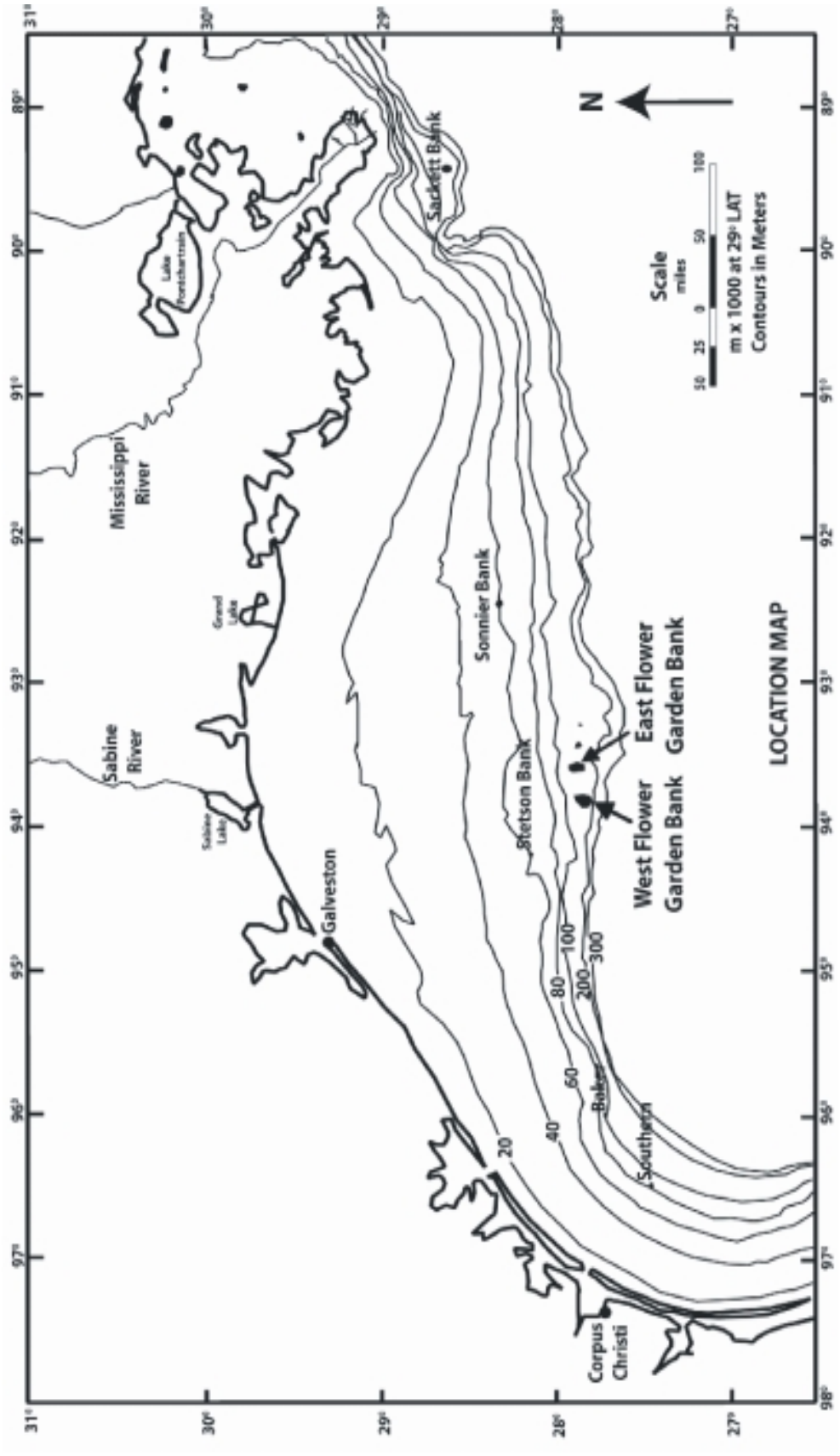

FIGURE 1. *Location of the East and West Flower Garden Banks in relation to the continental shelf and other topographic features of the northwestern Gulf of Mexico (Gittings et al. 1992).*

Quantification of coral disease

Data were collected during two cruises: 18-20 July, 2001, and 17-19 September, 2001. Following the methods described in Vogt (1995) and Ohlhorst et al. (1988), three transects were established at each bank on each cruise by tying one end of a transect tape to a common mooring block and extending it a distance of 50 m. Transect lines were oriented 120 degrees apart with the primary line running due north (0 degrees) at the West Bank and due south (180 degrees) at the East Bank. Digital video filming over the length of each transect commenced from the mooring block using a Sony TVR900™ camera in an Amphibico™ housing. A standard observation distance of 0.75 m was maintained above the reef surface by utilizing a metal bar attached to the camera housing. A standard measure gauge was included in each video frame via a 2 cm length of tape attached to the horizontally bent end of the distance bar.

The subject area was illuminated using artificial lights mounted to the exterior of the camera housing (Jaap 1995). During the videotaping of the transect at 60 degrees (East Bank), a large sand flat was encountered at 20 m. To avoid the large sand flat, the transect was shifted left to 90 degrees for 20 m. After 20 m, the transect was returned to 60 degrees for the duration of the run. During the videotaping of the transect at 300 degrees (East Bank), a 10 m long sand flat was encountered 15 m from the start of the transect. The sand flat was avoided by following the edge of the reef to the left for the length of the flat. After passing the flat, the original bearing was followed again. Digital still images were taken of representative disease manifestations within an 8-10 m path along the transect line, while swimming back towards the starting point. These

representative still images were not included in the statistical analysis of this project, but were captured as examples of various diseases found at the FGBNMS.

Digital video was analyzed by capturing still images downloaded and lightened using Corel Photo-Paint™ 8.0. The images were then uploaded into Sigma Scan™ 3.0, which was utilized to convert areas traced with a digital pad and pen into total surface area. Corresponding sections of digital video were viewed while analyzing the captured images in Sigma Scan™. Concurrent viewing of original video data while analyzing video captures ensured proper identification of species and diseases, which at times appeared with lower clarity in the capture format. In order to capture approximately 50 images (representing 50 quadrats per transect) the following procedure was utilized**:** As the duration for each transect varied, the total transect video time was transformed into total time in seconds. This value was then divided by 50 (the number of captures desired), resulting in the time interval in seconds between captures**,** or

$$\frac{\text{total transect time(seconds)}}{\text{\# captures desired (50)}} = \text{frame capture interval}$$

On average, a frame was captured approximately every ten seconds.

Observations of diseases and disease-like conditions were divided into the following categories: bleaching, black band disease, conditions of unknown etiology, ridge mortality, yellow blotch, dark spot, plague, and neoplasms. Diseases and disease conditions were identified using two different picture identification cards available to researchers, NOAA Disease and Predation Identification Cards (Bruckner and Bruckner 1998b) and Coral Disease Identification cards (HEED Global Change Program 1997). Coral species identifications were confirmed using Humann (1998).

Microbial Samples

Twenty-two water samples for microbiological analyses were collected along transects on the last day of sampling at each bank. In order to limit damage to corals, samples were taken directly above infected areas of individual corals. Control samples were collected directly (approximately 1 mm) above various coral species in apparently non-infected areas within the same transect. Samples were collected using 50 mL sterile syringes (Richardson 1997a; Fig. 2). An average of seven samples were collected along each transect. Once a diseased coral was located on the transect, the syringe tip was placed directly above the coral and the aspirator was pulled back, filling the syringe with a water sample.

Upon return to the research vessel, syringes were shaken, two drops of water from each syringe were transferred onto sterile glycerol artificial seawater plates (Ritchie and Smith 1995a) and two drops were used to inoculate sterile Zobell's 2216E plates (Austin 1988). Plates were stored and transported in an ice chest containing ice packs.

During the first collection cruise and after plate inoculation, syringes containing seawater were placed in an ice chest containing ice packs (Ritchie and Smith 1995b). Approximately two days after collection, samples were transported to the laboratory.

On both trips, duplicate water samples from separate syringes were preserved using gluteraldehyde (5mL gluteraldehyde per 45-50mL sample) for subsequent bacterial cell density counts (Garabetian et al. 1999). During the second collection cruise only preserved samples were retained.

FIGURE 2. *Water sample collection at reef-water interface using 50mL syringe.*

Bacterial isolation and enumeration

Transfers from plates inoculated on ship were made following procedures of Jensen and Fenical (1995). Triplicate transfers were made onto both glycerol artificial seawater and Zobell's plates. During the first collection cruise, it was observed that fewer colonies formed on the Zobell's 2216E medium than the glycerol artificial seawater plates. Therefore, only glycerol artificial seawater medium was used during the second collection cruise. Isolates from plates from which transfers were unsuccessful were transferred to artificial seawater broth prior to streak isolation upon solid media. Better growth appeared on the glycerol supplemented artificial seawater plates and further transfers were conducted using this medium. Upon isolation, bacteria were characterized by oxidase activity, Gram stain, and cellular morphology. Fourteen isolates were identified using the MicroLog™ microplate system after growth on Biolog Universal Growth plates (Biolog Incorporated, Hayward, California). Forty-four water samples preserved with gluteraldehyde were filtered and stained with acridine orange for subsequent enumeration using an epifluorescence microscope (Pettipher 1983; Kepner and Pratt 1994).

Statistical Analyses

Statistical analyses were conducted using SPSS version 10.0 (SPSS Inc. Headquarters, Chicago, Illinois) and MS Excel 2000 (Microsoft Corporation, Redmond, Washington) software. The primary dependent variable of investigation was surface area associated with individual coral species and diseases. Independent variables consisted of bank and coral species. Individual transects were considered statistical replicates. Three hypotheses were statistically evaluated in this study:

1) H_0= Bacterial cell density in the water column above different diseases is similar for all species of coral;

   H_a= Bacterial cell density in the water column above different diseases is different for all species of coral;

2) H_0= total area of coral covered by diseases is similar at each bank

   H_a= total area of coral covered by diseases is different at each bank

3) H_0= total area of coral covered by disease is similar for each species of coral

   H_a= total area of coral covered by diseases is different for each species of coral

Results from microbiological determination of cell density were transformed to natural logarithms prior to statistical analysis in order to accommodate high variation among samples. Two-way ANOVA with bank and species as independent variables was analyzed based on each individual disease. This allowed the effects of bleaching to be compared separately from other conditions of coral disease.

Coverage data were first normalized to allow for differences in the number of observations among transects. Transect results were then organized to focus on the data at three different levels. The first method utilized total areas of individual species of coral on each transect:

$$\frac{\text{species X area exhibiting disease Y}}{\text{total area of species X on transect A}}$$

The second method involved the division of the total for each species separated by diseases of coral by the total area surveyed in the respective bank:

$$\frac{\text{species X area exhibiting disease Y}}{\text{total area surveyed on bank A}}$$

The third normalization involved each observation to be divided by the total area of coral cover per bank:

$$\frac{\text{species X area exhibiting disease Y}}{\text{total area of coral cover on bank A.}}$$

Two-way ANOVAs with bank and species as independent variables were completed based on each individual disease and were then operationalized using each of the normalizations.

Statistical rankings of normalized values were also used as a secondary normalization technique to mitigate the effects of a non-normal data set. Ranking transformations allowed a proper account to be made of the multiple zero values present within the data set (not all species of coral exhibit all forms of disease, resulting in numerous zero values present within the data). These transformations involved assigning a numerical value (on a scale of 100) to each value in the original data set. Original data set values which were similar were assigned similar values. These rankings were then subjected to the same 2-way ANOVA involving bank and species on an individual disease basis.

Results

Bacterial Isolation and Enumeration

The water column above bleached areas of *Montastraea annularis* complex and *Millepora* sp. contained approximately equal bacterial cell densities; however, these

values were typically half those found in samples obtained from above *Diploria strigosa*. Water samples obtained from above *D. strigosa* displaying black band disease and bleaching contained the highest cell densities of bacteria, 47.75 and 34.26 cells/mL, respectively (Table 1). Water associated with bleaching of *Porites porites* and *Montastraea annularis* contained mean bacterial cell densities of 29.11 cells/mL and 15.53 cells/mL, respectively. Samples from all apparently uninfected corals showed a mean density of 6.0 cells/mL. Although mean cell density counts were numerically greater on the East Bank, the difference between these banks was not statistically significant for any disease. There was, however, a single significant difference between all species impacted by bleaching (P=0.0030).

Bacterial Speciation

Eleven water samples contained bacteria that could be identified by the Biolog™ system. Despite attempting different isolation techniques and numerous transfers, some cultures persisted in displaying mixed morphologies (e.g. rods and cocci); thus, rendering them inappropriate for speciation by the MicroLog™ microbial identification system. Other isolates yielded a color change in all the MicroLog™ microplate wells and were considered unidentifiable.

Only two species of common marine bacteria were identified: *Vibrio alginolyticus* and *V. proteolyticus*. The remaining identified nine isolates were bacteria common to areas of human activity (Table 2).

All bacterial isolates, excluding one, were oxidase positive. Eleven isolates were Gram positive, three were Gram variable, and six were Gram negative. In terms of cellular morphology, thirteen isolates were coccoid, one was of mixed morphology and

TABLE 1. *Mean bacterial cell densities (and natural log transformation) associated with the water column above diseased corals observed at each bank.*

Disease	Species	Mean cells/mL	ln cells/mL
Bleach	*Diploria strigosa*	34.26	3.53
	Montastraea annularis	15.53	2.74
	Montastraea cavernosa	3.47	1.24
	Porites asteroides	8.95	2.19
	Millepora species	15.60	2.75
	Copophilia natans	4.10	1.41
	Porites porites	29.11	3.37
Black Band Disease	*Montastraea annularis*	9.88	2.29
	Porites asteroides	47.75	3.87
Other	*Montastraea annularis*	18.40	2.91
Yellow Blotch	*Diploria strigosa*	15.02	2.71
	Montastraea cavernosa	4.16	1.43
	Copophilia natans	4.48	1.5
	Madracis mirabilis	3.56	1.27
	Porites porites	5.82	1.76
Apparently Healthy	*Diploria strigosa*	5.48	1.7
	Copophilia natans	6.55	1.88

TABLE 2. *Identified bacterial species and the most common environment in which they are found (Holt 1994).*

Species	Common Environment
Staphylococcus aureus anaerobius	skin, mucous, dust, water
Arthrobacter cumminsii	soil
Micrococcus luteus	mammalian skin, soil, air
Brevibacterium mcbrellneri	human skin, dairy products
Corynebacterium auris	mucous membranes
Streptococcus species	mouth, upper respiratory tract
Vibrio alginolyticus	marine
Vibrio proteolyticus	marine
Aeromonas veronii	sewage
Bacillus halodurans	varied
Kytococcus sedentarius	unknown

the others were rod shaped (Table 3).

Video Results

Digital video captures from the study site did not have the clarity of digital still images and required additional post-collection manipulation in order to more clearly identify the visual signs of disease.

Of the total area surveyed at both banks, 68.2% was covered by coral. The remaining area was composed of sand and algal patches, sponges, and bare substrate created by coralline algae.

The most prevalent coral species in the sample area was *M. annularis* complex, accounting for 31.1% of the total coral area observed (Table 4). The second and third most abundant species present at the study site were *D. strigosa* and *P. asteroides*, accounting for 18.8 and 6.7% of coral cover, respectively. These proportions are consistent with those previously reported for this site by Dokken et al. (2000).

Bleaching

Of the total coral observed, 6.52 m^2 was bleached. This condition was the most common indicator of stressed coral. Approximately 75.2% of all *Millepora* sp. (2.92% of the total area surveyed) observed were bleached (Fig. 3; Table 5). Of all *M. annularis* complex surveyed, 11.0% was bleached (3.43% of the total area). *D. strigosa* had 5.1% bleaching (0.97% of the total area). By both statistical normalization procedures used, there were significant differences between the total areas for bleached species (Table 6). *Millipora* sp. and *M. annularis* were the most substantially impacted species using ranking of species and *P. asteroides* was the species most significantly impacted based on

TABLE 3. *Characteristic of bacteria in water-coral interface waters.*

Coral Species	Disease	Gram Stain	Oxidase Test	Cellular Morphology	Associated Bacterial Species Identified
Porites asteroides	Black Band	positive	positive	coccus	none
Porites porites	Bleach	negative	positive	coccus	none
		variable	positive	coccus	none
		positive	positive	coccus	*Streptococcus species*
		positive	positive	rod	*Bacillus halodurans*
		positive	positive	coccus	*Staphylococcus aureus anaerobius*
	Yellow Blotch	variable	positive	coccus	none
Montastrea annularis	Bleach	positive	positive	coccus	*Arthrobacter cumminsii, Corynebacterium auris, Brevibacterium mcbrellneri, Staphylococcus aureus anaerobius, Micrococcus luteus*
Madracis species	Yellow Blotch	positive	negative	rod	none

TABLE 3. *Characteristic of bacteria in water-coral interface waters.* (continued)

Coral Species	Disease	Gram Stain	Oxidase Test	Cellular Morphology	Associated Bacterial Species Identified
Diploria strigosa	Bleach	positive	positive	coccus	none
		variable	positive	coccus	none
		positive	positive	coccus	none
	Unknown	positive	positive	coccus	none
		negative	positive	coccus	none
	Apparently healthy	negative	positive	rod	*Aeromonas veronii*
Colophylia natans	Yellow Blotch	positive	positive	mixed	none
	Unknown	positive	positive	coccus	none
Other Species	Unknown	negative	positive	coccus	none
	Apparently healthy	negative	positive	rod	*Vibrio alginolyticus*
		negative	positive	rod	*Vibrio proteolyticus*

TABLE 4. *Total area of coral species with normalized percentages.*

Species	Total area of coral (m^2)	% of total coral observed per bank	% of total area surveyed (coral and non-coral)
Montastraea annularis	24.93	45.63	31.09
Diploria strigosa	15.06	27.57	18.78
Porites asteroides	5.35	9.79	6.67
Montastraea cavernosa	4.24	7.76	5.29
Millepora species	3.11	5.69	3.88
Siderastrea siderea	1.10	2.01	1.37
Madracis mirabilis	0.22	0.40	0.27
Mussa angulosa	0.21	0.38	0.26
Colpophillia natans	0.16	0.29	0.20
Stephanocoenia mecheli	0.12	0.22	0.15
Madracis decatis	0.08	0.15	0.1
Siderastrea radians	0.04	0.07	0.05
Mycetophyllia danaana	0.01	0.02	0.01
Manicina aerola	0.00	0	0
Species Total	54.63	100	68.13
Picture Area Total	80.19		

FIGURE 3. *Millepora species bleaching at the Flower Garden Banks National Marine Sanctuary.*

TABLE 5. *Comparison of bleaching in different coral species.*

Species	Area of coral w/ bleaching (m^2)	% of total coral observed per bank	% of total area surveyed (coral and non-coral)
Montastraea annularis	2.75	5.03	3.43
Millepora species	2.34	4.28	2.92
Diploria strigosa	0.77	1.41	0.97
Porites asteroides	0.40	0.73	0.50
Montastraea cavernosa	0.17	0.31	0.22
Stephanocoenia mecheli	0.04	0.07	0.05
Madracis mirabilis	0.02	0.04	0.03
Colpophillia natans	0.02	0.04	0.03
Siderastrea siderea	0.01	0.02	0.01
Madracis decatis	0.00	0	0
Total	6.52	11.9	8.13

TABLE 6. *Results of Tukey's HSD post hock test for bleaching for coral species based upon rankings of normalization by species (top), total area surveyed (middle) and total coral area (bottom).*

Normalized per species

M. ae	M. an	S. ra	P. po	M. da	M. des	S. si	S. mei	C. na	M. mi	*D. st	M. ca	P. as	M. ann	Mill.
27.50	27.50	27.50	27.50	27.50	32.25	37.66	38.75	44.50	52.25	66.83	67.83	73.66	78.00	90.83

Normalized per total area

M. ae	M. ang	S. ra	P. po	M. da	M. des	S. me	S. si	C. na	M. mi	M. ca	P. as	D. st	Mill.	M. anns
27.50	27.50	27.50	27.50	27.50	32.41	34.92	37.50	38.75	44.25	69.66	75.50	77.66	88.00	89.66

Normalized per living coral

M. ae	M. ang	S. ra	P. po	M. da	M. de	S. me	C. na	S. si	M. mi	D. st	M. anns	M. ca	P. ass	Mill.
27.50	27.50	27.50	27.50	27.50	33.75	33.75	43.42	43.42	53.08	75.25	75.25	75.25	75.25	75.25

C. na = *Colpophillia natans*
D. st = *Diploria strigosa*
M. ae = *Manicina aerolata*
M. ang = *Mussa angulosa*
M. ann = *Montastraea annularis*
M. ca = *Montastraea cavernosa*
M. da = *Mycetophyllia danaana*
M. de = *Madracis decatis*
M. mi = *Madracis mirabilis*
Mill. = *Milleppora species*
P. as = *Porites asteroids*
P. po = *Porites porites*
S. me = *Stephanocoenia mechelini*
S. ra = *Siderastrea radians*
S. si = *Siderastrea siderea*

rankings of total coral area. A significant difference between the banks was observed when analyzing the rank of normalized per total coral coverage (P=0.0000).

Black Band Disease(BBD)

Black Band Disease (BBD) was the most prevalent disease at the FGBNMS. The total area of coral covered by BBD was 2.2 m^2 (2.72% of the total area surveyed) with *M. annularis*, *D. strigosa*, and *M. cavernosa* showing the highest areas infected by BBD (1.43, 0.81, and 0.25% respectively; Fig. 4; Table 7). The data indicate that there were significant differences between the normalized area values for both the banks and across species (P=0.0100 and 0.0002, respectively). The species with most extensive BBD were *D. strigosa* and *M. annularis*, based on rankings of species and total surveyed area. Based on rankings of total coral area, *M. cavernosa*, and *M. annularis* were most affected by BBD (Table 8). In the data there appears to be a significant interaction between species and bank (P=0.0002).

Conditions of Unidentified Disease

Approximately 1.9m^2 of the coral observed at the FGBNMS exhibited unidentifiable disease (2.33% of the total area surveyed). Of the total area surveyed, *M. annularis*, *D. strigosa*, and *M. cavernosa* had the highest levels of unknown disease (1.21, 0.49, and 0.34%, respectively; Fig. 5; Table 9). Results from ANOVA performed on the normalizations for unknown disease indicate a statistically significant difference between species (P=0.0090). *M. annularis* and *M. cavernosa* exhibited the most unidentified disease based on rankings of species, total area surveyed, and total coral area (Table 10). There was, however, no significant difference between banks or the interaction

Figure 4. *Black band disease on* Siderastrea siderea*; note skeleton colonized by algae behind the band.*

TABLE 7. *Black band disease (BBD) areas of coral species and normalized percentages each represent.*

Species	Area of BBD (m^2)	% of total coral observed per bank	% of total area surveyed (coral and non-coral)
Montastraea annularis	1.15	2.11	1.43
Diploria strigosa	0.65	1.19	0.81
Montastraea cavernosa	0.20	0.37	0.25
Siderastrea siderea	0.10	0.18	0.13
Porites asteroides	0.07	0.13	0.09
Millepora species	0.01	0.02	0.01
Coplophillia natans	0.00	0.00	0.00
Total	2.19	3.99	2.72

TABLE 8. *Results of Tukey's HSD post hock test for black band disease for coral species based upon rankings of normalization by species (top), total area surveyed (middle) and total coral area (bottom).*

Normalized per species

M. ae	M. mi	M. ang	M. de	S. me	S. ra	P. po	M. da	C. na	Mill.	S. si	P. as	M. ca	M. anns	D. st
39.50	39.50	39.50	39.50	39.50	39.50	39.50	39.50	43.71	46.42	48.75	62.08	71.50	71.66	72.16

Normalized per total area

M. ae	M. mi	M. ang	M. de	S. me	S. ra	P. po	M. da	C. na	Mill.	S. si	P. as	M. ca	D. str	M. anns
39.50	39.50	39.50	39.50	39.50	39.50	39.50	39.50	42.79	46.42	47.75	61.58	70.16	73.00	75.50

Normalized per total living coral

M. ae	M. mi	M. ang	M. de	S. me	S. ra	P. po	M. da	C. na	S. si	Mill.	P. as	D. st	M. ann	M. ca
39.50	39.50	39.50	39.50	39.50	39.50	39.50	39.50	43.29	47.08	48.58	63.75	71.33	71.33	71.33

C. na = *Colpophillia natans*
D. st = *Diploria strigosa*
M. ae = *Manicina aerolata*
M. ang = *Mussa angulosa*
M. ann = *Montastraea annularis*
M. ca = *Montastraea cavernosa*
M. da = *Mycetophyllia danaana*
M. de = *Madracis decatis*
M. mi = *Madracis mirabilis*
Mill = *Milleppora species*
P. as = *Porites asteroids*
P. po = *Porites porites*
S. me = *Stephanocoenia mechelini*
S. ra = *Siderastrea radians*
S. si = *Siderastrea siderea*

FIGURE 5. *Unidentified disease at the Flower Garden Banks National Marine Sanctuary.*

TABLE 9. *Comparison of extent of unidentified disease associated with each coral type.*

Species	Area of unidentified disease(m^2)	% of total coral observed per bank	% of total area surveyed (coral and non-coral)
Montastraea annularis	0.97	1.78	1.21
Diploria strigosa	0.39	0.71	0.49
Montastarea cavernosa	0.27	0.49	0.34
Porites asteroides	0.19	0.35	0.23
Colpophillia natans	0.04	0.07	0.05
Siderastrea siderea	0.01	0.02	0.01
Total	1.87	3.43	2.33

TABLE 10. *Results of Tukey's HSD post hock test for diseases of unidentified origin for coral species based upon rankings of normalization by species (top), total area surveyed (middle) and total coral area (bottom).*

Normalized per species

Mill.	M. ae	M. mi	M. ang	M. de	S. me	S. ra	P. po	M. da	S. si	C. na	D. st	P. as	M. ca	M. ann
38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	45.83	47.75	69.83	70.33	75.58	83.16

Normalized per total area

Mill.	M. ae	M. mi	M. ang	M. de	S. me	S. ra	P. po	M. da	S. si	C. na	P. as	D. st	M. ca	M. anns
38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	44.66	45.16	68.50	72.66	74.08	90.00

Normalized per living coral

Mill.	M. ae	M. mis	M. ang	M. de	S. me	S. ra	P. po	M. da	S. si	C. na	P. as	D. st	M. ca	M. ann
38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00	45.16	46.08	68.50	72.16	74.83	86.50

C. na = *Colpophillia natans*
D. st = *Diploria strigosa*
M. ae = *Manicina aerolata*
M. ang = *Mussa angulosa*
M. ann = *Montastraea annularis*
M. ca = *Montastraea cavernosa*
M. da = *Mycetophyllia danaana*
M. de = *Madracis decatis*
M. mi = *Madracis mirabilis*
Mill = *Milleppora species*
P. as = *Porites asteroids*
P. po = *Porites porites*
S. me = *Stephanocoenia mechelini*
S. ra = *Siderastrea radians*
S. si = *Siderastrea siderea*

of bank and species (P>0.0500).

Ridge Mortality (RMD)

The total area of coral area exhibiting ridge mortality disease (RMD) was 0.17 m^2 (0.2% of the total area surveyed). The most coverage was encountered on *Diploria strigosa* (0.15 m^2), followed by *M. annularis* (0.01 m^2). These values represented 0.2 and 0.02 %, respectively, of the total area surveyed (Fig. 6; Table 11). Significant differences in area of coral showing RMD were observed only between species (P=0.0001). The most ridge mortality was exhibited on *D. strigosa* and *P. asteroides*, based on rankings of species, total area surveyed and total coral area (Table 12).

Yellow Blotch (YB)

Only a small proportion of overall coral disease observed in this study could be attributed to YB (0.16 m^2, 0.3% of the total area surveyed). *Montastrea annularis* was most covered by this disease (0.11 m^2), nearly 5 times that of *M. cavernosa* (0.02 m^2, Figs. 7, 8; Table 13). The extent of the disease was greater for *Diploria strigosa* and *M. annularis* when analyzed using the rankings of normalizations and when applying the normalization to species, total area surveyed, and total coral area (Table 14).

Plague Disease

Plague disease was uncommon at the FGNMS and comprised only 0.15 m^2 of the total area surveyed (0.19%). Of the coral observed to have plague disease, *M. annularis* showed the greatest affected area. *Diploria strigosa* showed approximately half of this (0.12 and 0.05 % of the total area observed; Fig. 9; Table 15). Both these species were significantly affected by plague, based upon rankings of species, total area surveyed, and total coral area (Table 16; P=0.0000).

FIGURE 6. *Ridge mortality disease at the Flower Garden Banks National Marine Sanctuary.*

TABLE 11. *Ridge mortality areas of coral species and normalized percentages each represent.*

Species	Area of ridge mortality(cm^2)[a]	% of total coral observed per bank	% of total area surveyed (coral and non-coral)
Diploria strigosa	1525.89	0.29	0.20
Montastraea annularis	147.47	0.03	0.02
Colpophillia. natans	41.49	0.01	0.01
Porites asteroides	20.46	0.00	0.00
Total	1,735.31	0.33	0.22

[a] shown as cm^2 due to small values.

TABLE 12. *Results of Tukey's HSD post hock test for ridge mortality of coral species based upon rankings of normalization by species (top), total area surveyed (middle) and total coral area (bottom).*

Normalized per species

M. ca	Mill.	M. ae	M. mi	M. ang	M. de	S. si	S. me	S. ra	P. po	M. da	C. na	M. ann	P. as	D. st
44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	48.16	51.83	59.16	84.66

Normalized per total area

M. ca	Mill.	M. ae	M. mi	M. ang	M. de	S. si	S. me	S. ra	P. po	M. da	C. na	M. ann	P. as	D. st
44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	47.91	52.16	58.83	85.16

Normalized per living coral

M. ca	Mill.	M. ae	M. mi	M. ang	M. de	S. si	S. me	S. ra	P. po	M. da	C. na	M. ann	P. as	D. st
44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	44.00	47.87	51.75	60.25	84.25

C. na = *Colpophillia natans*
D. st = *Diploria strigosa*
M. ae = *Manicina aerolata*
M. ang = *Mussa angulosa*
M. ann = *Montastraea annularis*
M. ca = *Montastraea cavernosa*
M. da = *Mycetophyllia danaana*
M. de = *Madracis decatis*
M. mi = *Madracis mirabilis*
Mill = *Milleppora species*
P. as = *Porites asteroids*
P. po = *Porites porites*
S. me = *Stephanocoenia mechelini*
S. ra = *Siderastrea radians*
S. si = *Siderastrea siderea*

FIGURE 7. *Yellow Band ring (Photo by E. C. Peters; Copyright 2000, used with permission).*

FIGURE 8. *Yellow Band irregular (Photo by E. C. Peters; Copyright 2000, used with permission).*

TABLE 13. *Yellow blotch areas of coral species and normalized percentages each represent.*

Species	Area of yellow blotch(cm^2)[a]	% of total coral observed per bank	% of total area surveyed (coral and non-coral)
Montastraea annularis	1067.06	0.20	0.13
Montastraea cavernosa	235.69	0.05	0.03
Diploria. strigosa	107.61	0.02	0.01
Porites asteroides	92.98	0.01	0.01
Siderastrea. siderea	48.42	0.01	0.01
Total	1,551.76	0.30	0.20

[a] shown as cm^2 due to small values.

TABLE 15. *Other diseases, total areas of coral species with normalized percentages by total coral and total area.*

Species	Area of other diseases (cm^2)[a]	% of total coral observed per bank	% of total area surveyed (coral and non-coral)
Montastraea annularis	961.53	0.18	0.12
Diploria strigosa	411.07	0.08	0.05
Porites asteroides	96.86	0.02	0.01
Montastraea cavernosa	83.85	0.02	0.01
Total	1,553.31	0.30	0.19

[a] shown as cm^2 due to small values

Hyperplasia

Diploria strigosa was the only species of coral showing signs of hyperplasia (0.003 m^2, 0.00008% of the total area sampled). Incidence of hyperplasia was not significantly different when analyzed by either species, banks, or interaction of bank and species (Fig. 10; Table 17).

Temperature and Depth

Sea surface temperatures during the collection cruises remained between 29 and 30°C. These values are within previously observed values for late July-September (NOAA 2002b; buoy # 42019). Reef surface temperatures observed during videotaping were similar to those reported by Dokken et al. (2000; 2002).

Depth of the study site varied between banks and transects, and ranged from 20 m to over 25 m.

Discussion

Microbiology

The bacterial study was affected by problems encountered in the transport of samples including inadequate cold storage and lack of sterile work areas. The various sample transport methods used yielded mixed results. Use of an ice chest chilled by ice packs proved insufficient to maintain low temperatures due to the extensive length of transport time between the study site and the laboratory (2-3 days).

Coral with black band disease and “other” diseases showed the highest cell densities of bacteria associated with the overlying water columns (100.85 and 110.40 cells/mL, respectively). Unequal numbers of samples were collected from

different diseased areas. This prevented the data from being normally distributed and therefore caused it to be more difficult to analyze.

Future studies should include larger numbers of samples from each disease type. Multiple samples should also be included from each bank. These samples could provide an explanation for differences in cell densities observed above different corals. A higher incidence of disease was observed on the East Bank compared to the West Bank. The reason for this difference could potentially be associated with geographic placement of the bank and patterns of local currents around each individual location.

Only two of the fourteen bacterial species identified are common in marine waters. *Vibrio alginolyticus* and *V. proteolyticus* are Gram negative rods present in nearly all marine environments, often growing at temperatures less than 25°C (Holt ed. 1994). The remainder were species typically associated with human activity. Of the species identified, the majority were Gram positive, which contrast with previous studies that found primarily Gram negative bacteria typically associated with fecal contamination in the Florida Keys (Lipp et al. 2002).

Another potential explanation for samples containing a predominance of bacteria associated with human activities could be the close proximity of the FGBNMS to oil/gas platforms. To date, there is no evidence that these platforms have had any deleterious impact upon the site (Dokken et al. 2000; 2002); however, the potential for human waste-treatment spills exists. Daszak et al. (2001) has postulated that ballast water from international freighters could contain non-endemic bacteria which, if released into a new ecosystem, could facilitate the spread of disease and/or increase interspecific competition. Four of the five busiest ports in the United States are located in the Gulf of Mexico along

the Texas and Louisiana coast (USACE 2002). Two international "safety fairlanes" pass near the sanctuary. Until its designation as a national marine sanctuary, shipping vessels could use the FGBNMS as anchorage (NOAA 2000; IMO 2001). This could have been a factor in the introduction of exotic and pathogenic organisms to the site.

There are three main current circulation modes affecting the FGBNMS (Lugo-Fernandez et al. 2001): Cyclonic motion reaching from Mexico to the Texas Shelf; a continuous eastward flow from Mexico to Florida along 87.5°W; and a cross basin transport cycle ending in the Florida Keys. These currents could potentially play a role in the transport of bacteria around the Gulf of Mexico. .

Lipp et al. (2002) found that by analyzing the coral microsurface layer they were able to better identify bacteria specifically associated with coral. The microlayer appears to accumulate environmental microorganisms enhancing coral susceptibility to water-borne pathogens. Future analysis of bacteria associated with corals should therefore emphasize the coral microsurface layer.

To improve the accuracy and reproducibility of microbial field collections, the following modifications are suggested for future studies: 1) use of disposable or one-time use sterile sheets as work surfaces, and 2) portable mini-refrigerators for sample storage during transport if no usable on-ship refrigerator exists; portable coolers with replaceable ice packs proved inefficient at keeping a stable cool temperature over multi-day periods. Consistent temperature could increase the survival of bacteria within chilled water samples (Ritchie and Smith 1995a). Bacterial survival was greater on artificial seawater plates during transport; thus, this media should be utilized for transport of samples.

According to Le Campion-Alsumard et al. (1995), biochemical testing is inferior to molecular techniques in identifying marine pathogens due to limited information concerning marine bacteria and variable biochemical reactions from identical strains of bacteria. Molecular techniques are, however, more difficult and expensive to conduct which could potentially limit their application.

Multicellular endothelial isolates (MEI) of coral described by Kopecky and Ostrander (1999) could improve sample stability on future sampling cruises to the FGBNMS. These isolates are stable for 300 hr without additional medium exchange and could assist in storage of various tissues and bacteria present at the FGBNMS. In addition, MEI have provided excellent data for testing Koch's postulates (Kopecky and Ostrander 1999; Scully et al. 2001). This type of sampling could be incorporated into a future long-term disease-monitoring program at the FGBNMS in order to improve pathogen surveys.

Statistical analysis of bacterial cell density showed that water-column cell densities associated with species of coral or type of disease were not significantly different at either of the banks nor between the banks. Thus, the first hypothesis stating that cell densities above different coral, disease and banks are similar is accepted. This does not necessarily imply that cell densities were independent of these factors, nor does it indicate that bacterial cell densities above bleached areas were in some way dependent on the coral species over which they occurred.

Possible Bleaching Mechanisms at the FGBNMS

Most studies agree that coral bleaching is a symptom of stress and conditions contributing to stress could be bacterial in origin (Hagman and Gittings 1992; Ravindran

et al. 1999; Dokken et al. 2000). The higher incidence of bleaching of *M. annularis* could have been due to its high relative surface area at the site. The even higher percentage of bleaching observed on *Millepora* sp. was unexplained, but could have been due to the different physiology of the genera as compared to scleractinian coral species.

Water temperatures observed during this study were less than those commonly associated with temperature-induced bleaching events (Banin et al. 2000). Typically, a water temperature greater than 30°C is required to induce bleaching. Temperatures for 1997 fluctuated around 30-31°C, between June and October (NOAA 2002b). These values were higher than usual, and are near the minimum for temperature generated bleaching. Dokken et al. (2000; 2002) described water temperatures at reef level nearly 1-2°C cooler than sea-surface temperatures reported by NOAA buoys. The lower temperature of the reef when compared to the sea-surface could indicate an insulating effect by the water column. This lower temperature could possibly inhibit temperature-related bleaching episodes at the FGBNMS. In El Niño years, water temperatures during summer months are typically higher than in non-El Niño (i.e. La Niña) years. Although water temperature data was incomplete for the summer months of the El Niño event of 1991, the 1997 event did not show a substantial increase (NOAA 2002a, USACE 2002). Theoretically, although the reef-level temperatures at the FGBNMS are not high enough to induce bleaching alone, they are near the minimum threshold, and thus could be an additional stress experienced by the coral.

Bleaching at the FGBNMS could possibly be partially instigated by microbial infection and/or processes. Pathogenic microorganisms have been shown to increase virulence with increased water temperature (Kushmaro et al. 1996). For example, *Vibrio*

shiloi AK-1 was shown to be the causative agent of bleaching in *Oculina patagonica* in the Mediterranean Sea. *O. patagonica* exhibits an annual bleaching of approximately 80% once infected (Kushmaro et al. 1998). Banin et al. (2001) showed that *V. shiloi* can adhere to coral within 6 hr of exposure at specific receptor cells on the coral surface. Once it penetrates the tissues, it differentiates into a viable but non-culturable state and multiplies exponentially. The bacterium then produces an endotoxin which inhibits the photosynthetic rates of zooxanthellae (leading to their expulsion) and lyses zooxanthellae. At temperatures below 20°C *V. shiloi* does not adhere to *O. patagonica* and it has also been shown to express a ten-fold greater toxic reaction at a water temperature of 29°C vs. 16°C (Banin et al. 2000). Additional studies focusing on bacteria associated with bleached corals are warranted to help further elucidate the potential causes of bleaching symptoms at the FGBNMS.

Other *Vibrio* species have been shown to induce tissue necrosis and bleaching at warmer temperatures (Martin et al. 2002). *V. shiloi* was also observed to lyse within coral tissue at temperatures approximately 16°C as a result of intracellular coral defense mechanisms (Banin et al. 2000). Because *V. shiloi* was shown to reoccur each spring, it was concluded that "fresh" populations of bacteria are introduced to the coral environment on an annual basis. Studies are ongoing to identify the mechanisms of coral defense, as well as the origin of this pathogenic bacterium (Israely et al. 2001). Additionally, Harvell et al. (2001) identified *Scytonema* sp. in bleached samples of gorgonians. Evaluation of its pathogenicity has not been undertaken, although grafts of bleached coral have been shown to infect healthy coral in various experiments (Harvell et al. 2001).

Irrespective of the causative agent, bleaching is recognized as a symptom or condition of stress in corals (Dokken et al. 2000). Environmental stress has been associated with increased disease susceptibility (Bruckner et al. 1997a). Additional investigations are recommended during the colder months of February and March in order to provide a cold-water comparison of overall disease at the FGBNMS. This cold-water data could provide a more complete evaluation of bleaching events and rate of increase of this condition at the FGBNMS.

Future evaluations of bleaching at the FGBNMS should also incorporate National Oceanic and Atmospheric Administration (NOAA) guidelines (Ginsburg 2000) for visual observation and classification of bleaching: pale (discoloration of coral tissue), partly bleached (patches of fully bleached or white tissue), and bleached (tissue is totally white, no zooxanthellae visible). These guidelines would help identify in greater detail the level and severity of bleaching occurring at the site.

Solar irradiation (Catala-Stucki 1959), industrial and agricultural pollution such as phosphorus (Holdway 2002), and reduced salinity have been previously proposed as agents increasing stress in corals and, hence, increasing the incidence of bleaching. Dokken et al. (2000; 2002) surveyed levels of solar irradiation and water quality conditions present at the FGBNMS. Due to its depth and remote location, solar irradiation, as well as concentrations of industrial and agricultural impacts, were low suggesting these factors may not significantly influence disease at the FGBNMS. As the coral are located father from the surface of the water there is more water to act as a protective layer which could act as insulation against fluctuations in solar radiation that

the earth experiences regularly. The remote location, far from direct terrestrial impact could also potentially mitigate the effects of terrestrial anthropogenic effects.

Dokken et al. (2002) postulated that due to the physical conditions present at the FGBNMS, corals may be insulated from the fluctuations in temperature observed in shallow-water reefs. The insulative properties that could prevent large fluctuations of temperature at the FGBNMS could also minimize fluctuations in salinity observed in shallow water reefs.

Possible Etiology of Black Band Disease (BBD) at the FGBNMS

Black Band Disease has been studied extensively and is the only coral disease for which complete etiology has been determined (Carlton and Richardson 1995; Richardson 1997b). Around the world, BBD has been found to infect forty-two species of coral (Green and Bruckner 2000). At the FGBNMS, it was primarily associated with *M. annularis*, *D. strigosa*, and *M. cavernosa*. This disease is most often observed as a 1 mm thick necrotic band forming a boundary between living coral and calcified skeletons. It consists of an aggregation of various microbes analogous to the microbial mats found in sulfide rich, well-lit marine environments. The cyanobacterium, *Phormidium corallyticum*, the primary causative agent for tissue necrosis (Rutzler and Santavy 1983), was not identified in this study. Black Band Disease appears to be opportunistic, often associated with environmental stressors such as industrial pollution or agricultural runoff, notably the same stressors that are associated with bleaching (Bruckner et al. 1997). As there are no indications of industrial pollution, and the site is sufficiently isolated from agricultural runoff, the occurrence of BBD in the FGBNMS appears to be independent of these environmental stressors. Among clumped aggregations of colonies irrespective of

species, BBD has been show to spread rapidly, indicating that it can be highly infectious. Kuta and Richardson (1996) reported its rate of colonization to increase during the warmer months when water temperatures exceed 25°C. This observation is contrary to Edmund's study (1991) in the Caribbean Sea, which showed that though rates of infection decreased during winter months, those between neighboring colonies remained low even during the summer. Marano-Briggs et al. (2000) reported a strain of *Marichromatium purpuratum* which produces a thick biofilm within the consortium that could be partially responsible for the BBD mat formation. Research is currently being undertaken to identify whether this bacterium is present within dead portions of the mat. If so, it could possibly be a natural opportunistic pathogen present in the water column at all times.

Future studies should confirm the effect of water temperature on spread of BBD (and potentially other temperature influenced bacterial diseases) at the site. The presence of BBD and *P. coraliticum* associated with *M. annularis, D. strigosa,* and *M. cavernosa* could simply be explained by the prevalence of these corals within the study area. One water sample containing BBD and taken from above *P. asteroides* contained a high density of bacteria; however, overall mean cell density of bacteria above this species closely resembled that of the samples from apparently healthy coral. This result indicates that modifications in sampling methodology (e.g. from the mucous layer) could further assist in the identification of extant consortium species. Additionally, the interspecific spread of BBD should be monitored to better understand species specificity.

Potential Etiologies of Unidentified Disease at the FGBNMS

Areas of coral categorized as possessing conditions of unknown origin within this study were apparently consistent with those identified as "unknown" by the NOAA Disease and Predation Identification Cards (Bruckner and Bruckner 1998b), and as "rapid waste disease" by the Harvard Global Change Program Disease Identification Cards (HEED 1997). As with BBD, *M. annularis*, *D. strigosa*, and *M. cavernosa* represented the species most infected with unidentified disease at the FGBNMS. Currently, much debate exists as to the etiology of this disease. Arguments regarding origin range from the macrobiological (parrot-fish grazing hypothesis; Ogden 1997; Bruckner and Bruckner 1998a; 2000b), to the microbiological (as of yet unidentified microscopic pathogen; Cervino et al. 1997; Richardson 1998), to a combination of the two (i.e. parrotfish are cueing on some signal from the diseased but apparently healthy appearing coral). Cervino et al. (1997) renamed this condition "rapid wasting syndrome" (RWS). Cell densities of water samples taken from above conditions of unknown diseased areas were near the mean level of cells throughout the samples. This author did not observe concentrated focused feeding by multiple parrotfish on these corals; however, individual fish were feeding on areas of coral exhibiting this condition. Bruckner and Bruckner (1998a; 2000b; Bruckner et al. 2000) proposed that parrotfish were the causative factor of RWS. In cage exclusion studies, Bruckner and Bruckner (2000a) were able to show that RWS was not communicable to uninfected coral and that affected areas did not increase in size when parrotfish were excluded. To conclusively determine the etiology of this condition within the study area, a similar cage exclusion study should be conducted at the FGBNMS.

Ridge MortalityDisease (RMD) at the FGBNMS

Montastrea annularis, M. cavernosa, and *D. strigosa* were the species of coral most affected by this Ridge Mortality Disease (RMD). Ginsburg (2000) identified ridge mortality as a manifestation of predation, and thus, did not consider it a true disease. Other studies are ongoing to confirm the etiology of this condition (Ginsburg 2000). Should RMD indeed be a manifestation of predation, efforts to differentiate it from the early stages of other predatory conditions should be undertaken.

Potential Etiology of Yellow Blotch (YB) at the FGBNMS

Yellow blotch (YB) was originally identified in the literature as ring bleaching in the early 1970's (Cervino et al. 2001). It appears as an irregularly-shaped yellow patch on the surface of coral. Yellow blotch appeared to target *Montastraea* sp., and spreads at rates of 0.6 cm or more per month. The tissue in the center dies and begins to fill with sediment and is ultimately colonized by algae. This disease accounted for a total of 0.2% of disease coverage in the study area. In a recent study Cervino et al. (2001), showed that 90% of *M. annularis* at a site in the Florida Keys was infected with YB. Spread of a disease which progresses rapidly and preferentially impacts *M. annularis* (the dominant scleractinian coral species) could have a potentially severe negative impact upon the FGBNMS. Yellow Blotch is an apparently minor influence upon the site.

Potential Etiologies and Impacts of Plague Disease on the FGBNMS

Plague was the least observed disease at the FGBNMS study site. *Montastrea annularis, D. strigosa, P. asteroides* and *M. cavernosa* were the only coral species showing this disease and it comprised only 0.3% of the total coral cover. Reasons for its infrequency could include less than optimal environmental conditions and lack of viable

hosts. Plague was originally described in the late 1970s (Dustan 1977), and appeared to consist of three recognizable strains: Types I, II, and III. Type I can spread at rates of 3 mm per day (Dustan and Halas 1987). Type II, which was observed in the Florida Keys during the 1990s, appears to be far more virulent and can spread at a rate of 2 cm per day. It is capable of destroying complete coral colonies in less than 3 months at an overall mortality rate of 38% (Richardson 1998). Type III has only recently been identified and little is known about its rates of spread. The most dependable means of to differentiating among plague types is long-term visual observation (Green and Bruckner 2000).

Hyperplasms

Some coral tumors were observed during the course of this study. Tumors, or neoplasms, have been likened to cancer in that they are areas of abnormal tissue (i.e. coral) growth. Yamashiro et al. (2000) observed tumors on *Montipora informis* off the coast of Japan. These tumors were observed to have fewer polyps than corresponding sections of healthy skeletal tissue, lower zooxanthellae density levels and less-dense skeletal structure. Hyperplasms have been shown to contain reduced levels of lipids, wax, and triacylglycerol within their tissues (Yamashiro et al. 2001). This decreased level of lipids was found to be directly analogous to cancers in mammals in that the affected tissues have a higher energy demand and thus less availability for storage. Hyperplasms are more prone to bleaching than the normal tissue surrounding them (Yamashiro et al. 2001). The coral tumors exhibited higher levels of mortality during bleaching events.

With decreased levels of zooxanthellae in tissues of tumors, corals are more stressed, which partially explains their increased level of mortality. As stress upon the

coral organism increases their level of zooxanthellae decreases, which in turn increases stress, potentially leading to the death of coral. Causative agents of hyperplasms are still being evaluated (Yamashiro et al. 2000). The FGBNMS exhibited relatively low occurrence of neoplasmic tissues. Future investigations of neoplasms may reveal their relative abundance with respect to the levels of disease, and, hence, may eventually be observed as indicators of a colony or reef's overall health. Evaluations of hyperplasmic tissue should be undertaken during bleaching events at the FGBNMS in order to ascertain mortality associated with both healthy and tumored tissues.

Summary of Coral Disease at FGBNMS

"Disease" is identified by Webster's New World College Dictionary, 3rd edition (Neufeldt ed. 1997) as: "any departure from health." This definition as it pertains to corals, could include conditions such as bleaching and unknown origin. The most prevalent diseases and conditions of disease at the study site were bleaching (8.13%), black band disease (2.72%), and conditions of unidentified origin (2.33%). The extent of bleaching determined by this study should probably be considered the maximum area at present affected by bleaching for the year 2001. Bleaching coverage should be expected to decrease with a corresponding decrease in water temperature. Additional studies should be conducted during the colder spring months to establish minimum coverage levels. This comparison could possibly confirm, as elsewhere, that coral bleaching at the Flower Garden Banks National Marine Sanctuary is temperature-related and, therefore, a condition from which corals could possibly recover. The incorporation of cold-water monitoring data would enable observations and comparisons to be made of the rates of the spread of disease.

Microbial analyses were inconclusive in determining whether cell density counts increased in the water column immediately above diseased corals versus apparently healthy corals. Bacterial cell densities were different above corals exhibiting varying degrees of "health." However, this variation was probably influenced by micro-currents rather than being indicative of the health of the coral. The bacteria normally associated with humans which were found in the microbial samples were potentially the result of contamination rather than from sewage outflows as is prevalent in studies conducted near coastal zones. Mucoidal-layer bacterial sampling should be incorporated into the permanent photograph portion of site monitoring (Dokken et al. 2000; 2002) because it would enable long-term studies of bacterial colonization associated with known sites of disease at the site. Conclusive identification of bacteria could then possibly be utilized to determine origin of pathogenic bacteria.

Pathogen introduction from ballast water has been indicated as a major source of exotic, possibly pathogenic, bacteria in new areas (Daszak et al. 2001). At the FGBNMS international "safety fairlanes" pass near the sanctuary, and four of the busiest ports in the United States are located in adjoining Gulf states. These shipping lanes and ports near the sanctuary increase the chance that exotic bacteria can be introduced into the FGBNMS by ballast water discharge. Due to international convention, anchorage at the site has been prohibited since the initial designation of the site as a sanctuary in 1992 (NOAA 2000). This prohibition could potentially decrease the introduction of exotic and pathogenic species to the FGBNMS. The treatment of ballast water prior to release may inhibit the transfer of exotic and pathogenic species between environments around the world.

For disease studies at the FGBNMS, improvements in on-board handling of bacterial samples should be implemented. The use of a portable sterile work area would considerably improve efficiency of sample collection and a portable refrigerator would greatly facilitate proper sample storage.

The quantitative video portion of this project showed that coral disease and disease conditions were present at the FGBNMS. However, because of lower percentage coverage, they appear to play only a minor role in the overall ecosystem. These results are in contrast with recent studies conducted in the Florida Keys which indicate that the level of coral disease is much higher in other locations (Porter et al. 2001). The alternate hypotheses of the area covered by disease being significantly different between banks and significantly different between species was supported using multiple normalizations and transformations. The microclimate differences between banks such as localized currents within each bank (encountered around individual colonies), local faunal and nearby floral (algae) interaction with the coral, as well diver interaction could potentially explain the differences between the banks.

Bleaching, and the long-term effects of immune response induced by bleaching, could affect coral in numerous ways and should be carefully monitored. Future research should evaluate whether previously bleached and recovered corals become more susceptible to subsequent infection.

The effect of temperature on spread of BBD should be monitored in the future because its spread is reduced by low temperatures. Conditions of unknown origin could be subject to further evaluation by cage exclusion studies which could determine the possible etiology of this condition at the study site. Digital video sampling techniques

can be used for long-term monitoring programs. This technique provides a rapid sample methodology, allowing for longer study transects as data collection is facilitated over that of still images. A negative aspect of digital video sampling was the inferior resolution of video snapshot captures. Resolution of captures via digital video were approximately one-half of that produced by the same camera operating in digital still capture mode. However, digital video will likely continue to improve in resolution and, thus, should gain acceptance as a valuable sampling tool for long term monitoring projects.

Green and Bruckner (2000) stated that when evaluating coral disease, two conclusions form: 1) it is rare for diseases to be observed in a single colony and 2) complete tissue level recovery (i.e. complete recovery) has never been recorded. This adds impetus to the urgency for increased study and should aid in the incorporation of disease studies via long-term monitoring programs.

Large-scale Implications

Much research needs to be conducted to understand the etiology of the multitude of diseases and disease conditions of coral reefs around the world. Thirty-four mass mortalities of diverse groups of coral have been recorded (Green and Bruckner 2000) and these are becoming more prevalent. The mortalities range from large-scale bleaching coinciding with El Niño events (Nyström et al. 2000) to mass mortalities of marine invertebrates in the Mediterranean (Perez et al. 2000). During 1999, the northwestern Mediterranean Sea experienced a large-scale, multiple-species die off. This event has been compared to mass die off in tropical areas of the world (Perez et al. 2000). Over the past 30 years, disease events have been increasing in frequency and magnitude (Sherman

2000) and, as emphasized by the Perez et al. (2000) study, the numbers of these events could possibly continue to rise.

With 27% of the world's corals already destroyed, the trend towards mass coral extinction is projected to accelerate resulting in the worldwide death of all corals within 20 years (Wilkinson 2000). Fastidious monitoring of corals at the FGBNMS could help elucidate the progression of coral destruction in that relatively isolated location and provide a comparison with more coastally located reefs. There is still much debate on this topic, and though the exact amount of damage caused by disease remains unquantified, it is nevertheless a contributor. Coral in nontropical regions such as the FGBNMS may have a greater chance of limiting their exposure to temperature-related diseases due to recently lower sea temperatures. Ultimately, corals could be removed from the important ecological role they play in trophic hierarchies.

Literature Cited

Antonius, A. 1973. New observations on coral destruction in reefs (abstract). Page 17 *in* editor unknown. Tenth Meeting of the Association of Island Marine Laboratories of the Caribbean. Mayaguez, Puerto Rico, USA, July 1973. Caribbean Marine Biological Institute, Curacao, Netherlands Antilles.

Antonius, A. 1981. The 'band' diseases in coral reefs, in The Reef and Man. Pages 3-6 *in* Gomez, E.D. et al., editors. Proceedings of the Fourth International Coral Reef Symposium. Manila, Philippines, 18-22 May 1981. Great Barrier Reef Committee, Brisbane, Australia.

Antonius, A. 1999a. *Halofolliculina corallasia*, a new coral-killing ciliate in Indo-Pacific reefs. Coral Reefs 18:300.

Antonius, A. 1999b. *Metapeyssonnelia corallepida*, a new coral-killing red alga on Caribbean reefs. Coral Reefs 18:301.

Austin, B. 1988. Marine Microbiology. Cambridge University Press, Cambridge, England.

Awosika, L. and E. Marone. 2000. Scientific needs to assess the health of the oceans in coastal areas: a perspective of developing countries. Ocean Coastal Management 43:781-791.

Banin, E., Y. Ben-haim, T. Israely, Y. Loya and E. Rosenberg. 2000. Effect of the environment on the bacterial bleaching of corals. Water Air and Soil Pollution 123:337-352.

Banin, E., T. Israely, M. Fine, Y. Loya and E. Rosenberg. 2001. Role of endosymbioticzooxanthellae and coral mucus in the adhesion of coral-bleaching pathogen *Vibrio shiloi* to its host. FEMS Microbiological Letters 199(1):33-37.

Bright, T.J. and L.H. Pequegnat, editors. 1974. Biota of the West Flower Garden Banks. Gulf Publishing. Houston, Texas, USA.

Bright, T.J., G.P. Kraemer, G.A. Minnery and S.T. Viada. 1984. Hermatypes of the Flower Garden Banks, Northwestern Gulf of Mexico: A comparison to other western Atlantic reefs. Bulletin of Marine Science 34:461-476.

Bruckner, A.W. and R.J. Bruckner. 1997a. Outbreak of coral disease in Puerto Rico. Coral Reefs 16:260.

Bruckner, A.W. and R.J. Bruckner. 1997b. The persistence of black-band disease in Jamaica: impact on community structure. Page 601-60 *in* Lessios, H.A., Macintyre, I.G. editors. Proceedings of the Eighth International Coral Reef Symposium. Panama City, Panama, 24-29 June. 1996. Smithsonian Tropical Research Institute, Balboa, Panama.

Bruckner, A.W. and R.J. Bruckner. 1998a. Rapid wasting syndrome or coral predation by stoplight parrotfish? Reef Encounter 7/23:18-22.

Bruckner, A.W. and R.J. Bruckner. 1998b. Atlantic and Gulf Rapid Reef Assessment. 2000. AGRRA Disease and Predation Identification Cards. National Oceanic and Atmospheric Association. http://coral.aoml.noaa.gov.

Bruckner, A.W. and R.J. Bruckner. 2000a. Coral predation by *Sparisoma viride* and lack of relationship with coral disease. Ninth International Coral Reef Symposium Proceedings. Bali, Indonesia, 23-27 October, 2000. In press

Bruckner, A.W. and R.J. Bruckner. 2000b. Destruction of coral by *Sparisoma viride*. Coral Reefs 17:350.

Bruckner, A.W. R.J. Bruckner, and E.H. Williams. 1997. Spread of black-band disease epizootic through the coral reefs system in St. Ann's Bay, Jamaica. Bulletin of Marine Science 61(3):919-928.

Bruckner, A.W., R.J. Bruckner and P. Solins. 2000. Parrotfish predation on live coral: "spot biting" and focused biting". Coral Reefs 19:50.

Bythell, J.C., Z.M. Hillis-Starr and C.S. Rogers. 2000. Local variability but landscape stability in coral reef communities following repeated hurricane impacts. Marine Ecology Progress Series 204:93-100.

Carlton, R.G. and L.L. Richardson. 1995. Oxygen sulfide dynamics in a horizontally migrating cyanobacterial mat: Black-band disease of corals. FEMS Microbial Ecology 18:155-162.

Catala-Stucki, R. 1959. Fluorescence effects from corals irradiated with ultra-violet rays. Nature 183:949.

Cervino, J., T.J. Goreau, G. Smith, K. DeMeyer, I. Nagelkerken and R. Hayes. 1997. Fast spreading new Caribbean coral disease. Reef Encounters 22:16-18.

Cervino, J., T.J. Goreau, I. Nagelkerken, G.W. Smith and R. Hayes. 2001. Yellow band and dark spot in Caribbean corals: distribution, rate of spread, cytology, and effects on abundance and division rate of zooxanthellae. Hydrobiologia 460:53-63.

Chrost, R.J. and M.A. Faust. 1999. Consequences of solar radiation on bacterial Secondary production and growth rates in subtropical coastal waters (Atlantic coral reef off Belize, Central America). Aquatic Microbial Ecology 20:39-48.

Daszak, P., A.A. Cunningham and A.D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious disease in wildlife. Acta Tropica 78(2): 103-116.

Dokken, Q. R., I.R. MacDonald, J.W. Tunnell Jr., C.R. Beaver, G.S. Boland and D.K. Hagman. 2000. Long Term Monitoring at the East and West Flower Garden Banks, 1996-1997. U.S.D.I. M.M.S. Contract 1435-01-96-CT-30822.

Dokken, Q. R., I.R. MacDonald, J.W. Tunnell Jr., C.R. Beaver, G.S. Boland and D.K. Hagman. 2002. Long Term Monitoring at the East and West Flower Garden Banks, 1996-1997. U.S.D.I. M.M.S. Contract 1435-01-96-CT-30822.

Ducklow, H. and R. Mitchell. 1979. Obervations on naturally and artificially diseased tropical corals: a scanning electron microscope study. Microbial Ecology 5:215-223.

Dustan, P. 1977. Vitality of reef coral populations off Key Largo, Florida: recruitment and mortality. Environmental Geology 2:51-58.

Dustan, P. and J.C. Halas. 1987. Changes in reef-coral community of Caryfort Reef, Key Largo, Florida. Coral Reefs 6:91-106.

Edmunds, P.J. 1991. Extent and effect of black-band disease on a Caribbean reef. Coral Reefs 10:161-165.

Fisk, D.A. and T.J. Done. 1985. Taxonomic and bathymetric patterns of bleaching in corals, Myrmidon Reef (Queensland). Page 149-154 *in* Pirazzoli P.A.,editor. Proceedings of the Fifth International Coral Reef Congress. Tahiti, French Polynesia, May 27-June 1, 1985. Antenne Museum-EPHE, Moorea, French Polynesia, 1985.

Garabetian, F., M. Petit and P. Lavandier. 1999. Does storage affect epifluorescence microscopic counts of total bacteria in freshwater samples? Life Sciences 322:779-784.

Ginsburg, R.N. 2000. Coral Disease. http://coral.aoml.noaa.gov/agra/method/disease.html. (7-1-2002).

Gittings, S.R., G.S. Boland, K.J.P. Deslarzes, C.L. Combs, B.S. Holland and Y.J. Bright. 1992. Mass Spawning and reproductive viability of the reef corals at the East Flower Garden Bank, Northwest Gulf of Mexico. Bulletin Marine Science 51:420-428.

Gladfelter, W. 1977. Environmental studies of Buck Island Reef National Monument, St.Croix, US Virgin Islands, prepared for the National Park Services, Department of the Interior. Report for the National Park Services, Department of the Interior, Washington, D.C.,USA.

Gladfelter, W. 1982. White band disease in *Acropora palmata*: implications for the structure and growth of shallow reefs. Bulletin Marine Science 32:639-643.

Goreau T.J. and R.M. Hayes. 1994. Coral bleaching and ocean 'hot spots'. Ambio 23:176-180.

Green, E.P. and A.W. Bruckner. 2000. The significance of coral disease epizootiology for coral reef conservation. Biological Conservation 96(3):247-361.

Gulko, D. 1998. Hawaiian Coral Reef Ecology. Mutual Publishing, Honolulu, Hawaii.

Hagman, H.K. and S.R. Gittings. 1992 Coral bleaching on high latitude reefs at the Flower Garden Banks, NW Gulf of Mexico. Page 38-43 *in* Richmond, R., editor. Proceeding of the Seventh International Coral Reef Symposium. Mongmong, Guam, USA, 23 June 1992. University of Guam Press, Mongmong, Guam, USA

Harvell, D. K. Kim, C. Quirolo, J. Weir and G. Smith. 2001. Coral bleaching and disease: contributors to 1998 mass mortality in *Briareum asbestinum* (Octocorallia, Gorgonacea). Hydrobiologia 460:97-104.

HEED Global Change Program. 1997. Caribbean Coral Disease. HEED project, Center for Health and Global Environment. Harvard Medical School. Boston, Masachusetes, USA.

Hodgson, G. 1999. A Global Assessment of Human Effects on Coral Reefs. Marine Pollution Bulletin 38(5):345-355.

Hoegh-Guldberg O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research 50: 839-866.

Holdway, D.A. 2002. The acute and chronic effects of wastes associated with offshore oil and gas production on temperate and tropical marine ecological processes. Marine Pollution Bulletin 44:185-203.

Holt, J.G., editor. 1994. Bergey's manual of determinative bacteriology 9th edition. Williams and Wilkins. Maryland, USA.

Humann, P. 1998. Reef Coral Identification, Florida Caribbean Bahamas. New World Publications. Jacksonville, Florida, USA.

International Maritime Organization. 2001. http://www.imo.org/InfoResource.mainfram.asp?topic_id=112&doc_id=404. (5-15-2002).

Israely, T., E. Banin and E. Rosenberg. 2001. Growth, differentiation and death of *Vibrio shiloi* in coral tissue as a function of seawater temperature. Aquatic Microbial Ecology 24:8.

Jaap, W.C. 1995. Monitoring methods for assessing coral reef biota and habitat condition. Page 45-48 *in* Bradbury, R.H., editor. Proceedings of the Sixth International Coral Reef Symposium. Townsville, Australia, 8-12 August 1988. Springer-Verlag, New York, USA.

Jensen, P.R. and W. Fenical. 1995. The Relative abundance and seawater requirements of gram-positive bacteria in near-shore tropical marine samples. Microbial Ecology 29:249-257.

Kepner, R.L. and J.R. Pratt. 1994. Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiological Reviews 58(4):603-615.

Kinsey, D.W. and A. Domm. 1974. Effects of fertilization on a coral reef environment-primary production studies. Pages 49-66 *in* editors unknown. Proceedings of the Second International Coral Reef Symposium. M.V. Marco Polo cruising in the waters of the Great Barrier Reef Province, Australia, 22 June – 2 July 1973. Great Barrier Reef Committee, Brisbane, Australia.

Koh, E.G.L. 1997. Do scleractinian corals engage in chemical warfare against microbes? Journal of Chemical Ecology 23:379-398.

Koop, K., D. Booth, A. Broadbents, J. Brodie, D. Bucher, D. Capone, J. Coll, W. Dennison, M. Erdmann, P. Harrison, O. Hoegh-Guldberg, P. Hutchings, G.B. Jones, A.W.D. Larkum, J. O'Neil, A. Steven, E. Tentori, S. Ward, J. Williamson and D. Yellowlees. 2001. ENCORE: The effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Marine Pollution Bulletin 42(2):91-120.

Kopecky, E.J. and G.K. Ostrander. 1999. Isolation and Primary culture of viable multicellular endothelial isolates from hard corals. In Vitro Cellular Developmental Biology 35:616-624.

Kullenberg, G. 1999. Approaches to addressing the problems of pollution of the marine environment: an overview. Ocean and Coastal Management 42:999-1018.

Kushmaro, A., Y. Loya, M. Fine, and E. Rosenberg. 1996. Bacterial infection and coral bleaching. Nature 380:396.

Kushmaro, A., E. Rosenberg, M. Fine, Y. Ben Haim, and Y. Loya. 1998. Effect of temperature on bleaching of the coral Oculina patagonica by Vibrio AK-1. Marine Ecology Progress Series 171:131-137.

Kuta, K.G. and L.L. Richardson. 1996. Abundance and distribution of black band disease on coral reefs in the northern Florida Keys. Coral Reefs 15:219-223.

Le Campion-Alsumard, T., S. Golubic and P. Hutchings. 1995. Microbial endoliths in skeletons of live and dead corals: *Porites lobata* (Moorea, French Polynesia). Marine Ecology Progress Series 117:149-157.

Lipp, E.K., J.L. Jarrell, D.W. Griffin, J. Lukasik, J. Jacukiewicz and J.B. Rose. 2002. Preliminary evidence for human fecal contamination in corals of the Florida Keys, USA. Marine Pollution Bulletin 44:666-670.

Littler, M.M. and D.S. Littler. 1995. Impact of CLOD pathogen on Pacific coral reefs. Science 267:1356-1359.

Littler, M.M. and D.S. Littler. 1996. Black band disease in the South Pacific. Coral Reefs 15:20.

Lugo-Fernandez, A., K.J.P. Deslarzes, J.M. Price, G.S. Boland and M.V. Morin. 2001. Inferring probable dispersal of Flower Garden Banks Coral Larvae (Gulf of Mexico) using observed simulated drifter trajectories. Continental Shelf Research 21(1):46-67.

Marano-Briggs, K., R.B. Jonas, D.J. Ellis, D.K. Mills, P.M. Gillevet and S.W. Tully. 2000. A Novel Strain of *Marichromatium purpuratum* Isolated from Black Band-Disease on Boulder Coral, Andros Island, The Bahamas. Page 124 in Sliwa, J., editor. American Society for Microbiology 100th Meeting. Los Angeles, California, USA, 21-25 May 2000. American Society for Microbiology, Washington D.C., USA.

Martin, Y., J.L. Bonnefont and L. Chancerelle. 2002. Gorgonians mass mortality during the 1999 late summer in French Mediterranean coastal water: the bacterial hypothesis. Water Resources 36:779-782.

McClanahan, T.R., B.A. Cokos and E. Sala. 2002. Algal Growth and species composition under experimental control of herbirvory, phosphorus and coral abundance in Glovers Reef, Belize. Marine Pollution Bulletin In press.

Miller, I. 1996. Black band disease on the Great Barrier Reef. Coral Reefs 15:58. Journal of Sedimentary Petroleum 60:992-1007.

Mitchell, R. and I. Chet. 1975. Bacterial attack of corals in polluted seawater. Microbial Ecology 2:227-233.

Moberg, F. and C. Folke. 1999. Ecological goods and services of coral reef ecosystems. Ecological Economics 29:215-233.

National Oceanic and Atmospheric Association. 2000. www.noanews.noaa.gov/stories/s469.htm. (7-1-2002)
.

National Oceanic and Atmospheric Association. 2002a. www.noanews.noaa.gov/stories/s468.htm. (7-1-2002).

National Oceanic and Atmospheric Association. 2002b. www.ndbc.noaa.gov/station_page.phtml?station=42019. (7-1-2002).

Neufeldt, V., editor. 1997. Webster's New World College Dictionary, 3rd edition. Macmillan, New York, New York, USA.

Nyström, M., C. Folke and F. Moberg. 2000. Coral disturbance and resilience in a human dominated environment. Trends in Ecology and Evolution 15(10):413-417.

Ogden, J. 1997. Caribbean Coastal Marine Productivity (CARICOMP): A research and monitoring network of marine laboratories, parks, and reserves. Page 647-650 *in* Lessios, H.A., Macintyre, I.G. editors. Proceedings of the Eighth International Coral Reef Symposium. Panama City, Panama, 24-29 June 1996. Smithsonian Tropical Research Institute, Balboa, Panama.

Ohlhorst, S.L., W.D. Liddell, R.J. Taylor and J.M. Taylor. 1988. Evaluation of reef census techniques. Page 319-324 *in* Richmond, R. editor. Proceedings of the Sixth International Coral Reef Symposium. Townsville, Australia, 8-12 August 1988. Sixth International Coral Reef Symposium Executive Committee, Townsville, Australia.

Perez, T., J. Garrabou, S. Sartoretto, J-G. Harmelin, P. Francour and J. Vacelet. 2000. Mass mortality of marine invertebrates: an unprecedented event in the Northwestern Mediterranean. Comptes Rendus de L'Academie des Sciences-Series III-Sciences de la Vie 232(10):853-865.

Peters, E.C. 1998. Coral Disease Key. http://ourworld.compuserve.com/homepage/mccarty_and_peters/coraldis.htm. (7-1-2002).

Pettipher, G.L. 1983. The direct epifluorescent filter technique for the rapid enumeration of micro-organisms. Research Studies Press, New York, New York, USA.

Porter, J.W., P. Dustan, W.C. Jaap, K.L. Patterson and V. Kosmynin. 2001. Patterns of spread of coral disease in the Florida Keys. Hydrobiologia 460(1):1-24.

Ravindran, J., C. Raghukumar and S. Raghukumar. 1999. Disease and stress-induced mortality of corals in Indian reefs and observations on bleaching of corals in the Andamans. Current Science (Bangalore) 76:233-237.

Rezak, R., T.J. Bright and D.W. McGrail. 1985. Reefs and Banks of the Northwestern Gulf of Mexico: Their Geology, biological and physical dynamics. John Wiley and Sons. New York, USA.

Richardson, L.L. 1997a. Occurrence of the black band disease cyanobacterium on healthy corals of the Florida Keys. Bulletin Marine Science 61:485-490.

Richardson, L.L. 1997b. Ecology of the black band disease microbial consortium. Page 597-600 *in* Lessios, H.A., Macintyre, I.G. editors. Proceedings of the Eighth International Coral Reef Symposium. Panama City, Panama, June 24-29 1996. Smithsonian Tropical Research Institute, Balboa, Panama.

Richardson, L.L. 1998. Coral Disease: What is really known? Trends in Ecology & Evolution 13(11):438-443.

Riegl, B. and K.E. Luke. 1998. Ecological parameter of dynamited reefs in the northern Red Sea and their relevance to reef rehabilitation. Marine Pollution Bulletin 37(8-12):488-498.

Ritchie, K.B. and G.W. Smith. 1995a. Carbon-source utilization patterns of coral-associated marine heterotrophs. Journal Marine Biotechnology 3:107-109.

Ritchie, K.B. and G.W. Smith. 1995b. Preferential carbon utilization by surface bacterial communities from water mass, normal, and white-band diseases *Acropora cervicornis*. Molecular Marine Biological Biotechnology 4(4):345-352.

Ritchie, K.B. and G.W. Smith. 1998 Type II white-band disease. Revisions Biological Tropical 46:199-203.

Rubek, P.P. 1988. The need for conservation and management of Philippine coral reefs. Environmental Biology 23:141-154.

Rutzler, K. and D.L. Santavy. 1983. The black band disease of Atlantic reef corals. A description of the cyanophyte pathogen. Marine Ecology 4:301-319.

Scully, E.P., J. Prappas and G.K. Ostrander. 2001. Laboratory models for the study of coral pathologies. Hydrobiologia 460:91-95.

Sheppard, C., editor. 2000. In seas at the millennium: An environmental evaluation. vol. 2. Elsevier Amsterdam, Netherlands, EU.

Sherman, B.H. 2000. Marine ecosystem health as an expression of morbidity, mortality and disease events. Marine Pollution Bulletin 42(1-6):232-254.

Spalding, M.D. and G.E. Jarvis. 2002. The impact of the 1998 coral mortality on reef fish communities in the Seychelles. Marine Pollution Bulletin 44(4):309-321.

Unites States Army Corps of Engineers. 2002. Navigation Data Center. www.iwr.usace.army.mil/ndc/wcsc. (7-1-02).

Vogt, H. 1995. Video image analysis of coral reefs in Saudi Arabia: a comparison of methods. Beitr Palaont 20:99-105.

Wilkinson, C. 2000.Status of Coral Reefs of the World: 2000. www.coral.noaa.gov/gcrmn. (7-1-02).

Yamashiro, H., M. Yamamoto and R. van Woesik. 2000. Tumor formation on the coral *Motipora informis*. Diseases of Aquatic Organisms 41:211-217.

Yamashiro, H., H. Oku, K. Onaga, H. Iwasaki and K. Takara. 2001. Coral tumors store reduced levels of lipids. Journal of Experimental Marine Biology and Ecology 265:171-179.

Yentsch, C.S., C.M. Yentsch, J.J. Cullen, B. Lapointe, D.A. Phinney and S.W. Yentsch. 2002. Sunlight and water transparency: cornerstones in coral research. Journal of Experimental Marine Biology and Ecology 268(20):171-183.