ETIOLOGY AND HISTOPATHOLOGY OF THE WHITE POX DISEASE OF THE CARIBBEAN ELKHORN CORAL *ACROPORA PALMATA*

by

KATHRYN PATTERSON SUTHERLAND

(Under the Direction of James W. Porter)

ABSTRACT

White pox is a lethal disease of the Caribbean elkhorn coral, *Acropora palmata.* White pox was first reported in 1996 in the Florida Keys, and has since been observed throughout the Caribbean. Annual photographic monitoring at eight coral reef sites in the Florida Keys showed that *A. palmata* populations with white pox disease signs sustained losses averaging 87% between 1996 and 2002. Rate of tissue loss associated with white pox is rapid, averaging 2.5 $cm^2 \cdot day^{-1}$, and is greatest during periods of seasonally elevated temperature and rainfall.

The etiology of white pox was determined through examination of surface mucopolysaccharide layers (SML) from white pox-affected and -unaffected corals collected on reefs throughout the Caribbean. Metabolic profiles of pure bacterial strains isolated from SML were used to identify potential pathogens. Potential pathogens were experimentally inoculuted onto healthy *A. palmata.* Corals inoculated with isolate PDL100 showed white pox signs, and PDL100 was reisolated from these corals (satisfying Koch's postulates). 16S rDNA gene sequence analyses of PDL100 demonstrated a 100% identity to the enterobacterium *Serratia marcescens*. The source of the *S. marcescens* strain that causes white pox is uncertain. This

study begins the search for the origin of the pathogen through the screening of seawater collected in the Florida Keys using biochemical and restriction analyses. Thirteen bacterial isolates were identified as putatively belonging to the genus *Serratia*, and the majority of these isolates were from stations where human fecal contamination of seawater is probable.

White pox-affected and apparently healthy coral tissues were collected from white pox-diseased colonies of *Acropora palmata* in the Florida Keys. Tissues were processed for histopathology with light microscopy. White pox is associated with rounding of granular gland cells, necrosis, and atrophy. Most lesions were concentrated in the coenosarc tissue. Cellular and tissue degeneration, however, was observed in both apparently healthy and diseased coral tissues, and there was no significant difference in the number or type of abnormalities present in diseased versus apparently healthy specimens. These similarities may indicate that colonies affected by white pox sustain a whole-colony reaction to infection.

INDEX WORDS: Coral, Disease, White Pox, *Acropora palmata, Serratia marcescens*, Histopathology

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DEDICATION

To my grandfather, Archie Patterson,
Professor Emeritus
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CHAPTER 1

INTRODUCTION

Coral reefs are in severe decline. The most reliable estimates suggest that worldwide, 27% have already been lost, with another 16% at serious risk for loss (Wilkinson 2002). Coral disease is thought to be a major cause for this decline (Dustan 1999, Porter et al. 2001). Epizootics have been reported for several coral species (Goreau et al. 1998, Richardson 1998, Richardson et al. 1998a, Richardson et al. 1998b, Harvell et al. 1999, Harvell et al. 2001, Porter et al. 2001) and evidence is mounting of substantial declines in the biodiversity and abundance of reef-building corals worldwide (Hayes & Goreau 1998, Porter & Tougas 2001, Wilkinson 2002).

Since the mid-1990's, coral diseases have increased in number, species affected, and geographic extent. To date, 18 coral diseases, affecting at least 152 scleractinian, gorgonian, and hydrozoan zooxanthellate species, have been described from the Caribbean and the Indo-Pacific. These diseases are associated with pathogens including bacteria, cyanobacteria, fungi, and protists and with abiotic stressors including elevated seawater temperature, sedimentation, eutrophication, and pollution. Etiologies of only 5 of the 18 coral diseases have been determined through fulfillment of Koch's postulates. This document begins with a review of the described coral diseases, known etiologies, and efforts to determine unknown etiologies. Disease terms are defined, the limitations of Koch's postulates and alternative techniques for identifying disease-causing organisms are discussed, and immune mechanisms of scleractinian and gorgonian corals are reviewed.

White pox disease is a lethal disease of the Caribbean elkhorn coral *Acropora palmata. A. palmata* is one of the Caribbean coral community's most important shallow water framework builders, providing elevated rates of calcium carbonate deposition (Adey 1978) and the highly complex three-dimensional structure of the shallow water forereef. This species provides shelter and food for reef organisms and aids in the protection of coastal regions by serving as a buffer between land and sea. *A. palmata* was once the most common reef building coral in the Caribbean, but today this species is a candidate for protection under the Endangered Species Act (Diaz-Soltero 1999). In many locations throughout the Caribbean, all that remains of this majestic coral species are elkhorn graveyards of standing intact skeletons. On most reefs, loss of *A. palmata* is accompanied by an ecological phase shift from coral-dominated to algal-dominated reefs (Hughes 1994). *A. palmata* population declines, therefore, are changing the composition, structure, and function of Caribbean coral reef ecosystems (Hughes 1994, Aronson & Precht 1997, Aronson & Precht 2001, Aronson et al. 2002, Patterson et al. 2002).

Acropora palmata populations began to decline in the early 1980s, and early losses were attributed to hurricanes, bleaching, predation, and white band disease (Gladfelter 1982, Porter and Meier 1992, Bythell & Sheppard 1993, Hughes 1994, Aronson and Precht 2001). In 1996, white pox disease emerged on reefs throughout the Caribbean (Holden 1996, Porter et al. 2001, Rodríquez-Martínez et al. 2001, Santavy et al. 2001, Patterson et al. 2002), and decimated the already reduced *A. palmata* populations, with losses of this species in the Florida Keys National Marine Sanctuary (FKNMS) averaging 87% by 2002 (Patterson et al. 2002, Sutherland & Ritchie *in press*).

The surface mucopolysaccharide layers of white pox disesed and apparently healthy *Acropora palmata* were screened to elucidate the causal agent of white pox. Koch's postulates

were fullfilled and showed that white pox is caused by the fecal enterobacterium, *Serratia marcescens*. *S. marcescens* is found in the intestines of humans, insects, and other animals, and in freshwater, soil, and plants (Grimont and Grimont 1994). *S. marcescens* is pathogenic to humans, cows, goats, chickens, fishes, insects, and plants (Baya et al. 1992, Grimont and Grimont 1994). The prevalence of *S. marcescens* in the marine environment is unknown. The source of the *S. marcescens* strain that causes white pox is uncertain, and this study begins the search for the origin of the white pox disease pathogen through the examination of seawater samples collected within the FKNMS.

Histopathology of diseased corals is a valuable tool that can be used to identify cell and tissue abnormalities associated with diseases and to visualize microorganisms that may contribute to disease pathogenesis. Histopathological analyses have been conducted for only 5 of the 18 coral diseases documented to date in the global oceans (Cheng & Wong 1974, Morse et al. 1977, Morse et al. 1981, Peters et al.1983, Ramos-Flores 1983, Peters 1984, Peters et al. 1986, Coles & Seapy 1998, Richardson et al. 1998a, Richardson et al. 1998b, Yamashiro et al. 2000, Bythell et al. 2002). I report here on the histopathological examination of coral tissues from white pox diseased *Acropora palmata.*

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CHAPTER 2

DISEASE AND IMMUNITY IN CARIBBEAN AND INDO-PACIFIC ZOOXANTHELLATE CORALS[1]

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ABSTRACT

Since the mid-1990's, coral diseases have increased in number, species affected, and geographic extent. To date, 18 coral diseases, affecting at least 152 scleractinian, gorgonian, and hydrozoan zooxanthellate species, have been described from the Caribbean and the Indo-Pacific. These diseases are associated with pathogens including bacteria, cyanobacteria, fungi, and protists and with abiotic stressors including elevated seawater temperature, sedimentation, eutrophication, and pollution. Etiologies of only 5 of the 18 coral diseases have been determined through fulfillment of Koch's postulates. Corals and other invertebrates utilize innate immune mechanisms including physiochemical barriers and cellular and humoral defenses against pathogens. Here we review the described coral diseases, known etiologies, and efforts to determine unknown etiologies. We define disease terms, discuss the limitations of Koch's postulates, describe alternative techniques for identifying disease-causing organisms, and review coral immunology.

INTRODUCTION

Coral reefs are in severe decline. The most reliable estimates suggest that worldwide, 27% have already been lost, with another 16% at serious risk of loss (Wilkinson 2002). Coral disease is thought to be a major cause for this decline (Dustan 1999, Porter et al. 2001). Epizootics have been reported for several coral species (Goreau et al. 1998, Richardson 1998, Richardson et al. 1998a, b, Harvell et al. 1999, 2001, Porter et al. 2001) and evidence is mounting of substantial declines in the biodiversity and abundance of reef-building corals worldwide (Hayes & Goreau 1998, Porter & Tougas 2001, Wilkinson 2002). Within the Caribbean, populations of elkhorn and staghorn corals, *Acropora palmata* and *A. cervicornis,* are being decimated by disease (Gladfelter 1982, Bythell & Sheppard 1993, Aronson & Precht 1997, 2001, Aronson et al. 1998, 2002,

Greenstein et al. 1998, Miller et al. 2002, Patterson et al. 2002), with losses of *A. palmata* in the Florida Keys National Marine Sanctuary (FKNMS) averaging 87% (Fig. 2.1) or greater (Miller et al. 2002, Patterson et al. 2002, Sutherland & Ritchie *in press*). On most Caribbean reefs, loss of acroporids is accompanied by an apparent ecological phase shift from coral-dominated substrata to algal-dominated substrata (Hughes 1994). While severe population declines of Caribbean acroporid corals have led to the identification of *A. palmata* and *A. cervicornis* as candidates for inclusion on the Endangered Species List (Diaz-Soltero 1999), the impact of most diseases on coral populations is poorly understood.

Coral disease is becoming more widespread. The first coral disease was reported in 1965 and during the subsequent 3 decades, only 4 new diseases were reported (Table 2.1). Beginning in the mid-1990's, reports of novel coral diseases increased worldwide, and by 2002, 13 new diseases were described (Table 2.1, Fig. 2.2). In the FKNMS, number of locations exhibiting disease increased from 26 to 131 stations (404% increase) and number of coral species exhibiting disease increased from 11 to 36 (218% increase) between 1996 and 2000 (Porter et al. 2001). These disease increases parallel a 37% decline in living coral over the same time period and at the same stations (Porter et al. 2002).

Biodiversity of corals is much greater in the Indo-Pacific than in the Caribbean. Age and geographic extent of the Indo-Pacific region contributes greatly to its richness (Veron 1995). Whereas only 53 coral species are found in Discovery Bay, Jamaica (Wells 1973), 362 coral species are found in Milne Bay, Papua New Guinea (Werner & Allen 1998) and 350 coral species are found in the Great Barrier Reef, Australia (Veron 1985). Despite the greater species richness of the Indo-Pacific, the number of species affected by disease is proportionally much lower than in the Caribbean (Tables 2.2 & 2.3).

Of the 18 coral diseases described to date, 4 are reported globally: black band (BBD), white plague-like diseases (WPL-L), shut-down reaction (SDR), and skeletal anomalies (SKA); 9 are found exclusively in the Caribbean: white band Types I (WBD I) and II (WBD II), white plague Types I (WPL I), II (WPL II), and III (WPL III), aspergillosis (ASP), white pox (WPD), yellow blotch/band (YBL), dark spots (DSD); and 6 are apparently endemic to the Indo-Pacific: yellow band (YBD), skeleton eroding band (SEB), pink-line syndrome (PLS), fungal-protozoan syndrome (FPS), *Vibrio shiloi*-induced bleaching (VSB), *V. coralliilyticus*-induced bleaching and disease (VCB). Etiologies and mechanisms of tissue death of the majority of coral diseases are not understood (Richardson 1998).

Tissue loss or damage from predation is often impossible to distinguish from tissue loss or damage from disease. To the human eye, there are not many ways in which coral tissue can exhibit signs of stress. Corallivores, including fishes, gastropods, and other invertebrates, produce predation scars that are easily confused with disease signs. The best method to distinguish between predation and disease is to observe the progress of the condition in the absence of predation, via predator exclusion *in situ* or predator removal under laboratory conditions. A number of coral abnormalities, some of which have been described in the literature as coral diseases, are likely associated with predation rather than disease. For example, predation by the stoplight parrotfish *Sparisoma viride*, was initially described as a coral disease termed rapid wasting syndrome (Cervino et al. 1997). Subsequent investigations identified fish predation as the primary cause of skeletal loss (Bruckner & Bruckner, 2002), but the possible role of fungi in the dissolution of the skeleton (Cervino et al. 1998, Hayes & Goreau 1998) has not been fully vetted. Further, in some cases, disease signs attributed to coral disease (e.g. WPL, WBD, and WPD) may be difficult to distinguish from predation scars produced by corallivores

including the gastropod, *Coralliophila abbreviata*, and the fire worm, *Hermodice caruculata* (Patterson et al. 2002).

In order to facilitate an understanding of disease processes and causation in corals, it is necessary to understand general disease terminology and coral immunity. The objectives of this paper are to: (1) define disease terminology and relate these terms to coral disease, (2) discuss the process of proving disease causation, (3) review described coral diseases, known etiologies, and efforts to determine unknown etiologies, (4) illustrate each of the known Caribbean coral diseases, and (5) review coral immunology.

DISEASE TERMINOLOGY

A disease is any impairment (interruption, cessation, proliferation, or other disorder) of vital body functions, systems, or organs (Stedman 2000). The term syndrome is synonymous with disease (Stedman 2000). Etiology is analysis of causes, development, and consequences of a disease (Kinne 1980). Disease causation (i.e. etiology) may be attributed to pathogens, environmental stressors, or a combination of biotic and abiotic factors. Biotic diseases are caused by pathogenic microorganisms such as viruses, bacteria, fungi, and protists and are often species specific (Peters 1997) and infectious (Kinne 1980). Abiotic diseases result from both natural and human-induced environmental stressors including change in ambient conditions or exposure to pollutants. Biotic and abiotic diseases are often closely related. Biotic diseases may be associated with environmental stressors that (1) hinder the resistence of host organisms, (2) promote growth and virulence of pathogens, (3) trigger the pathogenic process, or (4) increase the rate of disease transmission (Kushmaro et al. 1996, 1998, Peters 1997, Toren et al. 1998, Ben-Haim et al. 1999, 2003b, Alker et al. 2001, Banin et al. 2001a, Israely et al. 2001, Ben-Haim

& Rosenberg 2002, Kuta & Richardson 2002, Richardson & Kuta 2003). Abiotic diseases may be exacerbated by secondary opportunistic infections (Peters 1997).

Etiologies of 11 diseases affecting scleractinian and gorgonian corals may involve pathogens including bacteria, cyanobacteria, fungi, and protists (Table 2.4). Ten diseases are associated with abiotic stressors including temperature extremes, sedimentation, eutrophication, and pollution (Table 2.5). VSB and VCB are examples of conditions with a combination of abiotic and biotic factors contributing to disease causation. Infections with *Vibrio shiloi* and *V. coralliilyticus* do not occur in the absence of elevated seawater temperature (Kushmaro et al. 1998, Toren et al. 1998, Ben-Haim et al. 1999, 2003b, Banin et al. 2001a, Israely et al. 2001, Ben-Haim & Rosenberg 2002).

An infectious disease is one in which the causal agent can be transmitted from one host individual to another (Kinne 1980, Peters 1997, Stedman 2000). Disease transmission can be either horizontal or vertical. Horizontal disease transmission is the transmission of infectious agents from an infected individual to a susceptible contemporary (Stedman 2000). Vertical disease transmission is the transmission of infectious agents from an infected individual to its offspring (Stedman 2000). There is no evidence to date to associate coral diseases with vertical transmisssion.

Horizontal disease transmisssion can be either direct or indirect. Direct transmission requires contact between infected and uninfected individuals. In terrestrial environments direct transmission may occur via physical contact or respiratory aerosols. For sessile marine invertebrates, including scleractinian, gorgonian, and hydrozoan corals, direct physical contact only occurs between close neighbors. Inanimate or living agents are required for indirect transmission. Vectors are living agents that transmit pathogens (Stedman 2000) and in the coral

reef environment may include predatory and/or herbivorous arthropods, annelids, mollusks, echinoderms, and fishes. The only known vector for a coral disease is the marine fireworm *Hermodice carunculata,* which transmits *Vibrio shiloi,* the causal agent of VSB, to its coral host (Sussman et al. 2003). Contaminated water is an inanimate mode of indirect disease transmission and waterborne pathogens are significant in marine ecosystems.

Infection begins with invasion and multiplication of microorganisms in host tissues and may result in cellular injury. Infection does not always impair the host and therefore is not synonymous with disease (Stedman 2000). For instance, intimate associations between 2 different genetic entities such as host and microorganism are known as symbioses (living together) and range from parasitism to commensalism. A parasite is an organism that grows in or on a host and imparts negative effects to the host (Kinne 1980). A pathogen is a parasite that causes damage to a host, resulting in disease and possibly mortality (Peters 1997). All biotic diseases are symbiotic relationships between a host and a pathogen (Kinne 1980).

Pathogenesis is the process by which infection leads to disease (Stedman 2000). Virulence is the capacity of a pathogen to cause disease (i.e. degree of pathogenicity, Stedman 2000) and is influenced by the interaction between the host, the pathogen, and the environment (Peters 1997). Virulence factors affecting the severity of coral diseases are poorly understood. However, elevated seawater temperature increases the virulence of *Vibrio shiloi* (Kushmaro et al. 1998, Banin et al. 2001a), *V. coralliilyticus* (Ben-Haim & Rosenberg 2002), *Aspergillus sydowii* (Alker et al. 2001) and the BBD microbial consortium (Table 2.5, Kuta & Richardson 2002, Richardson & Kuta 2003). Virulence factors affecting VSB include both heat-stable and heat-sensitive toxins that target the zooxanthellae and play a role in pathogenesis (Rosenberg et al. 1998, Ben-Haim et al. 1999).

Susceptibility is the capacity of a host to become infected (Kinne 1980). Host susceptibility may vary between species and within species or individuals and according to environmental stressors, nutrition, genetics, age, and developmental stage (Kinne 1980, Peters 1997). Resistance is a measure of susceptibility of a host to an invading organism, i.e. the ability of an organism to maintain its immunity to or to counteract a disease agent (Kinne 1980, Stedman 2000). Resistance and susceptibility of corals can vary with health status (Kim et al. 2000a) and size class (Dube et al. 2002, Kim & Harvell 2002, Nugues 2002) of the host. Corals are likely more susceptible to (i.e. less resistant to) disease when they are exposed to environmental stressors including sub- and supra-optimal temperature and salinity levels, or poor water quality associated with anthropogenic disturbances including eutrophication, sedimentation, and pollution (Mitchell & Chet 1975, Ducklow & Mitchell 1979a, Johnston et al. 1981, Glynn et al. 1984, Peters 1984, Hodgson 1990, Richmond 1993, Frias-Lopez et al. 2002). Physical stressors such as temperature-induced coral bleaching may also promote disease susceptibility (Kushmaro et al. 1997, Harvell et al. 1999).

Epizootiology is the study of the occurrence, distribution, and control of a disease in an animal population (Stedman 2000). The term is synonymous with epidemiology in human populations (Stedman 2000). Incidence is the number of individuals with new cases of a disease during a specified time period in a specified population (Stedman 2000). Prevalence is the number of cases of a disease in a population at a specific time (Stedman 2000). An epizootic is analogous to an epidemic in human populations and is defined as: (1) a disease occurence with a frequency in excess of the expected frequency in an animal population during a given time interval (Stedman 2000) or (2) an outbreak of an infectious animal disease within a localized region (Kinne 1980). Epizootics may result from: (1) introduction of a new pathogen into a

susceptible population, (2) increase in pathogen numbers or virulence, or (3) lowered resistance of the host population (Peters 1997). Corals and other marine organisms of the Caribbean have sustained epizootics in recent years. Two separate epizootics affecting the sea fans *Gorgonia ventalina* and *G. flabellum* have occurred since the 1980s (Garzon-Ferreira & Zea 1992, Nagelkerken et al. 1997a, b), and both have been attributed to ASP (Smith et al. 1996, Geiser et al. 1998). An epizootic affecting the long-spined sea urchin *Diadema antillarum* occurred in 1983 and resulted in catastrophic reductions in urchin populations (Lessios et al. 1984). WBD (type I) and WPD epizootics, which began in the mid-1970's and the mid-1990's, respectively, have decimated populations of acroporid corals on Caribbean coral reefs (Gladfelter 1982, Bythell & Sheppard 1993, Aronson & Precht 1997, 2001, Aronson et al. 1998, Patterson et al. 2002).

PROVING DISEASE CAUSATION

Since the 19th century, it has been generally accepted that in order to prove disease causation by a biotic agent, Koch's postulates must be fulfilled (Koch 1882). Koch's postulates require that: (1) the putative pathogen be found in every diseased individual, (2) the putative pathogen be isolated from a diseased individual and grown in pure culture, and (3) the disease be induced in experimental organisms by transferring the pathogen from the culture (Koch 1882). A fourth postulate, that: (4) the same pathogen be isolated from the experimental organism after the disease develops, was later added to Koch's list but was not required by Koch himself. Limitations of the postulates were immediately apparent, and Koch was never able to fulfill his own formulation for disease causation for cholera or leprosy. The accepted etiologic agent of

leprosy, *Mycobacterium leprae,* remains unproven by fulfillment of Koch's postulates (Fredericks & Relman 1996).

Although Koch's postulates have elucidated the etiologies of countless diseases, this method of proving disease causation has numerous limitations. Koch's third postulate cannot ethically be fulfilled for fatal diseases that exclusively affect humans (e.g. HIV). Koch's postulates cannot be fulfilled according to the strict definition of the procedure for diseases that: (1) are caused by unculturable bacteria, fungi, or viruses (2) are caused by a consortium of microorganisms, (3) are caused by abiotic stressors, (4) require a vector or a carrier state, (5) cause subclinical or latent infection, or (6) cause injury through systemic attack via virulence factors such as toxins (Fredericks & Relman 1996, US EPA 2000). In addition, Koch's postulates ignore the classic paradigm of disease causation through pathogen interaction with host and environment. Fulfillment of Koch's postulates unequivocally demonstrates disease causation by a specific pathogen; however, failure to fulfill these postulates does not eliminate the possibility that: (1) the putative pathogen does in fact cause the disease, nor that (2) the suspected pathogen is a commensal organism (Fredericks & Relman 1996).

Several scientists have formulated alternative postulates for disease causation that take into account the limitations of those established by Koch (Rivers 1937, Huebner 1957, Hill 1965, Johnson & Gibbs 1974, Evans 1976, Falkow 1988, Fredericks & Relman 1996). These revisions of Koch's postulates address viral etiology (Rivers 1937, Huebner 1957, Johnson & Gibbs 1974, Evans 1976), carcinogenesis (Evans 1976), antibody response (Huebner 1957, Evans 1976), epidemiology (Huebner 1957, Evans 1976), genetic diseases (Falkow 1988), abiotic diseases (US EPA 2000), and molecular techniques for determining disease causation (Fredricks & Relman 1996).

When Koch's 19th century postulates cannot be fulfilled due to characteristics of a disease that may be attributed to the nature of its etiology or the mechanisms of disease causation, then 21st century techniques can be employed to identify disease-causing microbes (Fredricks & Relman 1996, Ritchie et al. 2001). The genotype-based technique of 16S and 18S rRNA gene sequence amplification can be used to identify putative pathogens and to generate specific probes and primers for use with *in situ* nucleic acid hybridization. *In situ* hybridization identifies a putative pathogen within diseased tissues. Nucleic-acid amplification techniques are highly sensitive and may also target microbes that are insignificant to disease causation (e.g. commensals or contaminants). However, these techniques provide a means of associating putative pathogens with diseased tissues (Fredricks & Relman 1996).

Limitations of Koch's postulates have quickly become evident in the emerging field of coral disease etiology. Koch's postulates have been successfully fulfilled for only 5 (WPL II, WPD, ASP, VSB, VCB) of the 18 coral diseases identified to date in the global oceans (Table 2.4; Kushmaro et al. 1996, 1997, 1998, 2001, Smith et al. 1996, Geiser et al. 1998, Richardson et al. 1998a, b, Rosenberg et al. 1998, Ben-Haim & Rosenberg 2002, Patterson et al. 2002, Ben-Haim et al. 2003a, b, Denner et al. 2003). Attempts to fulfill Koch's postulates have been unsuccessful for other coral diseases. Although a microbial consortium, dominated by cyanobacteria, is widely accepted as the causal agent of BBD, Koch's postulates have not been, and technically cannot be, fulfilled for this disease. Koch's postulates assume that a single microorganism, which can be grown in pure culture, causes a disease. In the case of BBD, a microbial consortium is thought to be required to induce disease (Carlton & Richardson 1995, Richardson et al. 1997). WBD II is always associated with *Vibrio charcharia*, but attempts to

fulfill Koch's postulates with this bacterium have been unsuccessful to date (Ritchie & Smith 1995a).

Molecular techniques have the potential to increase our knowledge of coral disease processes that cannot be understood through the use of 19th century criteria for disease causation (Ritchie et al. 2001, Bythell et al. 2002, Cooney et al. 2002, Frias-Lopez et al. 2002, 2003). Community DNA can be isolated from coral tissue or its associated surface mucopolysaccharide layer (SML). Both diseased and apparently healthy corals have a bacterial community associated with SML, and this bacterial community is known to shift under conditions of stress (e.g. disease and bleaching, Ritchie & Smith 1995a, b). Total community DNA from SML and coral tissue can be isolated, amplified, cloned, and compared to known sequences in a database (e.g. GenBank). A phylogenetic tree can then be constructed and used to identify the evolutionary relationships of putative pathogens. Although these molecular techniques do not prove disease causation, their rapid and broad screening can establish an association between potential pathogens and disease, aiding in understanding of the infectious disease processes (Ritchie et al. 2001). To make significant advances in understanding coral disease etiology, alternative criteria for disease causation, in addition to those postulated by Koch, need to be developed, utilized, and accepted by coral disease researchers.

GLOBAL CORAL DISEASES

Black Band

Black band disease (BBD; Fig. 2.3A) affects corals worldwide (Rützler & Santavy 1983, Antonius 1985a, 1988, Edmunds 1991, Carlton & Richardson 1995, Littler & Littler 1996, Miller 1996, Bruckner & Bruckner 1997a, b, Bruckner et al. 1997, Green & Bruckner 2000, Al-

Moghrabi 2001, Dinsdale 2002). BBD is characterized by a darkly pigmented microbial mat, which forms a band (1 to 30 mm wide and ca. 1 mm thick) that separates living tissue from recently denuded skeleton (Fig. 2.3A, Rützler & Santavy 1983, Carlton & Richardson 1995).

The microbial consortium that composes the band is dominated by a filamentous cyanobacterium, the identity of which has long been believed to be *Phormidium corallyticum* (Rützler & Santavy 1983). However, recent studies utilized molecular techniques to characterize the BBD consortium, and, while a single cyanobacterium species was associated with the disease, this cyanobacterium was not a member of the genus *Phormidium* (Table 2.4; Cooney et al. 2002, Frias-Lopez et al. 2002, 2003). The 16S rRNA gene sequencing identified at least 3 different taxa of cyanobacteria associated with BBD and determined that these taxa vary between the Caribbean and Indo-Pacific (Frias-Lopez et al. 2003). In the Caribbean, the BBD mat is dominated by an unidentified cyanobacterium most closely related to the genus *Oscillatoria* (Cooney et al. 2002, Frias-Lopez et al. 2003). In the Indo-Pacific, the BBD cyanobacterium is most closely related to the genus *Trichodesmium* (Frias-Lopez et al. 2003), and this genus (specifically *T. tenue*) has also been isolated from BBD mats in the Caribbean (Frias-Lopez et al. 2002).

Since 16S rRNA gene sequencing did not conclusively identify the cyanobacteria associated with BBD, but rather provided a most homologous match (Frias-Lopez et al. 2003), there is currently a discussion about the identitiy of the BBD cyanobacteria. It is important to note that the only 2 studies targeting the physiology of BBD cyanobacteria in the laboratory (Taylor 1983, Richardson & Kuta 2003) both used cultures isolated from BBD that contained cyanobacteria identified as *Phormidium corallyticum* based on the morphology of this species

(Rützler & Santavy 1983). Future research will determine whether or not *P. corallyticum* is an essential component of the BBD microbial consortium.

Other microbes identified in the BBD consortium include sulfate-reducing bacteria *Desulfovibrio* spp. (Garrett & Ducklow 1975, Schnell et al. 1996, Cooney et al. 2002), sulfide oxidizing bacteria *Beggiatoa* spp. (Ducklow & Mitchell 1979b), a multitude of heterotrophic bacteria (Garrett & Ducklow 1975, Cooney et al. 2002, Frias-Lopez et al. 2002), and a marine fungus (Ramos-Flores 1983). Two molecular studies carried out to investigate BBD (Cooney et al. 2002, Frias-Lopez et al. 2002) each found over 50 bacterial species associated with BBD. These included a wide assortment of proteobacteria, *Cytophega* sp., and an α-proteobacterium closely related to the pathogen that causes juvenile oyster disease. The disease consortium, and not a single microorganism alone, is thought to be required for disease causation (Carlton & Richardson 1995, Richardson et al. 1997). Koch's postulates have not been fulfilled with any component of the consortium (Carlton & Richardson 1995).

BBD affects 19 (of 66) Caribbean shallow water scleractinian species (Table 2.2; Rützler et al. 1983, Kuta & Richardson 1996, Garzón-Ferreira et al. 2001, Porter et al. 2001, Wheaton et al. 2001) and 45 (of approximately 400) Indo-Pacific scleractinian species (Table 2.3; Antonius 1985a, b, Miller 1996, Green & Bruckner 2000, Al-Moghrabi 2001, Riegl 2002, Frias-Lopez et al. 2003). Six Caribbean gorgonian species are also affected by BBD (Table 2.2; Antonius 1981, 1985b, Feingold 1988). Caribbean corals most susceptible to BBD include *Diploria strigosa, D. labyrinthiformis, Montastraea annularis*, *M. cavernosa*, *M. faveolata, M. franksi* and *Colpophyllia natans* (Antonius 1981, Ramos-Flores 1983, Rützler et al. 1983, Kuta & Richardson 1996). These corals are massive species and the dominant reef-builders. *Acropora cervicornis, A. prolifera,* and *Porites porites* appear resistant to BBD infections (Antonius 1981).

BBD was recently reported affecting *A. palmata* in the Colombian Caribbean (Garzón-Ferreira et al. 2001). Prior to this report *A. palmata* was thought to be resistant to the disease. While BBD is rarely reported on acroporids in the Caribbean, this genus is among the most susceptible to BBD on the Great Barrier Reef, Australia (Miller 1996, Dinsdale 2002). A BBD epizootic affecting faviid corals occurred at Looe Key Reef, Florida in 1986 (Peters 1993). BBD most often affects fewer than 1% of coral colonies on any reef area at any one time (Edmunds 1991, Kuta & Richardson 1996), but the susceptibility of major frame-building species greatly enhances the threat that BBD poses to the reef community.

Infection with BBD usually begins on upper surfaces of a coral colony (Antonius 1981) as a small darkly-pigmented patch (1 to 2 cm diameter). The patch quickly forms a ring, the circumference of which rapidly increases as the band migrates horizontally across the coral. As the microbial mat migrates, it kills all tissue and leaves behind bare skeleton (Antonius 1981, Carlton & Richardson 1995). Horizontal movement of the band is greatest at the front of the band (adjacent to living tissue) during the day, and at the back of the band (adjacent to dead skeleton) at night. This migration pattern results in a widening of the microbial mat during the day and a contraction at night (Richardson 1996).

BBD progresses at an average rate of 3 mm d^{-1}, but is capable of advancing up to 1 cm d^{-1} (Antonius 1981, 1985a, Edmunds 1991, Carlton & Richardson 1995). This rapid rate of tissue loss, coupled with the slow growth rate of scleractinian corals, denudes living coral tissue quickly and allows for complete colony mortality. However, BBD may disappear before complete colony mortality occurs (Carlton & Richardson 1995). This cessation of BBD most often occurs with the onset of lower seawater temperatures (Carlton & Richardson 1995).

BBD has been widely reported as a seasonal phenomenon, with most active infections occurring during late summer and fall, and cessation occurring during winter (Antonius 1981, 1985a, Edmunds 1991, Carlton & Richardson 1995). Seasonality of BBD is related to summer seawater temperatures in excess of 25°C (Rützler et al. 1983, Edmunds 1991, Kuta & Richardson 2002, Richardson & Kuta 2003). When BBD disappears in the winter, remaining living coral tissue survives (Antonius 1981, Carlton & Richardson 1995). However, seasonal reappearance of warm seawater coincides with observations of reinfection, and reinfection makes complete colony mortality possible (Carlton & Richardson 1995, Kuta & Richardson 1996). It is important to note that BBD has been reported year-round, even at seawater temperatures as low as 20°C (Kuta & Richardson 1996), and that the cyanobacteria associated with BBD are capable of photosynthesis at temperatures as low as 18 and 20ºC (Taylor 1983, Richardson & Kuta 2003).

BBD has been proposed to be correlated with other environmental and physiological stressors, including terrestrial runoff (Littler & Littler 1996, Bruckner et al. 1997, Frias-Lopez et al. 2002), coral overgrowth by algae (Bruckner et al. 1997), eutrophication (Antonius 1981, 1985a, Kuta & Richardson 2002), and pollution (Antonius 1985a, Al-Moghrabi 2001), including human fecal contamination (Frias-Lopez et al. 2002, Table 2.5). However, very little quantitative data, and no definitive results, have supported a positive correlation. Pollution has been implicated in extending depth range, frequency, and severity of BBD (Antonius 1985a). BBD has been frequently reported from shallow depths (Rützler et al. 1983, Antonius 1985a, Kuta & Richardson 2002) and reefs with low coral species diversity (Bruckner & Bruckner 1997b, Bruckner et al. 1997, Kuta & Richardson 2002).

The mechanism by which BBD kills coral tissue is directly linked to dynamics of the microbial mat community, which produces a vertical zonation of oxygen and sulfide microenvironments that migrates on a diel basis. The dominant constituent of BBD, the cyanobacteria, forms the scaffolding of the band and is always present throughout the band. The cyanobacteria undergo oxygenic photosynthesis during the day, producing an oxygen supersaturated oxic zone in the top ½ to 2/3 of the band. The cyanobacteria adapt to the high sulfide environment of the disease band by performing oxygenic photosynthesis in the presence of sulfide (Richardson & Kuta 2003). The anoxic base of the band is dominated by sulfate-reducing bacteria *Desulfovibrio* spp. The oxic/anoxic interface contains sulfide-oxidizing bacteria *Beggiatoa* spp., and this zone migrates vertically on a diel basis in response to changes in light intensity (Viehman & Richardson 2002) and photosynthetic activity occurring within the band (Carlton & Richardson 1995, Richardson et al. 1997). Under low light conditions (e.g. shade, darkness) the surface community of BBD is dominated by cyanobacteria, but when light intensity increases, *Beggiatoa* spp. migrate to the band surface (Viehman & Richardson 2002). At night, in absence of oxygen production, *Desulfovibrio* spp. undergo sulfate reduction, increasing sulfide concentrations in the band. As a result, sulfide is present throughout the band at night and the oxic/anoxic interface migrates to the band surface (Carlton & Richardson 1995, Richardson et al. 1997).

Presence of sulfide and anoxia at the base of the band (adjacent to coral tissue) is thought to be the cause of tissue lysis and death (Carlton & Richardson 1995). BBD-induced coral mortality presumably releases inorganic nutrients (NH_4^+ and PO_4^{3-}) that support cyanobacterial photosynthesis. Furthermore, because the disease band directly overlies coral tissue that is being degraded, nutrients supplied by tissue mortality diffuse directly into the band, providing

concentrated nutrients to the microbial consortium, and fueling their growth and reproduction (Carlton & Richardson 1995). This process of tissue death may serve as the source for elevated nitrite levels associated with seawater immediately surrounding BBD-affected corals (Kuta & Richardson 2002).

The mechanism of BBD transmission remains unknown. However, BBD may be infectious (Edmunds 1991, Kuta & Richardson 1996) and transmitted in the water column (Kuta & Richardson 1996, Bruckner et al. 1997). Sediment patches on the surfaces of apparently healthy corals may serve as reservoirs of the cyanobacteria associated with BBD (Richardson 1997).

Disease signs similar to BBD were reported in 1983 (Rützler et al. 1983) and documented as a new disease termed red band (Richardson 1993). Red band reportedly affects *Gorgonia ventalina* in Belize, Puerto Rico and the Florida Keys (Santavy & Peters 1997), and *Montastraea annularis* and *M. faveolata* in the Colombian Caribbean (Garzón-Ferreira et al. 2001). Red band is characterized by a red-brown to brown-black microbial mat that forms a migrating band that separates recently denuded skeleton from living tissue (Santavy & Peters 1997). The condition was considered not to be BBD because *Phormidium corallyticum*, accepted at the time to be the dominant component of the BBD consortium, was not present in red band (Rützler et al. 1983). Instead, red band was dominanted by other species of cyanobacteria, identified as *Schizothrix calcicola*, *S. mexicana* (Rützler et al. 1983) and *Oscillatoria* spp. (Richardson 1993). The RBD microbial consortium may include other cyanobacteria, heterotrophic bacteria, the sulfur-oxidizing bacterium *Beggiatoa* sp., and the nematode *Araeolaimus* sp. (Santavy & Peters 1997). The recent discovery that BBD is associated with at least 3 different taxa of cyanobacteria (Frias-

Lopez et al. 2003) and the lack of new reports of red band disease since the early 1990s, suggest that red band is not a distinct disease, but rather BBD.

White Plague

White plague (WPL; Fig. 2.3 B,C) has affected Caribbean and Indo-Pacific corals since the late 1970s (Dustan 1977, Richardson et al. 1998a) and early 1980s (Antonius 1985a), respectively. In the Caribbean, the disease reported in the 1970s has been renamed WPL I to distinguish this condition from 2 new diseases with similar disease signs: WPL II (Richardson et al. 1998a) and WPL III (Richardson et al. 2001). WPL II is an infectious biotic disease (Richardson et al. 1998a) caused by a new genus and species of bacterium, *Aurantimonas coralicida* (Table 2.4; Richardson et al. 1998b, Denner et al. 2003). Pathogenesis and transmission of WPL II are not understood. Histopathology of WPL I affected tissues shows necrosis at lesion boundaries (Peters 1984, Bythell et al. 2002) accompanied by dense clusters of coccoid bacteria that do not resemble the rod-shaped *A. coralicida* from WPL II infections (Bythell et al. 2002). Similarly, 16S rDNA sequencing indicates that *A. coralicida* is not associated with a WPL-like disease affecting Caribbean corals (Pantos et al. 2003). These studies suggest that there is indeed more than one etiologic agent associated with the various WPL diseases in the Caribbean. The causal agent(s) of WPL I and III are unknown.

In the Indo-Pacific, WPL-like (WPL-L) disease signs have been reported. Authors that documented WPL-L disease in the Indo-Pacific referred to the disease as WBD (Antonius 1985a, Coles 1994, Riegl 2002). Species affected and disease signs reported for the Indo-Pacific (Antonius 1985a, Coles 1994) are indicative of a WPL-L disease. In the future, Indo-Pacific researchers must distinguish between WPL-L and WBD-like diseases, which are characterized,

respectively, by a sharp line of tissue loss that progresses across a coral colony and by a ring of tissue loss that progresses up or down acroporid coral branches. WPL-L disease affects 38 Indo-Pacific scleractinian species (Table 2.3; Antonius 1985a, Coles 1994, Riegl 2002).

WPL I progresses slowly (3.1 mm d^{-1} maximum) and is characterized by a sharp line of tissue loss where healthy tissue is immediately adjacent to recently denuded skeleton (Fig. 2.3B,C; Dustan 1977). WPL I affects at least 13 Caribbean scleractinian species (Table 2.2; Dustan 1977, Richardson et al. 1998a).

WPL II progresses rapidly (2 cm d^{-1} maximum) and is characterized by a sharp line of disease progression. At times a narrow band (2 to 3 mm) of bleached tissue separates healthy tissue and bare skeleton, but more commonly the disease line appears the same as for WPL I, i.e. healthy tissue is immediately adjacent to recently denuded tissue (Richardson et al. 1998 a, b). Another distinguishing characteristic of WPL II is that infection most often begins at the base of the coral colony and progresses upward in a concentric ring around the entire colony (Richardson et al. 1998a, b). Three major epizootics of WPL II have been reported in South Florida since the mid-1990s (Richardson et al. 1998a). The number of species affected by WPL II is increasing. In 1995, Richardson et al. (1998a, b) reported 17 species of scleractinian corals affected on reefs in the Florida Keys, and by 2000 this number had increased to 32 species (Weil et al. 2002). Highest disease prevalence of WPL II has been recorded for *Dichocoenia stokesii*, with mortality as high as 38% (Richardson et al. 1998a).

WPL III was first documented in 1999 in the northern Florida Keys. WPL III appears to exclusively affect large colonies (3 to 4 m diameter) of *Colpophyllia natans* and *Montastraea annularis* (Table 2.2). Tissue loss attributed to WPL III is extremely rapid and greatly exceeds loss rates attributed to WPL I and WPL II (Richardson et al. 2001).

Surveys of WPL prevalence conducted in Puerto Rico (Bruckner & Bruckner 1997a) and St. Lucia, West Indies (Nugues 2002) did not distinguish type. However, based on slow rate of disease progression (1.3 mm d^{-1} maximum) measured in St. Lucia (Nugues 2002) and rapid disease progression (1.4 cm d^{-1} maximum) measured in Puerto Rico (Bruckner & Bruckner 1997a) it is likely that the diseases surveyed were WPL I and II, respectively. Highest disease prevalence was recorded for *Diploria labyrinthiformis* (47% of colonies affected) in Puerto Rico (Bruckner & Bruckner 1997a) and for *Montastraea faveolata* (19% of colonies affected) and *Colpophyllia natans* (13% of colonies affected) in St. Lucia (Nugues 2002).

Nugues (2002) observed diseased individuals of 4 scleractinian species, *Isophylastrea rigida, Montastraea faveolata, Mussa angulosa,* and *Mycetophyllia* sp., not yet documented as susceptible to WPL I (Table 2.2). Surveys conducted within the FKNMS documented WPL (type not determined) on 14 additional species (Table 2.2; Porter et al. 2001).

Shut-Down Reaction

Shut-down reaction (SDR) is a condition that most often affects corals contained in aquaria and is rarely observed in natural coral reef environments. SDR occurs only on wounded corals (e.g. predation, diver contact) and is always associated with abiotic environmental stressors (e.g. temperature extremes, sedimentation, Table 2.5; Antonius 1977). SDR begins at and radiates from the interface between wound and healthy tissue. Tissue is sloughed off the affected colony at the rapid rate of 10 cm h^{-1}. Once SDR is triggered, complete colony mortality is inevitable. SDR is contagious, indicating presence of a pathogen, and can be both directly and indirectly transmitted from an infected colony to a stressed, but otherwise apparently healthy

colony, via physical contact between neighboring colonies and water transport, respectively (Antonius 1977). SDR cannot be transmitted to unstressed coral colonies (Antonius 1977).

SDR has been experimentally induced in aquaria for 6 Caribbean scleractinian species (Table 2.2; Antonius 1977). In the Caribbean, SDR has been reported affecting only 3 individual coral colonies in the field; the species affected were *Montastraea annularis* and *Acropora cervicornis* (Table 2.2; Antonius 1977). Prior to recent reports of SDR in the Red Sea (species affected not reported, Antonius & Riegl 1997, 1998), new cases of the condition had not been documented since the first report in the late 1970s (Antonius 1977).

Skeletal Anomalies

Skeletal anomalies include tumors, galls, nodules, and other abnormalities of coral tissue and skeleton. Skeletal anomalies of scleractinian corals have been observed on reefs throughout the world including the Florida Keys (Peters et al. 1986), Netherlands Antilles (Bak 1983), Hawaii (Squires 1965, Cheng & Wong 1974, Grygier & Cairns 1996, Aeby 1998, Hunter & Peters 1993), Guam and Enewetak (Cheney 1975), Oman (Coles & Seapy 1998), Japan (Yamashiro et al. 2000), and the Great Barrier Reef, Australia (Loya et al. 1984).

A tumor is an abnormal tissue proliferation (Sinderman 1990) and, in corals, is often associated with an abnormal skeletal growth (Yamashiro et al. 2000). Tumors result from neoplasia, hyperplasia, or hypertrophy. Neoplasia (neoplasm) is uncontrolled cell proliferation. Hyperplasia and hypertrophy are nonneoplastic controlled cell proliferation and nonneoplastic increase in cell size, respectively (Sinderman 1990). The terms tumor and neoplasia are considered by many to be synonymous (Stedman 2000).

The first suspected neoplasm of a scleractinian coral was documented in 1965 on *Madrepora kauaiensis* in the Hawaiian Islands (Table 2.3, Squires 1965). This condition, also observed on *M. oculata* (Table 2.3), has recently been reinterpreted as a polyp hypertrophy characterized by gall formation (i.e. a parasite-induced proliferation of tissues, Grygier & Cairns 1996). These lesions develop on *Madrepora* spp. when the crustacean *Petrarca madreporae,* an obligate endoparasite of corals, invades a normal coral polyp as a larva and matures within the polyp, causing development of an enlarged (hypertrophied) corallite with abnormal septae (Table 2.4; Grygier & Cairns 1996).

Other skeletal anomalies have been attributed to interactions with foreign organisms in the skeleton (Table 2.4). *Porites compressa* and *P. lobata* in Kaneohe Bay, Oahu, Hawaii develop grossly visible pink nodules in response to the encystment of the digenetic trematode *Podocotyloides stenometra* within the tentacles of the coral polyps (Table 2.3; Cheng & Wong 1974, Aeby 1998). The scleractinian corals *P. lobata, P. lutea, Manicina areolata*, and *Montastraea cavernosa* can detect invasion by endolithic fungi and respond by surrounding the site of fungal penetration within a layer of thickened calcium carbonate produced by hypertrophied calicoblasts (Tables 2.3 & 2.4; Le Champion-Alsumard et al. 1995, Ravindran et al. 2001, E.C. Peters pers. comm.). However, this defense mechanism fails to hinder fungal advancement, and, as hyphae penetrate the layer of calcium carbonate repair, the coral repeats the process, resulting in a calcareous skeletal protuberance composed of a number of carbonate layers (Le Champion-Alsumard et al. 1995). Nodules on the gorgonian coral *Gorgonia ventalina* are attributed to both infection with *Aspergillus sydowii*, the causal agent of aspergillosis (Dube et al. 2002), and to infestation with filamentous green algae of the Order Siphonales (Tables 2.2 & 2.4; Morse et al. 1977, 1981). The algal nodules are hyperplasias of the axis epithelial cells

that produce the endoskeletal gorgonin. Amoebocytes infiltrate the associated mesoglea and encapsulate the algal filaments (Morse et al. 1977, 1981). Aspergillosis-associated nodule formation may be a defense mechanism that sequesters fungal hyphae and limits spread of infection (Smith et al. 1998). Nodules on the gorgonian corals *Pseudoplexaura* spp. are the result of skeletal encapsulation of the marine microalgae, *Entocladia endozoica* (Tables 2.2 & 2.4; Goldberg et al. 1984).

Peters et al. (1986) and Coles & Seapy (1998) described the only known true neoplasms (tumors) of corals. Peters et al. (1986) observed neoplasms, termed calicoblastic epitheliomas, on *Acropora palmata* in the Florida Keys (Table 2.2). These calicoblastic epitheliomas result from proliferation of calicoblasts and associated tissues and are characterized by raised (up to 1 cm high), irregularly shaped, smooth, white lumps that develop on all parts of the coral colony. Mean growth rate of tumors is 0.12 mm d^{-1} or 25 to 44 mm yr^{-1} (Peters et al. 1986). Similar calicoblastic epitheliomas affect *A. valenciennesi* and *A. valida* in the Gulf of Oman, Indian Ocean (Table 2.3; Coles & Seapy 1998).

Coral skeletal anomalies are characterized by: (1) thinning of coral tissue covering anomalies (Peters et al. 1986, Coles & Seapy 1998), (2) increased porosity of coral skeleton (Peters et al. 1986, Coles & Seapy 1998, Yamashiro et al. 2000), (3) loss of mucous secretory cells and nematocysts (Peters et al. 1986, Coles & Seapy 1998), (4) loss of zooxanthellae (Bak 1983, Peters et al. 1986, Coles & Seapy 1998, Yamashiro et al. 2000), (5) loss, reduction, or degeneration of normal polyp structures (Bak 1983, Peters et al. 1986, Coles & Seapy 1998, Yamashiro et al. 2000), and (6) reduced fecundity (Yamashiro et al. 2000).

Skeletal anomalies pose a serious threat to affected corals. Loss of the primary defense mechanism, mucous secretory cells, inhibits removal of foreign material from the coral surface,

contributing to cell death and increasing susceptibility to invasion by filamentous algae. Porous skeletons may be more susceptible to storm-related damage (Peters et al. 1986). Loss of zooxanthellae reduces fecundity, skeletal growth, calcification rates, and nutrition (Bak 1983, Porter et al. 1989, Brown 1997a). Polyp destruction limits reproductive capacity (Peters et al. 1986, Yamashiro et al. 2000).

Skeletal anomalies (SKA; Fig. 2.3D) affect 17 Caribbean (Table 2.2) and 24 Indo-Pacific (Table 2.3) scleractinian species, 1 Caribbean hydrozoan, and at least 5 species of Caribbean gorgonians (Table 2.2; Squires 1965, Cheney 1975, Morse et al. 1977, 1981, Bak 1983, Goldberg et al. 1984, Loya et al. 1984, Peters et al. 1986, Hunter & Peters 1993, Le Champion-Alsumard et al. 1995, Grygier & Cairns 1996, Coles & Seapy 1998, Green & Bruckner 2000, Yamashiro et al. 2000, Ravindran et al. 2001, Dube et al. 2002). Acroporids appear to be the most susceptible to neoplasia (Peters et al. 1986, Coles & Seapy 1998), and this may be due to the rapid growth rates of this genus (Peters et al. 1986). *Acropora palmata* is capable of linear extension rates as high as 47 to 99 mm yr^{-1} (Gladfelter et al. 1978).

With the exception of microorganism-induced nodule or gall formation (Cheng & Wong 1974, Morse et al. 1977, 1981, Goldberg et al. 1984, Le Champion-Alsumard et al. 1995, Grygier & Cairns 1996, Aeby 1998, Ravindran et al. 2001, Dube et al. 2002), the etiology of coral skeletal anomalies is unknown (Peters et al. 1986, Coles & Seapy 1998, Yamashiro et al. 2000). Parasitic and commensal organisms have been ruled out as potential causal agents of the skeletal anomalies described by Peters et al. (1986) in the Florida Keys and by Yamashiro et al. (2000) in Japan. Solar UV radiation has been hypothesized as a possible initiator of neoplasia formation (Table 2.5; Peters et al. 1986, Coles & Seapy 1998).

CARIBBEAN CORAL DISEASES

Aspergillosis

Mass mortalities of the sea fans *Gorgonia ventalina* and *G. flabellum* were reported throughout the Caribbean during the 1980's (Garzón-Ferreira & Zea 1992). A second epizootic affecting *Gorgonia* spp. began in 1995 (Nagelkerken 1997a, b, Slattery 1999), which was less virulent but more widespread than the 1980s epizootic (Nagelkerken 1997a, b).

Both epizootics have been attributed to the fungus *Aspergillus sydowii* (Table 2.4; Smith et al. 1996, Geiser et al. 1998). *Aspergillus* disease, termed aspergillosis (ASP; Fig. 2.3E), destroys living tissue and degrades skeletal framework (Nagelkerken et al. 1997b). In addition to *Gorgonia ventalina* and *G. flabellum*, ASP signs have been observed on 6 additional gorgonian species from 5 genera (Kim et al. 2000b, Weil et al. 2002, Table 2.2). However, Koch's postulates have only been fulfilled, establishing *A. sydowii* as the causative agent, for disease cases affecting *G. ventalina* and *G. flabellum* (Smith et al. 1996).

ASP lesions are characterized by recession of rind tissue (coenenchyme) exposing the internal axial skeleton. *Aspergillus sydowii* hyphae are embedded in living tissue at the receding edge of the lesion (Smith et al. 1996). Lesions are often circumscribed by a purple halo indicative of an abundance of purple sclerites (Kim et al. 1997, Smith et al. 1998, Slattery 1999). Purple sclerite-dense nodules often erupt on affected sea fans (Kim et al. 1997, Smith et al. 1998, Dube et al. 2002). Sclerite recruitment and nodule formation may be methods of defense that sequester fungal hyphae and limit spread of infection (Smith et al. 1998). Mechanisms by which *A. sydowii* produces tissue degradation and nodule formation remain unknown, but virulence of the pathogen is known to increase with elevated seawater temperatures (30ºC, Table 2.5; Alker et al. 2001).

The genus *Aspergillus* is not commonly found in marine environments and most often inhabits terrestrial soils. However, *Aspergillus* spp. can easily cope with the salinity of seawater (Kendrick et al. 1982) and have been isolated from marine environments (Muntanola-Cvetkovic & Ristanovic 1980, Kendrick et al. 1982, Ravindran et al. 2001). *A. sydowii* has been shown to bioerode living stony corals (Kendrick et al. 1982).

Aspergillus sydowii is a terrestrial fungus. Delivery of *A. sydowii* to the marine environment may be associated with either local sediment runoff (Smith et al. 1996) or long distance transport (Table 2.5, Shinn et al. 2000). If local runoff is the source, then ASP may be linked to anthropogenic disturbance. Shinn et al. (2000) hypothesize that *A. sydowii*, and perhaps other coral disease-causing pathogens, are transported to the western Atlantic in African dust air masses. *A. sydowii* has been cultured from spores collected in the US Virgin Islands during African dust storm events. These *A. sydowii* isolates, when inoculated onto healthy sea fans, produced ASP signs (Weir et al. *in press*).

Terrestrial sources are a possible mode of primary transmission of *Aspergillus sydowii* hyphae and/or spores to unaffected sea fans in the marine environment. *A. sydowii* germinates but does not sporulate on sea fans. Hyphae must break free from an infected gorgonian and reach the surface of the water to produce spores (G.W. Smith, pers. comm.). Secondary transmission of ASP from infected to uninfected sea fans may occur through: (1) direct physical contact with an infected individual, i.e. a diseased sea fan may brush against a close neighbor (Smith et al. 1996, Jolles et al. 2002), (2) transport of fungal hyphae in the water column (Jolles et al. 2002), or (3) transport of fungal spores (produced at the sea surface from hyphae released from diseased sea fans) in the water column (G.W. Smith, pers. comm.).

Incidence and prevalence of ASP are greater at protected than at exposed sites and increase with depth in areas with low to moderate wave action, indicating that the more frequent mechanical swaying of colonies that occurs in shallow and more exposed areas may decrease sea fan susceptibility to disease (Nagelkerken 1997a). ASP is prevalent in the Florida Keys, affecting 43% of *Gorgonia ventalina* colonies Keys-wide (Kim & Harvell 2002). Poor water quality (i.e. increased turbidity and increased chl *a*) may play a role in the impact of ASP on sea fan populations (Table 2.5). Kim & Harvell (2002) found that severity of ASP in the Florida Keys was greatest near the city of Key West. With approximately 25500 residents and a high seasonal influx of tourists, Key West is by far the most populous town in the Florida Keys (US BOC 2000). Runoff of nutrients and pollutants into the marine environment is likely higher near Key West than in other less populated areas in the Florida Keys.

White Band

White band disease (WBD; Fig. 2.3F) has affected Caribbean scleractinian corals since the late 1970s (Antonius 1985a, Bythell & Sheppard 1993). The disease, as first described by Gladfelter (1982), has been renamed WBD I to distinguish the condition from WBD II (Ritchie & Smith 1998). WBD I and II exclusively affect branching acroporid corals. While WBD I affects both *Acropora palmata* and *A. cervicornis* Caribbean-wide (Gladfelter 1982, Peters 1984), WBD II has been reported only from the Bahamas, exclusively affecting *A. cervicornis* (Ritchie & Smith 1998; Table 2.2).

WBD type I has been implicated as the principal cause of mass mortalities of *Acropora cervicornis* and *A. palmata* that occurred in the 1980's and 1990's (Gladfelter 1982, Bythell & Sheppard 1993, Aronson & Precht 1997, 2001, Aronson et al. 1998, 2002). On most reefs, loss

of acroporids was accompanied by an ecological phase shift from a coral-dominated to an algal-dominated reef (Hughes 1994). However, on the Belizean Barrier Reef, the grazing urchin *Echinometra viridis* consumed fleshy and filamentous macroalgae and allowed for a wide scale (at least 500 km^2) coral community shift (Aronson et al. 2002). Following the WBD epizootic of the late 1980s, the previously dominant coral *A. cervicornis* was replaced by the thin-leaf lettuce coral *Agaricia tenuifolia* (Aronson & Precht 1997, Aronson et al. 2002). Examination of the fossil record indicates that the wide–scale *Acropora*-to-*Agaricia* shift is unprecedented in the last 3800 yr (Aronson & Precht 1997, 2001, Aronson et al. 2002). This evidence suggests that WBD is an emergent disease and not a natural cyclic phenomenon that has occurred on Caribbean reefs in the past.

WBD I progresses rapidly (2 cm d^{-1} maximum) and has the potential to cause extensive mortality (Antonius 1981, Gladfelter 1982, Peters et al. 1983). The disease is characterized by a white band of recently denuded skeleton adjacent to a necrotic front of normally pigmented living tissue (Fig. 2.3F; Gladfelter 1982, Peters et al. 1983). WBD I develops at the base of a coral colony or branch and progresses upward toward branch tips in a concentric ring (Gladfelter 1982).

WBD II was first documented in 1993 in the Bahamas (Ritchie & Smith 1995a, Ritchie & Smith 1998). WBD II is distinguished from WBD I by a band (2 to 20 cm wide) of living bleached tissue separating denuded skeleton from normally pigmented tissue. The bleaching of the tissue progresses more rapidly than does the margin of necrotic tissue and can arrest, allowing the necrotic margin to catch up to normal tissue. When this occurs, WBD II resembles WBD I (Ritchie & Smith 1998) and the 2 diseases cannot be distinguished from a single observation in the field. Like WBD I, WBD II can develop at the base of a coral colony and

progress upward, but WBD II is also capable of developing at tips of branches and progressing downward. When the disease begins at branch tips, tissue loss can be accompanied by skeletal degradation, i.e. dissolution and loss of branch tips (Ritchie & Smith 1998).

The causative agents of WBD I and II are unknown, however, efforts have been made to determine etiologies of these conditions. Tissues of WBD I-diseased and apparently healthy *Acropora palmata* and *A. cervicornis* at St. Croix, US Virgin Islands and at Bonaire were found to contain Gram-negative bacterial aggregates. Aggregates were more abundant in diseased corals than in apparently healthy corals. However, other apparently healthy and diseased acroporids do not contain aggregates (Peters et al. 1983). Examination of *A. cervicornis* from the Bahamas and the Florida Keys revealed WBD I-affected colonies both with and without bacterial aggregates (Peters 1984). Thus, the role of bacterial aggregates in WBD I is uncertain. Histopathology of WBD I tissues shows no signs of necrosis or clustering of microorganisms (Peters et al. 1983, Bythell et al. 2002).

WBD II is always associated with the bacterium *Vibrio charcharia* (Table 2.4; Ritchie & Smith 1995a). Attempts to fulfill Koch's postulates with *V. charcharia* were unsuccessful, and therefore the significance of the bacterium to the etiology of WBD II remains unknown.

White Pox

White pox disease (WPD; Fig. 2.4A,B), also termed acroporid serratiosis (Patterson et al. 2002) and patchy necrosis (Bruckner & Bruckner 1997a), was first documented in 1996 on reefs off Key West, Florida (Holden 1996). WPD has since been observed throughout the Caribbean (Porter et al. 2001, Rodríquez-Martínez et al. 2001, Santavy et al. 2001, Patterson et al. 2002). The disease

exclusively affects *Acropora palmata* (Table 2.2), and is caused by the common fecal enterobacterium *Serratia marcescens* (Table 2.4, Patterson et al. 2002).

Serratia marcescens is a Gram-negative bacterium classified as a coliform and a member of the Enterobacteriaceae family. It is found in feces of humans and other animals and in water and soil (Grimont & Grimont 1994). The prevalence of *S. marcescens* in the marine environment is unknown. However, this bacterium has been found in the marine environment in sewage–polluted estuaries. For example, *S. marcescens* has been linked to disease of white perch *Morone americanus* in the sewage-polluted Back River, Maryland (Baya et al. 1992).

Identification of *Serratia marcescens* as a coral pathogen marked the first time that a common member of the human gut microbiota was shown to be a marine invertebrate pathogen. While *S. marcescens* is ubiquitous, its noted association with human hosts prompts speculation that improperly treated sewage may be associated with white pox disease in corals. Human enteric bacteria and viruses are prevalent in coral SML and other marine environments of the Florida Keys (Griffin et al. 1999, Lipp et al. 2002). The origin and pathogenic mechanisms of the WPD pathogen are unknown (Patterson et al. 2002).

Coral colonies affected by WPD are characterized by irregularly shaped distinct white patches of recently exposed skeleton surrounded by a necrotic front of normally pigmented living tissue (Fig. 2.4B). Lesions range in area from a few square centimeters to > 80 cm^2 and develop simultaneously on all surfaces of the coral colony (Patterson et al. 2002). Lesions exhibit tissue loss along the perimeter and increase in area as tissue is lost from the leading edge of infection. Rate of tissue loss is rapid, averaging 2.5 cm^2 d^{-1}, and is greatest during periods of seasonally elevated temperature and rainfall (Table 2.5). WPD is highly contagious, with nearest neighbors most susceptible to infection (Patterson et al. 2002). The disease spread rapidly within

and between reefs in the Florida Keys during the mid-1990s (Porter et al. 2001, Patterson et al. 2002).

WPD has been implicated as the principal cause of mass mortality of *Acropora palmata* within the FKNMS (Patterson et al. 2002). Between 1996 and 2002, average loss of A. palmata Keys-wide was 87% (Fig. 2.1; Patterson et al. 2002, Sutherland & Ritchie *in press*). Losses of *A. palmata* at Eastern Dry Rocks Reef, FL (24º 27.715' N, 81º 50.801'W) between 1994 and 2002, and at Looe Key Reef, FL (24º 33' N, 81º 24' W) between 1983 and 2000, were 97 and 93%, respectively (Miller et al. 2002, Patterson et al. 2002, Sutherland & Ritchie *in press*). These severe population declines of the coral community's most important primary producer and shallow water framework builder have led to the identification of *A. palmata* as a candidate for inclusion on the Endangered Species List (Diaz-Soltero 1999). Diseases of acroporid corals (WPD and WBD) are changing the composition, structure, and function of Caribbean coral reef ecosystems (Hughes 1994, Aronson & Precht 1997, 2001, Aronson et al. 2002, Patterson et al. 2002).

Yellow Blotch/Band

Yellow blotch/band (YBL; Fig. 2.4C,D) disease has been reported throughout the Caribbean since 1994 (Santavy & Peters 1997, Santavy et al. 1999, Cervino et al. 2001, Garzón-Ferreira et al. 2001, Toller et al. 2001, Weil et al. 2002). YBL affects 9 scleractinian coral species (Table 2.2; Garzón-Ferreira et al. 2001), but most often affects *Montastraea annularis* (Cervino et al. 2001). The causal agent of YBL is unknown.

YBL lesions are characterized by circular to irregularly shaped patches (Fig. 2.4C) or bands (Fig. 2.4D) of discolored coral tissue. Lesions can develop on all areas of the coral colony, but are most common on upper surfaces (Santavy et al. 1999). A patch of exposed

skeleton is often present at the center of each circular to irregularly shaped lesion and at the edge of each band-shaped lesion. This dead zone is surrounded by rings or bands of yellow translucent tissue, which may in turn be surrounded by rings or bands of pale brown to bright-white bleached tissue. Apparently healthy tissue surrounds the outer regions of YBL lesions (Santavy et al. 1999, Toller et al. 2001). Tissue loss rate associated with YBL is approximately 0.6 cm mo^{-1} (Cervino et al. 2001).

YBL-affected coral tissues have approximately 40% fewer algal symbionts than do apparently healthy tissues, and these zooxanthellae appear vacuolated and lack organelles (Cervino et al. 2001). In San Blas (Panama) zooxanthellae within yellow and normal tissues from YBL-affected *Montastraea annularis, M. faveolata,* and *M. franksi,* were found to be of different taxa of the genus *Symbiodinium* (Toller et al. 2001). Normal (unaffected) tissues were dominated by *Symbiodinium* C, the taxon common in healthy corals at depth of collection (1 to 10 m). Yellow (affected) tissues predominately contained *Symbiodinium* A, the source of the characteristic yellow color of YBL lesions (Toller et al. 2001).

Dark Spots

Dark spots disease (DSD; Fig. 2.4E,F) was first documented in the early 1990s in Colombia and today is found throughout the Caribbean (Goreau et al. 1998, Cervino et al. 2001, Garzón-Ferreira et al. 2001, Gil-Agudelo & Garzón-Ferreira 2001, Weil et al. 2002). DSD reportedly affects 11 scleractinian species (Table 2.2; Goreau et al. 1998, Cervino et al. 2001, Garzón-Ferreira et al. 2001, Gil-Agudelo & Garzón-Ferreira 2001).

DSD is characterized by irregularly shaped dark spots of purple, maroon, or brown coloration on normal tissue (Goreau et al. 1998). DSD may be associated with tissue necrosis

and/or depression of the colony surface (Cervino et al. 2001). When DSD is associated with tissue loss, a spot may expand into a characteristic dark ring separating dead skeleton from living tissue (Goreau et al. 1998).

Dark pigmentation of DSD-affected *Stephanocoenia michelinii* extends into, and stains, the skeleton, and may be attributed to zooxanthellae, which appear darker in pigment in diseased corals of this species. In *Siderastrea siderea* affected with DSD, skeletal staining and zooxanthellae of darker pigmentation are not evident, but zooxanthellae do appear swollen and necrotic. These observations suggest that similar disease signs observed on *S. michelinii* (Fig. 2.4E) and *S. siderea* (Fig. 2.4F) may represent 2 different diseases (Cervino et al. 2001).

In the Colombian Caribbean, DSD is correlated with depth and temperature. DSD is more prevalent at depths less than 6 m and during summer when seawater temperature is highest (Table 2.5). Depth distribution of DSD may be due to high disease prevalence (94%) on 2 coral species that occupy shallow depths, *Siderastrea siderea* and *Montastraea annularis*. Distribution of DSD on Colombian reefs is clumped, indicating that the disease may be contagious and therefore of biotic origin (Gil-Agudelo & Garzón-Ferreira 2001). The causal agent of DSD is unknown.

INDO-PACIFIC CORAL DISEASES

Vibrio-Induced Bleaching

Coral bleaching is a global phenomenon that occurs when symbiotic algae (zooxanthellae) and/or their pigments are lost from host coral tissues, resulting in pale brown to translucent living tissue. This condition reduces reproductive output, skeletal growth, calcification rates, and nutrition (Porter et al. 1989, Brown 1997a), and therefore is a sign of

disease. Bleaching is most often correlated with elevated seawater temperature, but may be associated with salinity extremes (Coles & Jokiel 1992), light irradiance extremes (Brown et al. 1994, 2000, Brown 1997b, Shick et al. 1996), and pollutants (Peters et al. 1981, Harland & Brown 1989). Bleaching may result in coral mortality, but most often the coral regains its algal symbionts, resulting in full recovery (Fitt et al. 1993).

Coral bleaching, once regarded exclusively as an abiotic condition, has recently been associated with 2 bacterial pathogens of the genus *Vibrio*. In the Mediterranean Sea, bleaching of the coral *Oculina patagonica* can be induced by an infection with the bacterium *V. shiloi* (Tables 2.3 & 2.4; Kushmaro et al. 1996, 1997, 1998, 2001, Rosenberg et al. 1998). Bleaching and lysis of tissue of the coral *Pocillopora damicornis* in Zanzibar, Indian Ocean and Eilat, Red Sea are induced by infection with *V. coralliilyticus* (Tables 2.3 & 2.4; Ben-Haim & Rosenberg 2002, Ben-Haim et al. 2003a, b). *Vibrio*-induced bleaching and lysis are always associated with elevated seawater temperatures in excess of 24.5ºC (Kushmaro et al. 1998, Toren et al. 1998, Ben-Haim et al. 1999, 2003b, Banin et al. 2001a, Israely et al. 2001), and therefore are examples of diseases with etiologies associated with both abiotic and biotic factors.

Vibrio shiloi-Induced Bleaching

Vibrio shiloi-induced bleaching (VSB) of *Oculina patagonica* is agueably the most characterized coral disease in terms of etiology and mechanism of pathogenesis. Koch's postulates were fullfilled and showed that bleaching can be induced by infection with V. *shiloi* in apparently healthy corals maintained at elevated seawater temperatures (25 to 29ºC, Kushmaro et al. 1998). Elevated seawater temperatures increases the virulence of *V. shiloi* (Table 2.5;

Kushmaro et al. 1998, Toren et al. 1998, Ben-Haim et al. 1999, Banin et al. 2001a, Israely et al. 2001).

Under conditions of elevated seawater temperatures, infection begins with adhesion of *Vibrio shiloi* to a β-galactoside-containing receptor in mucus on the surface of the host coral (Toren et al. 1998, Banin et al. 2001b). Photosynthetically active zooxanthellae inside the host coral produce the β-galactoside-containing receptor. Only *V. shiloi* that are grown at elevated seawater temperatures are capable of adhesion (Toren et al. 1998). Therefore, adhesion only occurs under conditions of elevated temperature and if photosynthetically active zooxanthellae are present (Banin et al. 2001b).

The second step in the infection process is penetration of *Vibrio shiloi* into the epidermis of the host coral. Once *V. shiloi* are inside the tissues they multiply and transform into a viable but non-culturable state (VBNC). In other words, intracellular *V. shiloi* are not capable of forming colonies on media that support growth of extracellular *V. shiloi*. Intracellular bacteria are protected against effects of treatment with the antibiotic gentamicin. Gentamicin kills extracellular *V. shiloi* associated with coral mucus, but has no effect on *V. shiloi* that have successfully penetrated host tissue (Banin et al. 2000). At elevated temperatures, VBNC *V. shiloi* infect healthy coral and induce bleaching (Israely et al. 2001).

Following penetration into the host coral, *Vibrio shiloi* produces both heat-stable and heat-sensitive toxins that target zooxanthellae and play a role in pathogenesis. The heat-sensitive toxin bleaches and lyses symbiotic algal cells isolated from the host coral (Rosenberg et al. 1998, Ben-Haim et al. 1999). The extracellular heat-stable toxin, termed Toxin P, is a proline-rich dodecapeptide that binds to zooxanthellae and inhibits photosynthesis in the presence of ammonia (Rosenberg et al. 1998, Ben-Haim et al. 1999, Banin et al. 2001a). Inhibition of

photosynthesis damages zooxanthellae and contributes to coral bleaching (Banin et al. 2001a). Toxin P is produced only under conditions of elevated seawater temperature (25 to 30ºC, Banin et al. 2001a). *V. shiloi* require another virulence factor, superoxide dismutase, in order to survive inside the host coral. High levels of superoxide dismutase are produced by *V. shiloi* under conditions of elevated seawater temperature (30ºC, Israely et al. 2001).

During summer in the Mediterranean Sea, *Vibrio shiloi* are present in all bleached colonies of *Oculina patagonica.* However, during winter, when water temperature drops to 16ºC, intracellular *Vibrio shiloi* lyse and die, and corals affected by VSB recover. *V. shiloi* outside the coral survive the temperature shift, indicating that the coral host may play a role in seasonal demise of the pathogen (Israely et al. 2001). Reinfection of *O. patagonica* colonies the following summer is facilitated, at least in part, by the marine fireworm *Hermodice carunculata,* which serves as a reservoir and a vector of *V. shiloi*. During winter, *V. shiloi* is present in the VBNC state inside *H. carunculata,* and during summer, when the worm feeds on *O. patagonica,* the VSB pathogen is transmitted to a few coral colonies. This indirect transmission serves to restart the infection process, and the infectious VSB then spreads from colony to colony (Sussman et al. 2003).

Vibrio coralliilyticus-Induced Bleaching and Disease

Vibrio coralliilyticus-induced bleaching and disease (VCB) affects the scleractinian coral *Pocillopora damicornis*. VCB is characterized by bleaching (Ben-Haim et al. 2003b) and lysis of coral tissue (Ben-Haim & Rosenberg 2002, Ben-Haim et al. 2003b). Koch's postulates were fulfilled and showed that VCB is caused by a new species of bacterium, *V. coralliilyticus* (Table 2.4, Ben-Haim & Rosenberg 2002, Ben-Haim et al. 2003a, b). Bleaching and tissue loss

associated with *V. coralliilyticus* is temperature dependent. Infection with *V. coralliilyticus* induces bleaching at seawater temperatures of 24.5 and 25.0°C, and tissue lysis and colony death at seawater temperatures of 27 and 29°C (Ben-Haim et al. 2003b). Bleaching does not preceed lysis at temperatures greater than 27°C (Ben-Haim et al. 2003b). VCB is infectious and can be transmitted through direct contact between an infected coral colony and an uninfected neighbor (Ben-Haim & Rosenberg 2002). *V. coralliilyticus* produces an extracellular protease that may play a role in pathogenesis, and production of this enzyme increases at temperatures greater than 24°C (Ben-Haim et al. 2003b). Elevated seawater temperature increases either pathogen virulence or host susceptibility, or both (Ben-Haim & Rosenberg 2002)

Skeleton Eroding Band

Skeleton eroding band (SEB) disease was first documented in 1988 in Papua New Guinea (Antonius & Lipscomb 2000). SEB has since been observed in the Red Sea, Indian Ocean, and the Great Barrier Reef, Australia. Attempts to locate the disease in the Caribbean have been unsuccessful. Although Koch's postulates have not been fulfilled, SEB is believed to be caused by the protozoan *Halofolliculina corallasia* (Table 2.4). SEB affects 24 Indo-Pacific scleractinian species (Table 2.3; Antonius & Lipscomb 2000).

Halofolliculina corallasia is a folliculinid heterotrich ciliate that is sessile in a secreted black sac-like test called a lorica. Dense clusters of protozoans, with basal portions of their loricae embedded in host coral skeleton, form the characteristic black band of SEB. SEB advances across a coral by asexually producing migratory larval stages that move ahead of the band in masses and locate a site to settle and secrete loricae. A combination of chemicals

associated with production of pseudochitinous loricae, and drilling of loricae into coral skeleton, result in skeletal erosion and tissue death (Antonius & Lipscomb 2000).

SEB resembles BBD in gross disease signs, except that SEB is not associated with a microbial mat that can be lifted from the coral surface. SEB affected corals are characterized by a black band ranging in width from less than 1 mm to 80 cm, depending on species affected. The line migrates horizontally across affected coral at a rate that may be as slow as 1 mm wk^{-1} or as fast as 1 mm d^{-1}, leaving dead skeleton in its wake. Denuded skeleton is flecked with tiny black spots composed of clusters of empty loricae, a characteristic that clearly distinguishes SEB from BBD, which leaves behind bright white skeleton (Antonius & Lipscomb 2000).

SEB is found at depths ranging from 0 to 35 m and is most common at 0.5 to 3 m. The disease does not appear to be correlated with seasonal seawater temperatures. SEB can be directly transmitted from a diseased colony to a healthy colony only via direct physical contact. Attempts to transmit the disease via seawater in enclosed aquaria were unsuccessful (Antonius & Lipscomb 2000).

Yellow Band

Yellow band disease (YBD) was first observed in the Gulf of Oman, Arabian Sea in the late 1990s. The disease affects 12 Indo-Pacific scleractinian coral species (Table 2.3; Korrûbel & Riegl 1998, Riegl 2002). YBD is not the same disease as Caribbean yellow blotch/band disease (YBL).

YBD is characterized by a broad band of yellow-pigmented tissue (Korrûbel & Riegl 1998) and a yellow-pigmented microbial mat (B. Riegl pers. comm.) that migrate horizontally across the coral, producing a margin of decaying tissue adjacent to healthy tissue and leaving

behind dead skeleton that often retains a yellow pigmentation (Korrûbel & Riegl 1998). The presence of a microbial mat suggests that YBD may be a variant of BBD (B. Riegl pers. comm.). Rate of tissue loss is correlated with seasonally elevated temperature and is greater in summer (19.7 mm wk^{-1}) than in winter (9.4 mm wk^{-1}, Table 2.5,; Riegl 2002). YBD is transmissible from colony to colony (Riegl 2002).

Pink-Line Syndrome

Pink-line syndrome (PLS) was first reported in 2001 affecting the scleractinian corals *Porites compressa* and *P. lutea* of Kavaratti Island, Indian Ocean (Table 2.3). PLS is characterized by a band of pink-pigmented tissue separating dead skeleton from apparently healthy tissue. This band may begin as a small ring and progress outward horizontally across a coral colony. As with other coral diseases, the zone of dead skeleton is bright white in appearance, indicating a relatively rapid rate of disease progression (Ravindran et al. 2001). PLS is associated with the cyanobacterium *Phormidium valderianum,* which induces pink coloration of coral tissue, hypothesized by increasing levels of pCO_2 (Ravindran & Raghukumar 2002).

Fungal-Protozoan Syndrome

Epizootics affecting 6 gorgonian species, 1 scleractinian species (Table 2.3), 1 zoanthid (*Parazoanthus axinellae*), sponges, and encrusting coralline algae occurred in the Liguarian Sea, north-western Mediterranean, in late summer 1999. Losses of gorgonian corals were estimated to be in the millions (Cerrano et al. 2000).

Gorgonian corals affected by the syndrome, here termed fungal-protozoan syndrome (FPS), are characterized by an increase in mucus production, loss of pigmentation, and loss of

coenenchyme tissue. Spicules in the coenenchyme lose the thin outer layer of epidermal cells and become disorganized. Coenenchyme tissues are colonized by fungi and coral polyps are colonized and consumed by protozoan ciliates. Fungi associated with FPS were most commonly of the genus *Trichoderma,* but *Clodosporium, Penicillum,* and *Humicola* were also common (Table 2.4; Cerrano et al. 2000).

Fungi and protozoa associated with FPS may be opportunistic rather than primary pathogens. FPS epizootics of 1999 were correlated with elevated seawater temperature (Table 2.5). The etiology of FPS may stem from a combination of an abiotic stressor (i.e. elevated seawater temperature) and a biotic attack by opportunistic microorganisms (Cerrano et al. 2000).

CORAL IMMUNITY

Immunity is the protection against infectious disease and can be innate or acquired (Stedman 2000). Knowledge of the immune systems of zooxanthellate corals and other invertebrates is limited; however, these organisms are known to possess highly efficient defense mechanisms against infection. In order to fully understand coral immunity, it is helpful to first briefly review the immune mechanisms of invertebrates in general.

Invertebrates are limited to innate immunity (Roch 1999), defined as a non-specific general ability of certain cells to resist most pathogens (Stedman 2000). Vertebrates, on the other hand, utilize both innate immunity and acquired immunity (Roch 1999, Stedman 2000), a specific and highly sophisticated mechanism for developing resistance to individual pathogens (Stedman 2000). Components of the acquired immune system, including antibodies, antigen presenting cells, interactive lymphocytes, and lymphoid organs are absent in invertebrates (Sinderman 1990).

Invertebrate and vertebrate immune responses are similar in that both types of organisms employ physiochemical barriers, cellular defenses, and humoral defenses against pathogens (Roch 1999). Physiochemical barriers serve as the first line of defense against invaders and include epidermis, mucus, cuticles, tests, shells, and gut barriers (Roitt et al. 1996, Peters 1997). Cellular (whole cell) defenses depend on the ability of the organism to distinguish non-self from self, and in invertebrates include: (1) coagulation and wound healing, (2) hemocytosis, (3) phagocytosis, (4) encapsulation, and (5) immunological memory (Kinne 1980, Sinderman 1990, Roitt et al. 1996). Invertebrates employ both natural and inducible humoral (cell product) defenses (Sinderman 1990, Roitt et al. 1996) that provide the lytic properties of phagocytic cells and hemolymph (blood, Sinderman 1990).

There is evidence for vertebrate defense mechanisms in invertebrates including cytokines, complement, and immunological memory (Roitt et al. 1996). Invertebrate cytokine-like molecules regulate invertebrate host defenses by activating phagocytosis and encapsulation. Invertebrates known to produce cytokine-like molecules include protozoa, annelids, echinoderms, tunicates, and arthropods (Roitt et al. 1996). Complement participates in control of inflammation, bacterial lysis, microbial killing, and phagocytosis (Roitt et al. 1996). Phagocytosis is enhanced when complement molecules, termed opsonins, bind to invading microorganisms or other foreign material (Bayne 1990, Roitt et al. 1996). Opsonin-dependent phagocytosis is found in a number of invertebrates including echinoderms, crustaceans, mollusks, annelids, and insects (Bayne 1990, Roitt et al. 1996). Immunological memory is a mechanism by which a host can distinguish non-self from self and resist infection by previously encountered pathogenic microorganisms (Sinderman 1990). Memory has been observed in scleractinian and gorgonian corals (Theodor 1970, Hildemann et al. 1975, 1977a, 1980a, Raison

et al. 1976, Johnston et al. 1981, Bak & Criens 1982, Neigel & Avise 1983), sponges (Hildemann et al. 1979, 1980b, Hildemann & Linthicum 1981, Bigger et al. 1982, 1983, Johnston & Hildemann 1983), echinoderms (Karp & Hildemann 1976, Hildemann et al. 1979), mollusks, crustaceans (Anderson 1986, Sinderman 1990), tunicates (Lakshma Reddy et al. 1975, Raftos et al. 1987), nemerteans (Langlet & Bierne 1982), and annelids (Hildemann et al. 1979).

Coral Physiochemical Barriers

Scleractinian and gorgonian corals utilize mucus production and a protective epidermis as physiochemical barriers (Peters 1997, Santavy & Peters 1997, Hayes & Goreau 1998). Sloughing of mucus is an important defense mechanism of corals against attachment of potentially pathogenic bacteria to surface tissues (Ducklow & Mitchell 1979a, Rublee et al. 1980). However, there are examples of mucus aiding in attachment of pathogenic microorganisms to the surface of corals. Adhesion of *Vibrio shiloi*, the pathogen that causes VSB, to the coral surface requires a β–galactoside-containing receptor in the mucus (Toren et al. 1998). Further, Lipp et al. (2002) demonstrated that potentially pathogenic enteric bacteria and viruses are concentrated on the surface mucus layers of scleractinian corals under natural near-shore conditions in the Florida Keys.

Coral Cellular Defenses

Cellular defenses depend on the ability of the coral to distinguish non-self (e.g. sediment, pathogens) from self. Cellular defenses documented for scleractinian and gorgonian corals include: (1) wound healing (Bigger & Hildemann 1982, Meszaros & Bigger 1999), (2) phagocytosis (Bayne 1990, Sinderman 1990, Peters 1997), and (3) immunological memory

(Theodor 1970, Hildemann et al. 1975, 1977a, 1980a, Raison et al. 1976, Johnston et al. 1981, Bak & Criens 1982, Neigel & Avise 1983).

Wound healing involves the infiltration of granular amoebocytes to immobilize invading microorganisms (Bigger & Hildemann 1982, Sinderman 1990). Coelenterate invertebrates, including scleractinian and gorgonian corals, have the ability to repair wounds, i.e. regenerate lost tissue (Patterson & Landolt 1979, Meszaros & Bigger 1999). Wound healing in coelenterates is carried out by mobile phagocytic cells, termed amoebocytes, that migrate from uninjured tissue, accumulate at the site of injury, and arrange into interconnected cell cords that form the healing front (Patterson & Landolt 1979, Meszaros & Bigger 1999). Amoebocytes also function to recognize, engulf, and destroy microbial invaders through a process termed phagocytosis (Bigger & Hildemann 1982, Bayne 1990, Sinderman 1990, Meszaros & Bigger 1999). For corals and other tropical marine invertebrates, phagocytosis is the principal cellular defense (Sinderman 1990, Peters 1997).

Immunological memory in corals is a component of allogenic recognition, the ability to distinguish self from non-self in tissue grafts. Corals accept isogenic (intracolony) grafts through complete fusion of tissue and skeleton (Hildemann et al. 1975, 1977a, Raison et al. 1976, Bak & Criens 1982, Neigel & Avise 1983, Jokiel & Bigger 1994), but reject allogenic (intraspecies) and xenogenic (interspecies) grafts through cytotoxic interaction and necrosis (Theodor 1970, Hildemann et al. 1975, 1977a, b, 1980a, b, Raison et al. 1976, Johnston et al. 1981, Jokiel & Bigger 1994). This aggression is accelerated for repeat grafts, indicating the presence of specific induced alloimmune memory (Theodor 1970, Raison et al. 1976, Hildemann et al. 1977a, 1980a, b, Johnston et al. 1981), an immune response generally reserved for vertebrates. However, in contrast to long-term vertebrate memory, coral alloimmune memory is short-term, lasting for a

period of 2 to 4r wk (Raison et al. 1976, Hildemann et al. 1977a, 1980a, b). Cytotoxic reactions to both primary and secondary allografts are accelerated under conditions of seasonally elevated seawater temperatures (27ºC, Bigger & Hildemann 1982, Johnston et al. 1981), suggesting that decreased temperatures (21ºC) may result in immunosuppression (Johnston et al. 1981), reducing a coral's ability to defend itself against pathogenic microorganisms.

Corals probably evolved mechanisms for cytotoxic attack due to the constant threat of overgrowth by adjacent benthic organisms. Scleractinian corals compete for space on the reef by utilizing a variety of defense mechanisms including: (1) rapid growth rate to overgrow neighbors (Lang 1973, Porter 1974, Maguire & Porter 1977), (2) extrusion of mesenterial filaments to attack neighboring species, killing coral tissue via extracoelenteric digestion (Lang 1973, Porter 1974), and (3) allelochemical attack, resulting in tissue necrosis (Theodor 1970, Hildemann et al. 1975, Raison et al. 1976, Hildemann et al. 1977a, b, 1980a, b, Johnston et al. 1981).

Corals that grow rapidly (e.g. acroporids) and therefore can overgrow and out compete neighbors for space, possess less potent mesenterial filaments and allelochemicals than do slower growing corals (Lang 1973, Porter 1974, Bak & Criens 1982). Intracolony isografts of *Acropora cervicornis* (Neigel & Avise 1983) and *A. palmata* (Bak & Criens 1982) are characterized by complete fusion of tissue and skeleton. Intercolony allografts of these 2 species result in overgrowth of tissues of either graft over recipient colony or recipient colony over graft (Bak & Criens 1982, Neigel & Avise 1983), and fusion of the skeleton is evident in *A. cervicornis* allografts (Neigel & Avise 1983). Xenografts of *A. palmata* and *A. cervicornis* are characterized by overgrowth where *A. palmata* consistently grows over *A. cervicornis* (Bak & Criens 1982). Fusion of skeleton and overgrowth of tissues of allografts and xenografts indicate that acroporid

corals utilize minimal antagonistic intraspecific and interspecific defenses (Bak & Criens 1982, Neigel & Avise 1983).

A number of cellular immune responses have been observed in scleractinian and gorgonian corals affected by coral disease. ASP-affected Caribbean gorgonian corals *Gorgonia* spp. utilize sclerite recruitment and nodule formation to sequester *Aspergillus sydowii* fungal hyphae and limit spread of infection (Smith et al. 1998). Similarly, Caribbean gorgonian corals *Pseudoplexaura* spp., with skeletal nodules caused by encapsulation of the algae *Entocladia endozoica,* produce many more sclerites than do healthy corals and these sclerites surround and encapsulate the infected skeleton (Goldberg et al. 1984). *Pseudoplexaura* spp. with algal nodules produce large numbers of granular amoebocytes when algal filaments extend beyond the skeletal lesion and invade host mesoglea. These amoebocytes coat invading algae with a layer of mesoglea-like material, and coated algae are then encapsulated by host skeleton (Goldberg et al. 1984). Indo-Pacific scleractinian corals *Porites* spp. detect invasion by endolithic fungi and respond by surrounding the site of fungal penetration within layers of repair calcium carbonate (Le Champion-Alsumard et al. 1995, Ravindran et al. 2001).

Coral Humoral Defenses

Corals employ both natural and inducible humoral defenses. Lysozyme and lysosomal enzymes are natural humoral defenses used by corals (Sinderman 1990). Lysozyme is an antimicrobial lysin found in phagocytic cells that destroys susceptible bacteria by elevating levels of lysosomal enzymes (Sinderman 1990). Lysosomal enzymes are contained within membrane-bound vesicles (lysosomes) and are granules containing bactericidal and hydrolytic substances that play a critical role in killing phagocytized microorganisms (Bayne 1990,

Sinderman 1990). Antimicrobial activity is both natural and inducible in scleractinian and gorgonian corals. Corals utilize antibiotic compounds or noxious chemicals to repel potentially pathogenic or parasitic organisms (Kim 1994, Slattery et al. 1995, 1997, Jensen et al. 1996, Koh 1997, Peters 1997, Kelman et al. 1998, Kim et al. 2000a, b).

Jensen et al. (1996) and Kelman et al. (1998) tested antibiotic activity (i.e. inhibition of bacterial growth) of secondary metabolites from gorgonian corals against marine bacteria and concluded that gorgonians lack potent broad-spectrum chemical defenses. However, gorgonians do possess species-specific chemical defenses against potentially pathogenic microorganisms (Kim 1994, Kelman et al. 1998).

Kelman et al. (1998) examined extracts from various reproductive and developmental stages of the Red Sea gorgonian *Pareythropodium fulvum fulvum* against bacteria isolated from: (1) SML of *P. fulvum fulvum*, (2) apparently healthy and necrotic tissue of *P. fulvum fulvum*, (3) reef substrates adjacent to test corals, and (4) seawater in the vicinity of test corals. *P. fulvum fulvum* possessed species-specific chemical defenses against the potentially pathogenic bacterium *Vibrio* sp. isolated from necrotic tissue as well as against bacteria isolated from reef substrates and seawater, but lacked antimicrobial activity against commensal bacteria associated with SML and healthy coral tissue (Kelman et al. 1998).

Koh (1997) measured antimicrobial activity of extracts from 100 species (44 genera, 13 families) of scleractinian corals from the Great Barrier Reef against 6 marine bacteria isolated from Australian waters and one terrestrial bacterium. All 100 species exhibited antimicrobial activity against 1 marine cyanobacterium: *Synechococcus* sp. Significant activity against the other 5 marine bacteria and the terrestrial bacterium was detected in only 6 and 11 coral species,

respectively, indicating that the majority of the corals did not employ chemical defenses against these potentially pathogenic bacterial species (Koh 1997).

Kim et al. (2000a) provide the first evidence of chemical defense mechanism against a known coral pathogen. Gorgonian corals possess inducible chemical defenses to resist infection with *Aspergillus sydowii*, the causal agent of ASP. Antifungal agents in crude extracts from *Gorgonia ventalina* and *G. flabellum* inhibit germination of *A. sydowii* spores (Kim et al. 2000a, Dube et al. 2002). Elevated summer seawater temperature (30ºC) reduces potency of *Gorgonia* crude extracts and promotes growth of *A. sydowii* (Alker et al. 2001).

Antifungal activity associated with ASP varies with health status and size class of host gorgonians and with lesion location on diseased individuals (Kim et al. 2000a, Dube et al. 2002, Kim & Harvell 2002). Increased antifungal activity is inducible in ASP-affected gorgonians with lesions at the colony center (Kim et al. 2000a) and in diseased individuals that are larger and more mature (Dube et al. 2002). Prevalence and severity of ASP is greatest on the largest size class of affected sea fans (Dube et al. 2002, Kim & Harvell 2002). Larger and/or older colonies may be more susceptible to *Aspergillus sydowii* as indicated by reduced potency of anti-fungal agents in their crude extracts (Kim & Harvell 2002). Small colonies exhibit higher anti-fungal activity than do large colonies and are more resistant to ASP (Dube et al. 2002). Antifungal activity is greater in healthy than in diseased gorgonians, and this activity is concentrated at colony edges. Greater concentrations of antifungal agents in crude extracts from healthy gorgonians indicate that antifungal activity influences resistance to infection (Kim et al. 2000a).

CONCLUSION

Eighteen coral diseases, affecting at least 152 scleractinian, gorgonian, and hydrozoan zooxanthellate species, have been described from the Caribbean and the Indo-Pacific (Tables 2.1, 2.2, & 2.3). Despite the greater species richness of the Indo-Pacific, the number of species affected by disease is proportionally much lower than in the Caribbean. Of the approximately 400 coral species in the Indo-Pacific, only 98 (25%) have been documented with 1 or more diseases, while at least 54 of the 66 (82%) Caribbean coral species are known to be susceptible to disease. Worldwide, 22 coral species are affected by 4 or more different diseases (Tables 2.2 & 2.3), and 14 of these species belong to 4 genera (*Acropora, Diploria, Colpophyllia,* and *Montastrea*) that represent the most common, frame-building coral species. The susceptibility of these species to a wide array of diseases has the potential to change the composition, structure, and function of coral reef ecosystems.

In the Caribbean, WPL (40), BBD (25), SKA (23), DSD (11), and YBL (9) affect the greatest number of coral species (Table 2.2). Diseases affecting the greatest number of Indo-Pacific coral species include BBD (45), WPL-L (37), SKA (24), SEB (24), and YBD (12; Table 2.3). Caribbean and Indo-Pacific scleractinian corals are highly susceptible to plague-like diseases, including WPL I, WPL II, WPL III, WPL-L, WBD I, and WBD II (Tables 2.2 & 2.3). It is important to note that the gross disease signs used to identify plague-like diseases in the field (i.e. coral tissue loss and exposed white skeleton, Pantos et al. 2003) may simply be indicative of coral death. Further, in the Caribbean, gross signs of WBD differ from those of WPL only in species affected (i.e. branching species). Future research will indicate whether plague-like signs on Caribbean and Indo-Pacific coral species represent a single disease condition, caused by a single pathogen (e.g. *Aurantimonas coralicida*, Richardson et al. 1998a, b,

Denner et al. 2003), or if similar disease signs documented for the plague-like diseases represent different diseases caused by a variety of pathogens.

Accumulating evidence suggests that human activity in the watershed may be causally related to coral decline. Increases in the number of both new diseases (Fig. 2.2) and species affected may be directly linked to human-induced alterations in coral reef environments both in terms of land-based sources of pollution as well as global climate change issues such as global warming (Table 2.5). Elevated seawater temperature may be associated with the etiologies of at least 9 coral conditions and is likely the most common abiotic stressor influencing disease pathogenicity (Table 2.5). Further, 5 coral disease-causing pathogens, including the BBD cyanobacteria*, Aurantimonas coralicida* (WPL II)*, Aspergillus sydowii* (ASP)*, Vibrio shiloi* (VSB), and *V. coralliilyticus* (VCB), are most virulent at seawater temperature at or above 29ºC (Kushmaro et al. 1998, Alker et al. 2001, Banin et al. 2001a, Israely et al. 2001, Ben-Haim & Rosenberg 2002, Kuta & Richardson 2002, Ben-Haim et al. 2003b, Richardson & Kuta 2003). Seawater temperature normally increases during late summer, but all current models of global climate change suggest that, on average, ocean temperatures will rise over the next century (Kleypas et al. 1999). Elevated temperature is a stressor in corals, causing thermally induced breakdown in the coral-zooxanthellae host-symbiont relationship (Porter et al. 1989, Fitt et al. 1993, Brown 1997b). Elevated temperature also promotes growth and virulence of pathogens (Kushmaro et al. 1996, 1998, Toren et al. 1998, Alker et al. 2001, Banin et al. 2001a, Israely et al. 2001, Ben-Haim & Rosenberg 2002, Kuta & Richardson 2002, Ben-Haim et al. 2003b, Richardson & Kuta 2003) and reduces immune response in host corals (Toren et al. 1998, Alker et al. 2001).

Nutrient and sediment loading may deliver potentially pathogenic organisms to the marine environment. Transport of the terrestrial fungus *Aspergillus sydowii*, the causal agent of ASP, to the marine environment may be associated with local sediment run-off from land or long-distance atmospheric transport (Smith et al. 1996, Shinn et al. 2000). Increasing evidence suggests that the health of reef organisms is affected by sewage pollution. BBD and WPD may be associated with fecal contamination of possible human origin (Frias-Lopez et al. 2002, Patterson et al. 2002). Human enteric bacteria and viruses are prevalent on coral surfaces and in nearshore, offshore, and canal waters in the Florida Keys (Lapointe et al. 1990, Paul et al. 1995a, b, 1997, Griffin et al. 1999, Lipp et al. 2002). The 1983 epizootic affecting the Caribbean long-spined sea urchin, *Diadema antillarum,* caused catastrophic reductions in urchin populations (Lessios et al. 1984). It has been suggested that the fecal bacterium *Clostridium perfringens* may have been involved in the urchin die-off (Bauer & Agerter 1987, 1994). Since 1996, populations of the sewage consuming reef sponge *Cliona delitrix* have increased by a factor of 10 on reefs in the Florida Keys (Ward-Paige & Risk 2003) while, concurrently, corals have declined by 37% (Porter et al. 2002).

Etiologies of only 5 coral diseases have been determined through fulfillment of Koch's postulates, but several other disease conditions have been linked to specific biotic organisms (Table 2.4). The application of Koch's postulates for the identification of coral disease has severe drawbacks, and in order for the study of coral disease etiology to advance, alternative techniques for identifying disease-causing pathogens and abiotic stressors must be accepted and implemented. Knowledge of coral disease reservoirs, transmission, pathogenesis, and epizootiology is limited, and significant advances remain to be made in the field of coral immunology.

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Fig. 2.1. *Acropora palmata.* Population decline (% cover) by 2002 on each of 7 reefs surveyed in the Florida Keys National Marine Sanctuary: (square) Western Sambo Reef: 91%; (circle) Sand Key Reef: 100%; (upward triangle) Molasses Reef: 79%; (diamond) Carysfort Reef: 89%; (oval) Rock Key Reef: 58%; (rectangle) Grecian Rocks Reef: 95%; (downward triangle) Sombrero Reef: 100%. Data in graph are mean ± SD

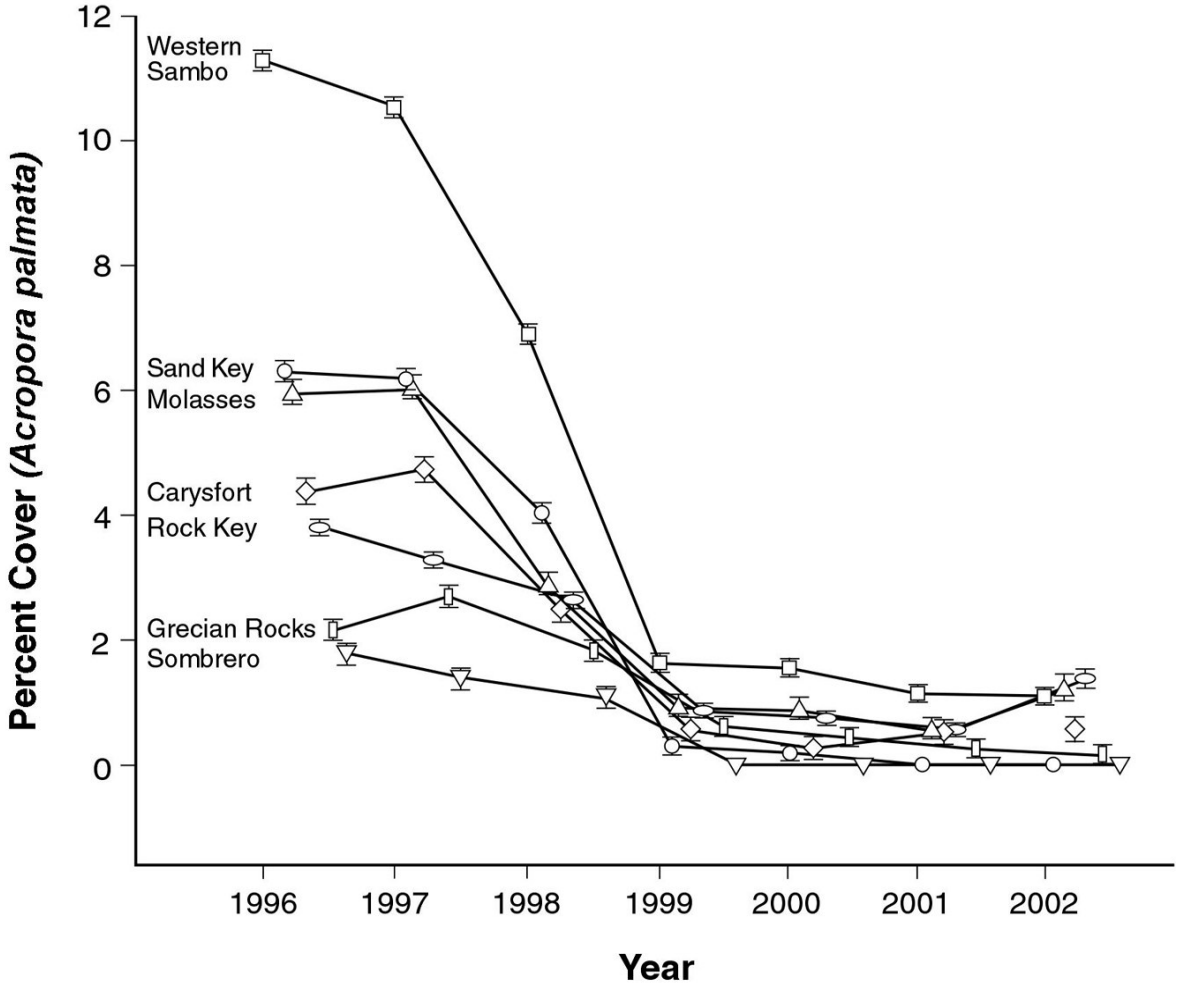
Percent Cover (Acropora palmata)
12
10
8
6
4
2
0
Western
Sambo
Sand Key
Molasses
Carysfort
Rock Key
Grecian Rocks
Sombrero
1996
1997
1998
1999
2000
2001
2002
Year

Table 2.1. First report of coral diseases in the Caribbean and the Indo-Pacific.

Disease	**Abbreviation**	**Year**	**Source**
skeletal anomalies	SKA	1965	Squires (1965)
black band	BBD	1973	Antonius (1973)
white plague type I	WPL I	1977	Dustan (1977)
shut-down reaction	SDR	1977	Antonius (1977)
white band type I	WBD I	1982	Gladfelter (1982)
aspergillosis	ASP	1996	Smith et al. (1996)
white pox	WPD	1996	Holden (1996)
Vibrio shiloi-induced bleaching	VSB	1996	Kushmaro et al. (1996)
yellow blotch/band	YBL	1997	Santavy & Peters (1997)
white plague type II	WPL II	1998	Richardson et al. (1998a)
white band type II	WBD II	1998	Ritchie & Smith (1998)
yellow band	YBD	1998	Korrûbel & Riegl (1998)
dark spots	DSD	1998	Goreau et al. (1998)
skeleton eroding band	SEB	2000	Antonius & Lipscomb (2000)
fungal-protozoan syndrome	FPS	2000	Cerrano et al. (2000)
white plague type III	WPL III	2001	Richardson et al. (2001)
pink-line syndrome	PLS	2001	Ravindran et al. (2001)
Vibrio coralliilyticus-induced bleaching and disease	VCB	2002	Ben-Haim & Rosenberg (2002)

Fig. 2.2. Exponential increase in the number of described coral diseases since the first report of disease in 1965. Although increased awareness and increased observational time might explain some of this increase, many diseases, such as white plague, are so common and so distinctive that their first description may reasonably be assumed to mark their first appearance as a phenomenon influencing the population dynamics of corals

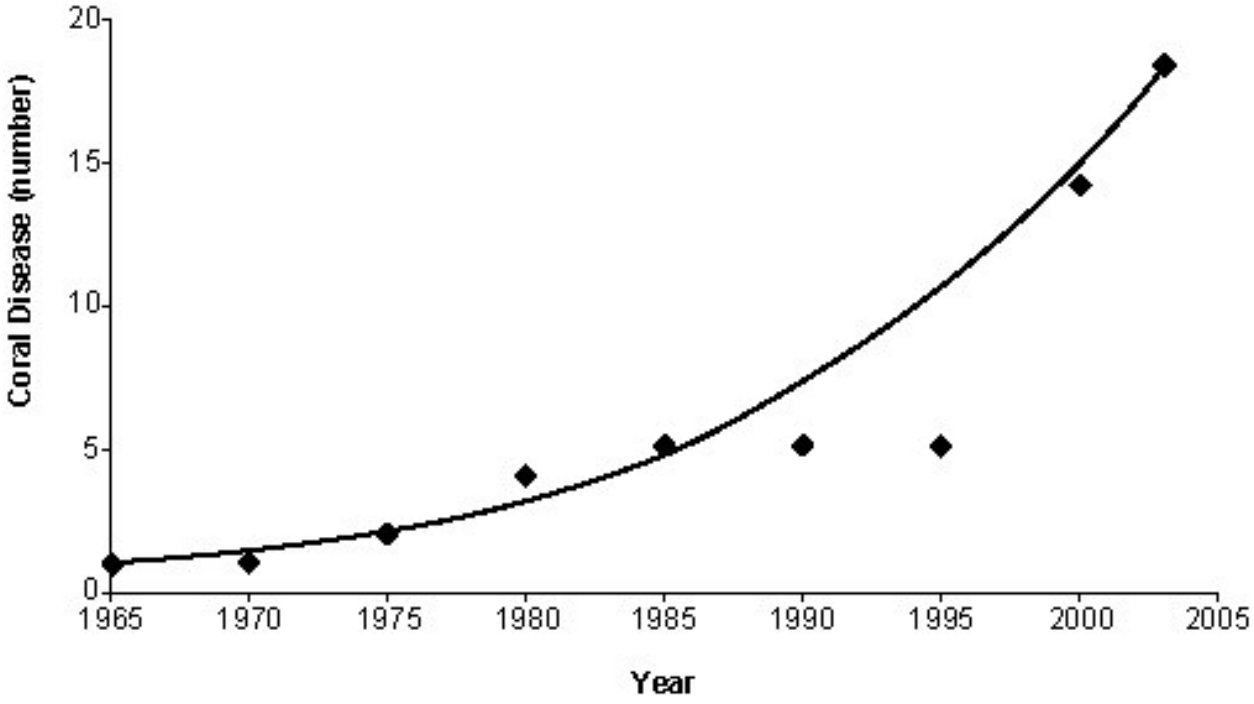
Coral Disease (number)
20
15
10
5
0
1965
1970
1975
1980
1985
1990
1995
2000
2005
Year

Table 2.2. Caribbean scleractinian, hydrozoan, and gorgonian coral species affected by diseases (total number of reported species affected by each disease and total number of reported diseases affecting each species). For WPL and WBD: I = Type I, II = Type II, III = Type III, X = type not determined. See Table 1 for disease definitions.

	BBD	WPL	SDR	SKA	ASP	WBD	WPD	YBL	DSD	No. of diseases
Scleractinians										
Agaricia agaricites		I, II						X	X	4
Agaricia fragilis		X								1
Agaricia lamarcki		II		X						2
Agaricia tenuifolia		X								1
Acropora cervicornis			X	X		I,II				4
Acropora palmata	X		X	X		I	X			5
Colpophyllia natans	X	I,II,III		X				X	X	7
Cladocora arbuscula		X								1
Dendrogyra cylindrus		II								1
Dichocoenia stokesi	X	II	X	X						4
Diploria clivosa	X	X								2
Diploria labyrinthiformis	X	I,II		X				X	X	6
Diploria strigosa	X	II		X				X		4
Eusmilia fastigiata		II								1
Favia fragum	X	X		X				X		4
Isophyllastrea rigida		I							X	2
Isophyllia sinuosa		X								1
Leptoseris cucullata		X								1
Madracis decactis	X	II								2
Madracis formosa				X						1
Madracis mirabilis	X	II								2
Manicina areolata		II		X						2
Meandrina meandrites	X	II							X	3
Meandrina labyrinthica				X						1
Montastraea annularis	X	I,II,III	X	X				X	X	8
Montastraea cavernosa	X	I,II		X					X	5
Montastraea faveolata	X	I						X	X	4
Montastraea franksi	X	X						X	X	4
Mussa angulosa		I								1
Mycetophyllia aliciae		X								1
Mycetophyllia danaana		X								1
Mycetophyllia ferox		I								1
Mycetophylia lamarkiana		I								1
Oculina diffusa		X								1
Porites astreoides	X	I		X				X		4
Porites porites		X		X						2
Scolymia cubensis		X								1
Siderastrea radians	X	X		X						3
Siderastrea siderea	X	I,II	X	X					X	6

	BBD	WPL	SDR	SKA	ASP	WBD	WPD	YBL	DSD	No. of diseases
Solenastrea bournoni		II								1
Solenastrea hyades	X	X	X							3
Stephanocoenia michelini	X	I,II							X	4
total scleractinan species	**19**	**38**	**6**	**17**	**0**	**2**	**1**	**9**	**11**	
Hydrozoans										
Millepora alcicornis		II		X						2
Millepora complanata		X								1
total hydrozoan species	**0**	**2**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	
Gorgonians										
Gorgonia flabellum	X				X					2
Gorgonia ventalina	X			X	X					3
Gorgonia sp.					X					1
Plexaura flexuosa	X									1
Plexaura homomalla	X									1
Plexaura sp.					X					1
Plexaurella sp.					X					1
Plexaurella homomalla				X						1
Plexaurella flexuosa				X						1
Pseudoplexaura spp.				X	X					2
Pseudoplexaura porosa				X						1
Pseudopterogorgia acerosa	X									1
Pseudopterogorgia americana	X				X					2
total gorgonian species	**6**	**0**	**0**	**5**	**7**	**0**	**0**	**0**	**0**	
total species number	**25**	**40**	**6**	**23**	**7**	**2**	**1**	**9**	**11**	

Table 2.3. Indo-Pacific scleractinian and gorgonian coral species affected by diseases (total number of reported species affected by each disease and total number of reported diseases affecting each species). See Table 1 for disease definitions.

	BBD	WPL-L	SKA	VSB	VCB	SEB	YBD	PLS	FPS	No. of diseases
Scleractinians										
Acropora aspera						X				1
Acropora capillaris		X								1
Acropora clathrata	X	X	X			X	X			5
Acropora cytherea	X									1
Acropora downingi	X	X				X	X			4
Acropora florida	X	X				X	X			4
Acropora formosa	X		X			X				3
Acropora gemmifera	X									1
Acropora hemprichi		X								1
Acropora humilis	X	X				X				3
Acropora hyacinthus	X	X				X				3
Acropora intermedia	X									1
Acropora microclados	X									1
Acropora microphthalma	X									1
Acropora millepora	X									1
Acropora monticulosa	X									1
Acropora nobilis	X	X	X			X				4
Acropora palifera	X	X								2
Acropora pharaonis	X	X					X			3
Acropora robusta	X									1
Acropora sarmentosa	X									1
Acropora squarrosa		X								1
Acropora tenuis						X	X			2
Acropora valenciennesi			X							1
Acropora valida		X	X			X	X			4
Acropora variabilis		X								1
Acropora virgata			X							1
Alveopora gigas	X									1
Astreopora myriophthalma	X									1
Cladocora caespitosa									X	1
Coscinarea monile		X								1
Cyphastrea chalcidicum						X				1
Cyphastrea microphthalma							X			1
Cyphastrea serailia						X				1
Echinophyllia aspera	X									1
Echinopora gemmacea		X								1
Enallopsammia rostrata			X							1
Favia favus	X	X								2
Favia matthaii	X									1
Favia pallida	X	X								2
Favia stelligera	X	X				X				3

	BBD	WPL-L	SKA	VSB	VCB	SEB	YBD	PLS	FPS	No. of diseases
Favia valenciennesii			X							1
Favites abdita						X				1
Favites pentagona	X	X								2
Goniastrea pectinata	X	X								2
Goniastrea retiformis	X	X				X				3
Goniopora columna	X									1
Goniopora somaliensis	X									1
Goniopora sp.	X									1
Hydnophora microconos	X	X				X				3
Leptastrea purpurea						X				1
Leptoria phrygia	X	X								2
Leptoseris explanata						X				1
Leptoseris glabra		X								1
Leptoseris mycetoseroides		X								1
Lobophyllia corymbosa		X								1
Madrepora kauaiensis			X							1
Madrepora oculata			X							1
Montipora aequituberculata	X	X								2
Montipora ehrenbergi		X								1
Montipora florida	X									1
Montipora foliosa			X							1
Montipora informis			X							1
Montipora monasteriata						X				1
Montipora patula			X							1
M*ontipora verrucosa*	X		X							2
Montipora sp.	X		X							2
Mycedium elephantotus		X								1
Oculina patagonica				X						1
Pavona gigantea			X							1
Pachyseris gemmae	X									1
Pachyseris rugosa						X				1
Platygyra daedalea		X								1
Platygyra lamellina	X	X								2
Platygyra pini			X							1
Platygyra sinensis			X							1
Pocillopora damicornis	X	X			X	X				4
Pocillopora eydouxi						X				1
Pocillopora verrucosa	X	X				X				3
Pocillopora meandrina			X							1
Podabacia crustacea		X								1
Porites sp.	X									1
Porites compressa			X					X		2
Porites harrisoni							X			1
Porites lichen							X			1

	BBD	WPL-L	SKA	VSB	VCB	SEB	YBD	PLS	FPS	No. of diseases
Porites lobata			X							1
Porites lutea	X	X	X				X	X		5
Porites nodifera							X			1
Pratzia mirabilis			X							1
Stylophora erthyaea			X							1
Stylophora pistillata	X	X				X				3
Symphyllia radians		X								1
Turbinaria mesenterina	X									1
Turbinaria reniformis		X					X			2
Verrillofungia concinna			X							1
total scleractinan species	**45**	**38**	**24**	**1**	**1**	**24**	**12**	**2**	**1**	
<u>Gorgonians</u>										
Corallium rubrum									X	1
Eunicella cavolini									X	1
Eunicella singularis									X	1
Eunicella verrucosa									X	1
Leptogorgia sarmentosa									X	1
Paramuricea clavata									X	1
total gorgonian species	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	
total species number	**45**	**38**	**24**	**1**	**1**	**24**	**12**	**2**	**7**	

Fig. 2.3. Caribbean coral diseases: (A) black band (BBD) on *Montastraea annularis* complex; (B) white plague Type II (WPL II) on *Dichocoenia stokesii;* (C) WPL II on *Dendrogyra cylindrus;* (D) skeletal anomaly (SKA) on *Acropora palmata;* (E) aspergillosis (ASP) on *Gorgonia* sp.; (F) white band Type I (WBD I) on *A. palmata.* Photographs by J.W.P. and C.T.)

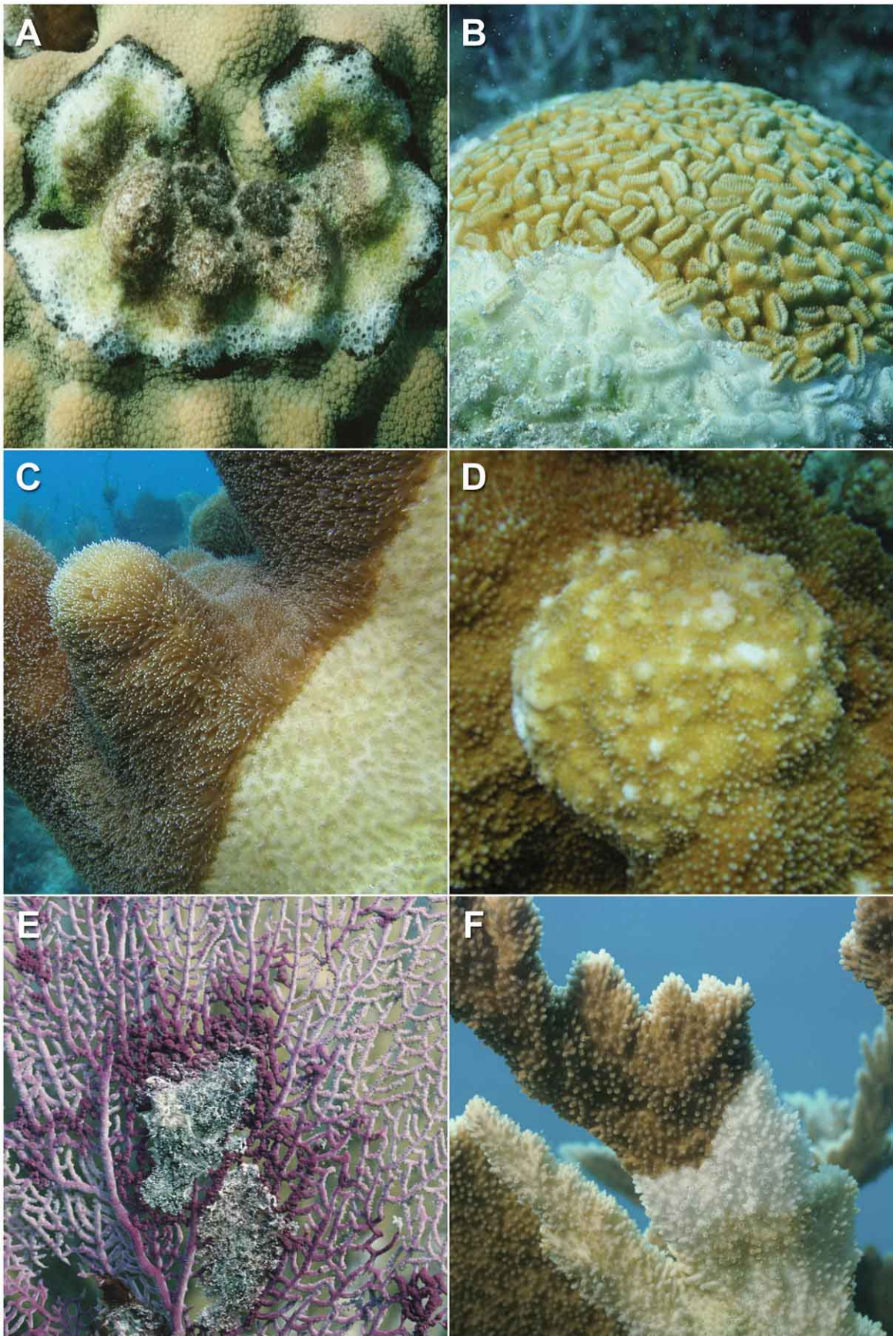
A
B
C
D
E
F

Fig. 2.4. Caribbean coral diseases: (A) white pox (WPD) on *Acropora palmata* ; (B) WPD lesions on *A. palmata* ; (C) yellow blotch/band (YBL) circular-shaped lesion and (D) YBL band-shaped lesion on *Montastraea annularis* complex; (E) dark spots (DSD) on *Stephanocoenia michelini;* (F) DSD on *Siderastrea siderea.* Photographs: (A) and (E) by J.W.P., (B) by K.P.S., (C) and (D) by C. Quirolo, (F) by J.W.P. and C.T.

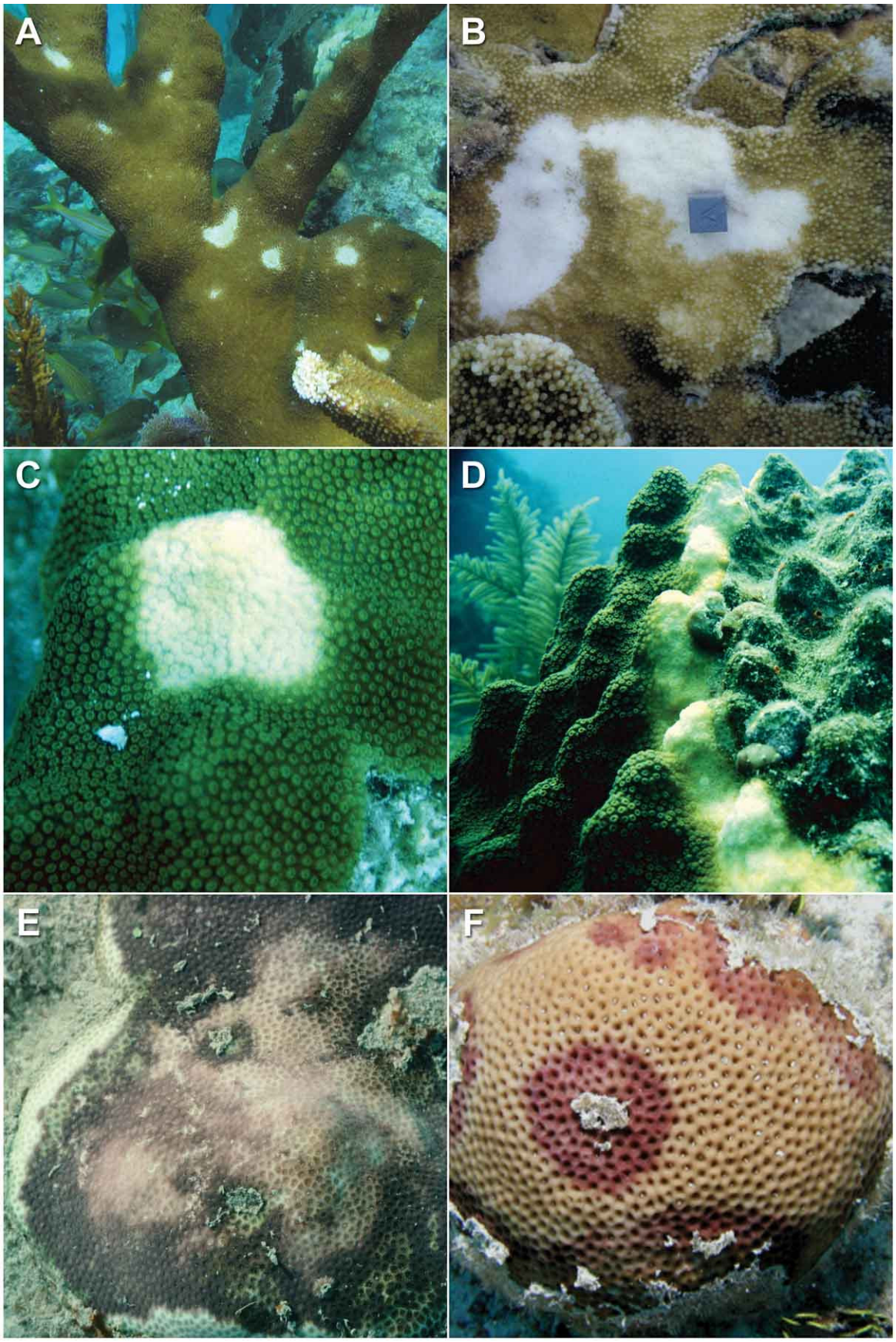
A
B
C
D
E
F

Table 2.4. Caribbean and Indo-Pacific coral diseases associated with biota. Koch's postulates have been fullfilled for only 5 diseases. See Table 1 for disease definitions.

Disease	Biota	Koch's Postulates	Source
BBD	*Phormidium corallyticum* (cyanobacterium)	no	Rützler & Santavy (1983)
	Trichodesmium spp. (cyanobacteria)		Frias-Lopez et al. (2002, 2003)
	cyanobacterium		Cooney et al. (2002), Frias-Lopez et al. (2003)
	Desulvovibrio spp. (bacteria)		Garrett & Ducklow (1975), Schnell et al. (1996), Cooney et al. (2002)
	Beggiatoa spp. (bacteria)		Ducklow & Mitchell (1979b)
	heterotrophic bacteria		Garrett & Ducklow (1975), Cooney et al. (2002), Frias-Lopez et al. (2002)
	marine fungus		Ramos-Flores (1983)
WPL II	*Aurantimonas coralicida* (bacterium)	yes	Richardson et al. (1998a, b) Denner et al. (2002)
SKA	*Petrarca madreporae* (crustacean)	no	Grygier & Cairns (1996)
	Podocotyloides stenometra (trematode)		Cheng & Wong (1974), Aeby (1998)
	endolithic fungi		Le Champion-Alsumard et al. (1995), Ravindran et al. (2001)
	Aspergillus sydowii (fungus)		Smith et al. (1998), Dube et al. (2002)
	Order Siphonales (algae)		Morse et al. (1977, 1981)
	Entocladia endozoica (algae)		Goldberg et al. (1984)
WBD II	*Vibrio charcharia* (bacterium)	no	Ritchie & Smith (1995a)
WPD	*Serratia marcescens* (bacterium)	yes	Patterson et al. (2002)
ASP	*Aspergillus sydowii* (fungus)	yes	Smith et al. (1996), Geiser et al. (1998)
VSB	*Vibrio shiloi* (bacterium)	yes	Kushmaro et al. (1996, 1997, 1998, 2001) Rosenberg et al. (1998)
VCB	*Vibrio coralliilyticus* (bacterium)	yes	Ben-Haim & Rosenberg (2002), Ben-Haim et al. (2003a, b)
SEB	*Halofolliculina corallasia* (protozoan)	no	Antonius & Lipscomb (2000)
PLS	*Phormidium valderianum* (cyanobacterium)	no	Ravindran & Raghukumar (2002)

Disease	Biota	Koch's Postulates	Source
FPS	*Trichoderma* spp. (fungi)	no	Cerrano et al. (2000)
	Clodosporium spp. (fungi)		Cerrano et al. (2000)
	Penicillum spp. (fungi)		Cerrano et al. (2000)
	Humicola spp. (fungi)		Cerrano et al. (2000)
	ciliate (protozoan)		Cerrano et al. (2000)

Table 2.5. Caribbean and Indo-Pacific coral diseases associated with abiotic stressors. See Table 1 for disease definitions.

Disease	Abiotic Stressors	Source
BBD	elevated temperature	Antonius (1981, 1985a), Rützler et al. (1983), Edmunds (1991), Carlton & Richardson (1995), Kuta & Richardson (2002), Richardson & Kuta (2003)
	eutrophication	Antonius (1981, 1985a), Kuta & Richardson (2002)
	sedimentation	Littler & Littler (1996), Bruckner et al. (1997), Frias-Lopez et al. (2002)
	pollution	Antonius (1985a), Al-Moghrabi (2001)
	fecal contamination	Frias-Lopez et al. (2002)
SDR	elevated temperature	Antonius (1977)
	sedimentation	Antonius (1977)
SKA	solar UV radiation	Peters et al. (1986), Coles & Seapy (1998)
ASP	elevated temperature	Alker et al. (2001)
	sedimentation	Smith et al. (1996), Shinn et al. (2000), Weir et al. (in press)
	poor water quality	Kim & Harvell (2002)
WPD	elevated temperature	Patterson et al. (2002)
	fecal contamination	Patterson et al. (2002)
	precipitation	Sutherland & Ritchie (in press)
DSD	elevated temperature	Gil-Agudelo & Garzón-Ferreira (2001)
VSB	elevated temperature	Kushmaro et al. (1998), Toren et al. (1998), Ben-Haim et al. (1999), Banin et al. (2001a), Israely et al. (2001)
VCB	elevated temperature	Ben-Haim & Rosenberg (2002), Ben-Haim et al. (2003b)
YBD	elevated temperature	Riegl (2002)
FPS	elevated temperature	Cerrano et al. (2000)

CHAPTER 3

THE ETIOLOGY OF WHITE POX, A LETHAL DISEASE OF THE CARIBBEAN ELKHORN CORAL, *ACROPORA PALMATA*[1]

[1]Patterson, K.L., J.W. Porter, K.B. Ritchie, S.W. Polson, E. Mueller, E.C. Peters, D.L. Santavy, and G.W. Smith. 2002. *Proceedings of the National Academy of Sciences* 99: 8725-8730.

ABSTRACT

Populations of the shallow-water Caribbean elkhorn coral, *Acropora palmata*, are being decimated by white pox disease, with losses of living cover in the Florida Keys typically in excess of 70%. The rate of tissue loss is rapid, averaging 2.5 $cm^2 \cdot day^{-1}$, and is greatest during periods of seasonally elevated temperature. In Florida, the spread of white pox fits the contagion model, with nearest neighbors most susceptible to infection. In this report, we identify a common fecal enterobacterium, *Serratia marcescens*, as the causal agent of white pox. This is the first time, to our knowledge, that a bacterial species associated with the human gut has been shown to be a marine invertebrate pathogen.

INTRODUCTION

Epizootics have been reported for several coral species (1-6) and evidence is mounting of substantial declines in the biodiversity and abundance of reef-building corals (7). The greatest losses within the Caribbean are among the branching elkhorn and staghorn corals, *Acropora palmata* and *Acropora cervicornis*, for which losses have been documented in St. Croix (8), Belize (9), Jamaica (10-12), Florida (13), and the Bahamas (14). Since the mid 1990s, observations of new coral diseases have been on the rise (1, 6). The Florida Keys National Marine Sanctuary has sustained an ecosystem wide increase in the number of coral species exhibiting disease as well as the number of reef sites with diseased corals (6). The etiologies and mechanisms of tissue death of the majority of coral diseases are not understood (2), and epidemiological data regarding the losses to coral reef communities due to these diseases are scarce. In this paper, we fulfill Koch's postulates and describe the etiology of white pox disease.

We also quantify substantial population losses suffered by white pox-affected colonies of *Acropora palmata* in the Florida Keys.

White pox disease was first documented in 1996 on Eastern Dry Rocks Reef (24^{o} 27.715' North, 81^{o} 50.801' West) off Key West, FL (15). The disease has since been observed on reefs throughout the Caribbean (6, 16-18). White pox exclusively affects the elkhorn coral, *A. palmata*, an important Caribbean shallow water species that provides elevated rates of calcium carbonate deposition (19) and the highly complex three-dimensional structure of the shallow water fore reef. Coral colonies affected by white pox disease are characterized by the presence of irregularly shaped white lesions where tissue has disappeared from the skeleton (Fig. 3.1). Lesions range in area from a few square centimeters to greater than 80 cm^2 and can develop simultaneously on all surfaces of the coral colony. The distinct white patches and the potential for tissue loss everywhere on the coral colony distinguish this disease from white-band disease (8), which develops at the base of a coral branch and progresses upward toward the branch tip in a concentric ring. Disease signs also clearly differ from coral bleaching and predation scars produced by the corallivorous snail, *Coralliophila abbreviata* (20, 21). Newly bared calcium carbonate skeleton of white pox-affected *A. palmata* is rapidly colonized by a variety of turf algae.

METHODS

Lesion Growth Measurements

White pox lesions on nine *A. palmata* colonies located at Looe Key Reef (24º 32.7' N; 81º 24.4' W) in the Florida Keys National Marine Sanctuary were photographed on August 6, 1998, and again on August 20, 1998. Photographs were stored on a Kodak CD and analyzed by

using Image-Pro Plus Software (Version 1.3, Media Cybernetics, Silvers Springs, MD). Each lesion was traced three times to obtain average measurements of area and perimeter. A square pin (1.61 cm^2), permanently positioned in the center of the lesion, was used to calibrate the tracing software (Fig. 3.1).

Inoculation Experiments

Syringe samples from the surface mucopolysaccharide layers of *A. palmata* were taken from white pox-affected and -unaffected (healthy) tissue from Florida (Looe Key Reef), the Bahamas (Gaulin's Reef, San Salvador Island 24º 2.1' N; 74º 36.1' W), the U.S. Virgin Islands (St. John 18º 21' N; 65º 15' W), and Caribbean Mexico. Surface mucopolysacharide layer samples were collected from the live tissue margins of white pox disease lesions (affected) and unaffected live tissue areas on either healthy or diseased coral colonies (unaffected). Unaffected samples were collected from healthy areas on diseased coral colonies when unaffected *A. palmata* colonies could not be located at the reef collection sites. Subsamples (0.1 and 0.01 ml) were plated onto glycerol artificial seawater media (22). Plates were incubated at 28 ºC for 24-48 h. Individual colonies (differing in colonial or cellular morphology or Gram stain) were then re-plated to pure culture. Pure cultures of each isolate (221 strains) were exposed to 95 different carbon sources on GN1 microplates (Biolog Inc., Haywood, CA) to obtain metabolic profiles (23) by using the methods as stated in Ritchie *et al.* (24). Microplates were incubated for 72 h at 28 ºC. Results were scored on a Biolog automated microplate reader (ML3 software, version 3.5). Metabolic profiles were compared among the isolates and reference strains (25). Isolates from metabolic groupings that occurred in much higher frequency (>85%) on affected tissue than on healthy tissue were selected for further analysis as potential pathogens.

Each potential pathogen was grown to a concentration of 10^9 colony-forming units/ml in glycerol artificial seawater broth. Volumes of the culture were then centrifuged (15 min, 3,500 x g, 4 °C), washed with artificial seawater, centrifuged again, and resuspended in an equal volume of artificial seawater. Two milliliters of the bacterial suspension was mixed with 0.5 g of sieved (1-mm internal diameter) calcium carbonate sediment and occasionally shaken for 3 h at 28 °C to allow the bacteria to be absorbed by the porous sediment particles. Inoculations were performed by depositing 0.5 g of the absorbed sediment directly onto the coral. *In situ* inoculations were performed near San Salvador, Bahamas, on an apparently healthy *A. palmata* colony recently fragmented by a storm. Each of four potential pathogens and a media control were inoculated in duplicates (10 inoculations total) on areas of the colony containing healthy tissue. The control inoculum consisted of sterile artificial seawater absorbed by sieved calcium carbonate. Laboratory experiments were performed using a 1/4 x 1/8-m fragment from an *A. palmata* colony collected approximately 4 m from the site of *in situ* experimentation. This apparently healthy fragment was transported to flow-through seawater tanks, allowed to acclimate for 3 days, and inoculated as described for field inoculations. After white pox disease signs developed on the experimentally inoculated corals, isolate PDL100 was reisolated from diseased tissue and characterized using the Biolog system.

Genomic DNA Extraction, Amplification, and Sequencing of 16S rRNA Genes

Before inoculation experiments, the 16S rRNA gene sequence of all likely pathogens was determined for identification purposes. Genomic DNA was isolated by growing pure cultures in liquid glycerol artificial seawater media overnight at 30 °C. Ultracentrifugation was performed on 1.5 ml of the cell suspensions for 10 min at 10,000 rpm (Eppendorf 5415C centrifuge). The

bacterial pellets were washed in 500 µl of Tris-EDTA (pH 8.0), resuspended in 360 µl of Tris-EDTA, 40µl lysozyme (40 mg/ml), and 10 µl RNase (10 mg/ml), and incubated at 37 °C for 10 min. Cell lysis was completed by the addition of 50µl of 10% SDS and DNA was purified by the addition of 100 µl of 5 M $NaClO_4$, followed by phenol/chloroform/isoamyl extraction. Chromosomal DNA was precipitated by the addition of –80 °C ethanol and spooled using a glass loop. Genomic DNA was resuspended in buffer containing Tris-EDTA plus 5 mM NaCl and subsequently used for PCR amplification. Isolates were identified by PCR sequence analysis of a 772-bp region of the 16S rRNA gene. PCR amplification was carried out selectively on genomic DNA with oligonucleotide forward primer R1n, corresponding to position 22-41 of the *Eschericia coli* 16S rRNA and reverse primer U2 corresponding to complementary position 1085-1066 (26). The conditions for PCR amplification are as follows: 10 ng of genomic DNA, 10 µl 10X reaction buffer, 1 unit *Taq* Polymerase (Roche/Boehringer), 200 nm each of the four deoxyribonucleotides (Gibco/BRL), and 20 pM each primer were combined in a total volume of 100 µl. As negative controls, reactions lacking DNA template were carried out. Reaction mixtures were overlayed with mineral oil (Sigma) and incubated in a thermal cycler (model 480, Perkin-Elmer Cetus). The cycling program was as follows: initial denaturation at 95 ºC for 5 min; 25 cycles of 94 ºC for 60 s, 40 ºC for 60 s, and 72 ºC for 60 s; a final extension step at 72 ºC for 8 min. Amplified PCR products were analyzed by Tris-borate-EDTA-agarose gel electrophoresis (27). Negative controls showed no amplification. PCR products were purified by using the Qiagen PCR purification kit (Valencia, CA) and directly sequenced by using the Applied Biosystems PRISM 377 automated sequencer (Retrogen, San Diego). Sequencing reactions were carried out by primer extension, by using the Dynamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia/Pharmacia), with oligonucleotide forward primers

corresponding to *E. coli* positions 336-358 and 515-536, and reverse primers corresponding to *E. coli* positions 930-907 and 1085-1066 (26). A GenBank BLAST search (28) was performed by using an unambiguous 779-bp consensus sequence (GenBank accession no. AF389108).

Microbiological Characterizations

Analyses of basic microbial characteristics were performed using standard methods as detailed by Smibert and Krieg (29). These analyses included, but were not limited to, assays for the following characteristics: antibiotic resistances and susceptibilities, arginine dihydrolase, caseinase, catalase, cellular morphology, citrate utilization (Simmons), colony morphology, DNase, esculin hydrolysis, fermentation of various carbon sources, growth at high NaCl concentrations, gas production from glucose, gelatinase, Gram reaction, hydrogen sulfide production from thiosulfate, indole production, lecithinase, lipase (Tween 80), lipase (egg yolk agar), lysine carboxylase, malonate utilization, motility, ornithine carboxylase, oxidase, starch hydrolysis, urease (Christensen), and Voges-Proskauer reaction. Utilization of 95 carbon sources was assayed by using the standard methods for the Biolog GN1 system (23) by using cultures grown on tryptic soy broth agar (TSBA). Analysis of cellular fatty acid methyl esters was performed by using the Microbial Identification System (MIS-TSBA, MIDI, Newark, NJ). All test results were compared with results for *Serratia marcescens* (ATCC 8100).

Scanning electron microscopy was performed on a Hitachi (Tokyo) S-3500N scanning electron microscope. Samples were prepared by fixing intact colonies of the isolate in 3.5% gluteraldehyde for 18 h. The samples were dehydrated by using a series of 30-min immersions in ethanol solutions (50, 70, 85, 95, 100, and 100%). Dehydrated samples were critical point dried (CO_2), mounted, and coated with palladium.

Coral Cover Measurements

Forty coral reef sites within the Florida Keys National Marine Sanctuary were selected by using a stratified random design (30). Seven of these reef sites contained *A. palmata*: Carysfort Reef (25° 13.205' N; 80° 12.628' W), Grecian Rocks Reef (25° 06.450' N; 80° 18.410' W), Molasses Reef (25° 00.525' N; 80° 22.589' W), Rock Key Reef (24° 27.285' N; 81° 51.589' W), Sand Key Reef (24° 27.119' N; 81° 52.650' W), Sombrero Reef (24° 37.531' N; 81° 06.624' W), and Western Sambo Reef (24° 28.771' N; 81° 42.970' W). At each site, four permanently marked stations containing three belt transects, each 0.5 m wide x 20 m long, were videotaped annually from 1996 to 2000. The video transect was filmed from a distance of 40 cm above the reef substratum. Abutting images were grabbed from this videotape to create a library of approximately 60 nonoverlapping images per transect. The percent cover for *A. palmata* and nonliving substratum (n = 12 transects per reef site) were calculated by counting 10 random points per image by using Point Count for Coral Reefs (30). Point Count for Coral Reefs retrieves video images from the CD-ROM, displays them on a computer screen, and then overlays each image with a unique set of random points.

Coral reef monitoring station data at the seven sites containing *A. palmata* was used to test the degree of dispersion for white pox disease through the use of an extra dispersion statistic (λ) (31). A dispersion value (λ) significantly greater than 1.0 implies that the spread is in a nonrandom, under-dispersed fashion. Such a clumped distribution is consistent with an epidemiological model of contagion that predicts disease spread from one diseased colony to its nearest neighbor.

Coral growth at Eastern Dry Rocks was monitored photographically on an annual basis beginning in 1994 by using the photostation survey method (13). The photostation was recorded

on color slide film in July 1994, July 1995, October 1996, June 1997, September 1997, May 1998, September 1998, August 1999, and December 2000. A total of 36 color slides, each covering 0.375 m^2, were required to survey the entire 13.5 m^2 photostation. The slides were scanned and stored in CD-ROM format. A random point count analysis was conducted for each photostation frame using Point Count for Coral Reefs (30). Seventeen random points were applied to each image. Counts were analyzed for percent live cover of *A. palmata*, percent cover of active white pox disease, and percent cover of nonliving substratum.

RESULTS AND DISCUSSION

Lesion Growth Measurements

White pox lesions enlarge along the perimeter. Photographic time series data show that white pox lesions are capable of increasing in area up to 10.5 $cm^2 \cdot day^{-1}$ with an average rate of tissue loss of 2.5 $cm^2 \cdot day^{-1}$ (± 2.7 SD; n = 36). The majority (60%) of the lesions increased in area by less than 2 $cm^2 \cdot day^{-1}$ (mean = 0.85 $cm^2 \cdot day^{-1}$ ± 0.56 SD), but this size class represents only 20% of the total tissue loss during the 15-day period (Fig. 3.2). The less numerous (9%) but largest pox lesions (8-12 $cm^2 \cdot day^{-1}$) account for 31% of all tissue loss. Intermediate-sized lesions constitute the remaining 49% of the tissue loss. Thus the fastest-growing lesions, although less common, are more important to coral tissue death than the more numerous lesions that increase in area at a slower rate. The time series data also show a high variability of the tissue loss rate between affected colonies. In a paired comparison of lesions of similar area, those lesions with higher perimeter-to-area ratios grew faster than similarly sized lesions with smaller perimeter-to-area ratios (Sign Test; $p < 0.05$; n = 23). In this respect, white pox is like other coral perimeter

diseases (white plague type II, black band), which exhibit tissue loss at the leading edge of the infection.

Inoculation Experiments and Sequencing of 16S rRNA Genes

A. palmata areas inoculated with isolate PDL100, both in the field (after 1 mo) and in aquaria (after 1 week), showed disease signs similar to white pox. Areas inoculated with the remaining strains and with the control inoculum remained healthy. Because three of the four candidate pathogens tested did not cause disease signs, these three inocula serve as bacterial negative controls. Isolate PDL100 was reisolated from diseased tissue on the experimentally inoculated corals that showed white pox disease signs (satisfying Koch's postulates). 16S rDNA sequence analyses of the bacterium (isolate PDL100) demonstrated a 100% identity to *S. marcescens*. This identification was supported by microbiological characterization and carbon source utilization patterns using the Biolog GN1 system (23).

S. marcescens (γ Proteobacteria) is ubiquitous and can be found as a fecal enteric bacterium in humans; it is also an opportunistic pathogen causing a variety of disease conditions in humans (32). This species can also be found as part of the intestinal microbiota of other animal species and as a free-living microbe in both water and soil (32). Enteric bacteria associated with human feces have recently been shown to be concentrated in the surface mucopolysaccharide layers of corals in the Florida Keys (33). However, to our knowledge, this report, establishing *S. marcescens* as the causal agent of white pox, is the first time that a bacterial species associated with the human gut has been shown to be a marine invertebrate pathogen.

Serratia species are known to cause disease in both marine and freshwater fishes (34) and to pose a serious threat as an opportunistic pathogen to marine organisms (35). *S. marcescens* has been linked to disease of white perch (*Morone americanus*) in the sewage-polluted Back River, Maryland (36). Sewage may serve either as the source of *S. marcescens* or as a stressor of fish, increasing susceptibility to disease. The *S. marcescens* strain (PDL100) isolated from white pox-affected *A. palmata* may also be associated with pollution of fecal origin. However, at present, the origin, pathogenic mechanisms, and host range of the white pox disease isolate (PDL100) are unknown and under investigation.

Microbiological Characterization

Standard microbiological testing revealed that isolate PDL100 was a Gram-negative motile rod (Fig. 3.3). The isolate was Vogues-Proskauer, lysine decarboxylase, ornithine decarboxylase, DNase, lipase, and catalase positive. Oxidase, urease, and arginine dihydrolase activities were not detected. Hydrogen sulfide was not produced from glucose, and indole was not produced. Acetate, citrate, D-fructose, D-glucose, maltose, D-sorbitol, and sucrose were used; L-arabinose, α–keto butyrate, cellobiose, α-D-lactose, malonate, propionate, D-raffinose, and L-rhamnose were not. The red pigment, prodigiosin, was not produced. Growth occurred in media with 7.5% NaCl, but not 10%. Antiobiotic resistances included cephalothin and ampicillin; sensitivities included gentamicin, pipericillin, and nalidixic acid. Gelatin, casein, lecithin, esculin, and starch were hydrolyzed. Whole cell fatty acid content as determined by GC-FAME analysis was consistent with bacteria of the family Enterobacteriaciae (MIS-TSBA, MIDI, Newark, DE). Results of GN1 Biolog testing were consistent with expected results for *S. marcescens*.

Coral Cover Measurements

White pox disease is highly contagious. The nonrandom under-dispersed (clumped) distribution and spread of the disease on Floridian coral reefs fits the nearest-neighbor contagion model well (extra dispersion value $\lambda = 1.34$; $p < 0.01$). Once white pox appeared on a reef, it spread to all four stations on that reef within 1 year. Movement between reefs was also rapid. By 1997, 1 year after the first documentation of the disease on Floridian reefs, white pox was found at all surveyed reefs in Florida that had *A. palmata* (30) (Fig. 3.4A). Signs of active white pox disease were observed on all seven of these reefs in 1997, 1998, and 1999. Between 1996 and 1999, the average loss of *A. palmata* at these reef sites was 85% (Page's Test; $p < 0.001$), approximately double the rate of loss caused by a different disease on *Dichocoenia stokesii* colonies in the Florida Keys (4, 5). The catastrophic declines of *A. palmata* documented in this study are comparable to the losses documented for this same species in St. Croix because of white-band disease (8).

Living cover of *A. palmata* at Eastern Dry Rocks Reef decreased by 82% between July 22, 1994 and September 9, 1998 (Fig. 3.4B and 3.5). This dramatic loss in *A. palmata* occurred before both Hurricane Georges (September 25, 1998) and the mass-bleaching event that occurred on reefs throughout the Florida Keys in late September 1998 (37). Hurricane or bleaching damage may have contributed to the further decline in live *A. palmata* that occurred at this site between September 1998 and December 2000. However, it is important to note that the first posthurricane/postbleaching survey (August 1999) actually showed a 3% increase in percent live cover of *A. palmata* (Fig. 3.4B).

Data for the years in which the photostation at Eastern Dry Rocks was surveyed both in early and late summer (October 1996-September 1998) demonstrate the effect of winter and

summer seawater temperatures on white pox disease progression (Fig. 3.4B). There appeared to be a correlation between month of survey and percent change in living cover of *A. palmata*. Percent living cover of *A. palmata* declined by 39% between our July 1995 survey and our first recognition of white pox in October 1996. During the winter months between October 1996 and June 1997, percent cover of *A. palmata* increased slightly. Some regrowth of coral tissue over lesions visible in 1996 was observed in the June 1997 photostation images and may account for the 1% increase in living *A. palmata*. Similar cessation of tissue loss followed by tissue regeneration over bare skeleton has been observed in several cases of white pox disease on reefs in the Florida Keys. By late summer 1997, Eastern Dry Rocks had sustained a further 37% decline in *A. palmata* cover, a 59% decrease since 1994. This pattern of reduced loss of living coral during the winter months followed by accelerated loss during the summer months repeats in the winter of 1997/1998 and the summer of 1998.

Eastern Dry Rocks Reef exhibited a striking contrast between high death rate of corals and lack of juvenile recruitment. By December 2000, *A. palmata* constituted only 0.49% of living coral cover at Eastern Dry Rocks (Fig. 3.4B and 3.5D), down from 23.9% in 1994. During the 7 years of the photostation survey, living cover of *A. palmata* decreased by 98%. Coral recruitment was not observed within the photostation during the 7-year survey.

CONCLUSION

We propose renaming white pox disease acroporid serratiosis, as this new terminology more accurately reflects the etiology of the disease. Research is continuing to determine if white pox disease signs on *A. palmata* from other regions beyond those investigated are also caused by *S. marcescens*. We are examining the metabolic characteristics of PDL100 that differ from other

environmental *Serratia* isolates and may make PDL100 pathogenic to *A. palmata*. We are also exploring the possibility that the host range of PDL100 is not exclusive to *A. palmata* but instead extends to other coral species on which disease signs are manifested differently. Because the etiologies of the majority of the coral disease conditions described to date are unknown (2), the extent of the pathogenicity of PDL100 warrants examination.

Our data demonstrate that rate of tissue loss due to white pox disease correlates with seasonal conditions of elevated temperature. Normally this occurs in late summer, but all current models of global climate change suggest that, on average, ocean temperatures will rise over the next century (38). Elevated temperature is a stress in corals, causing a thermally induced breakdown in the coral-zooxanthellae host-symbiont relationship (39), promoting accelerated growth of pathogens (40, 41), and reducing the potency of the host's immune system (42, 43).

Several coral disease organisms, including the *Phormidium corallyticum* consortium that causes black band (44, 45) and *Vibrio* AK-1, which induces bleaching in the coral *Oculina patagonica* (40), grow faster at elevated temperatures (41). Alker *et al.* (43) demonstrate a significant reduction in the potency of *Gorgonia ventalina* crude extracts against *Aspergillus sydowii* fungal infection when assayed at 30 vs. 25 ^{o}C. They speculate that this reduction at the higher temperature may be due to the inactivation of the host's antifungal compounds. Several coral bleaching events have been followed by coral disease outbreaks (46, 47). Although there is little understanding of how bleaching and mortality are causally linked, we propose that this causal linkage is due to elevated incidence of disease via opportunistic infections. It is also possible that the predicted increase in frequency and intensity of future bleaching events (48) may cause an increase in the frequency and severity of coral disease outbreaks such as white pox. One of the effects of global warming, therefore, may be to lengthen the disease season.

We have identified severe population declines for the coral community's most important primary producer and shallow water framework builder. These changes are especially important given the longevity and slow recruitment of this species. Our study demonstrates that in the beginning years of the 21st Century, disease is changing the composition, structure, and probably function of the Florida Keys coral reef ecosystem. Studies elsewhere in the Caribbean suggest that this generalization may be true for other acroporid coral reef ecosystems as well (8, 10, 11, 49). The rapid loss of acroporid corals in some locations, such as on Jamaican coral reefs (9), has been accompanied by ecological phase shifts from coral-dominated substrata to algal-dominated substrata. Hypotheses to explain the increase in algae include the loss of herbivores (both grazing fish and urchins), that is, by "top-down" controls (50, 51), and an increase in nutrients (both phosphorous and nitrogen), that is, by "bottom-up" controls (52). Although it is true that either grazer loss or nutrient increase can produce an increase in algal biomass, neither of these mechanisms addresses the role of coral disease in creating substratum for algal colonization.

A. palmata reproduce almost exclusively by fragmentation (53). While vegetative reproduction may be well adapted to recolonization following mechanical disturbances such as hurricanes, colony fragmentation is ineffectual following severe population declines due to disease, which frequently kills the entire coral colony (Fig. 3.5). Declining population numbers may also make *A. palmata* especially vulnerable to predation by the corallivorous snail, *C. abbreviata*, which preferentially feeds on this coral species (20, 21). If colonies are too rare or too far apart for high fertilization success, then *A. palmata* may be experiencing an Allee effect (53, 54), making rapid recovery of this species in the Florida Keys impossible.

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Fig. 3.1. White pox disease lesion on *A. palmata* at Looe Key Reef, FL, August 1998. The square pin (1.61 cm^2) was used to calibrate the image tracing software (photograph by K.L.P.).

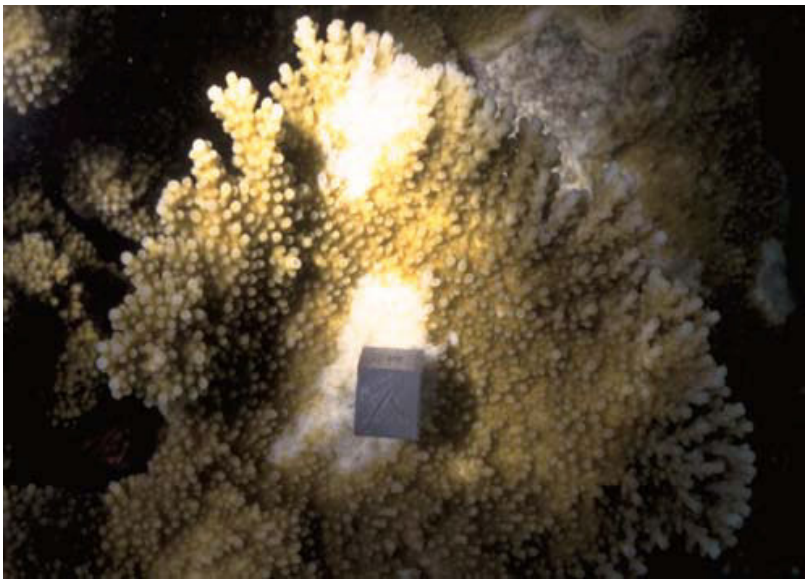


Fig 3.2. The size class distribution of white pox disease lesions and percent total tissue loss per size class on *A. palmata* colonies at Looe Key Reef, FL, after 15 days (August 6-20, 1998) *in situ*.

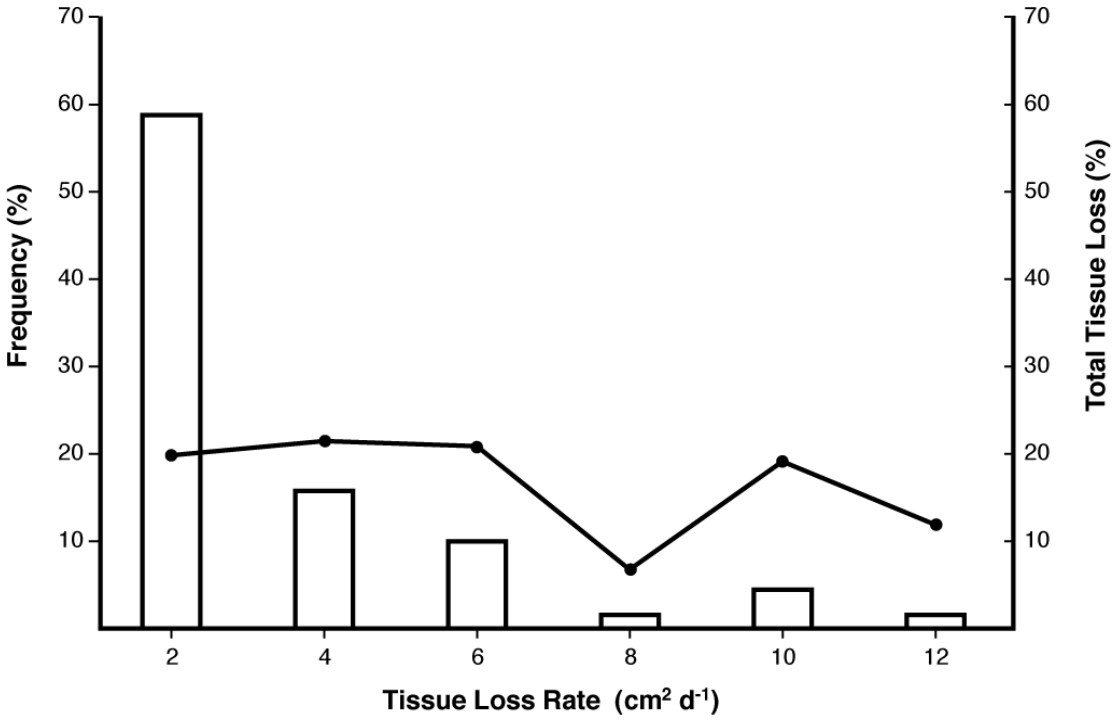

70
60
50
40
30
20
10
Frequency (%)
Total Tissue Loss (%)
2
4
6
8
10
12
Tissue Loss Rate (cm² d⁻¹)

Fig. 3.3. Scanning electron micrograph of the white pox pathogen (PDL100). The bacterium was identified as *S. marcescens* by 16S rRNA gene sequencing, carbon source utilization patterns, and standard microbiological testing (photograph by S.W.P.).

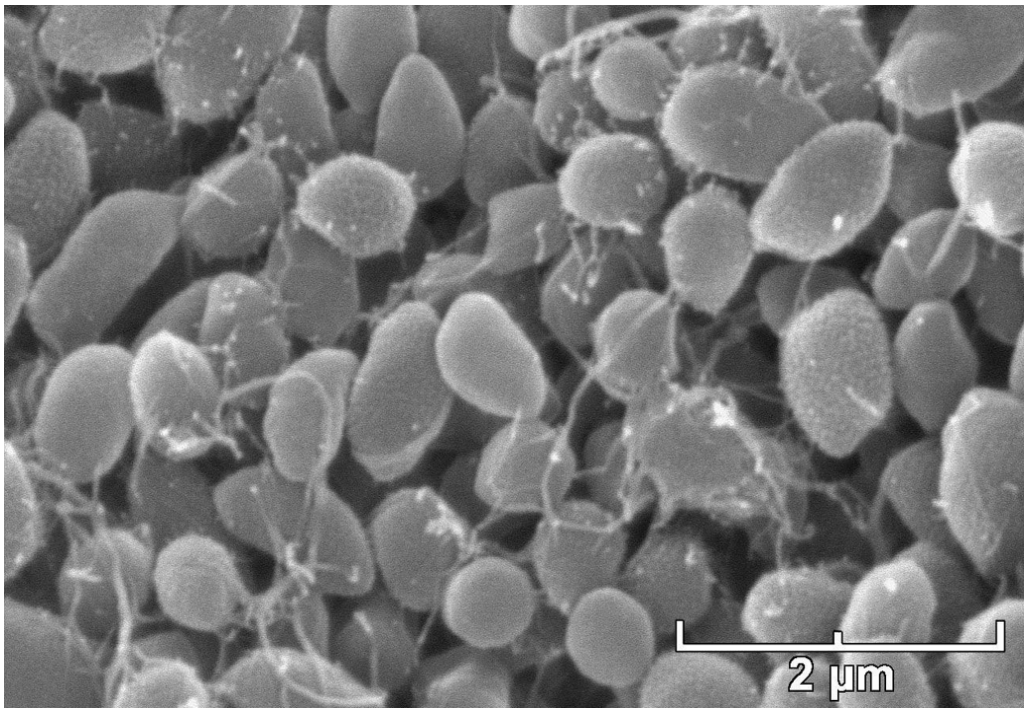
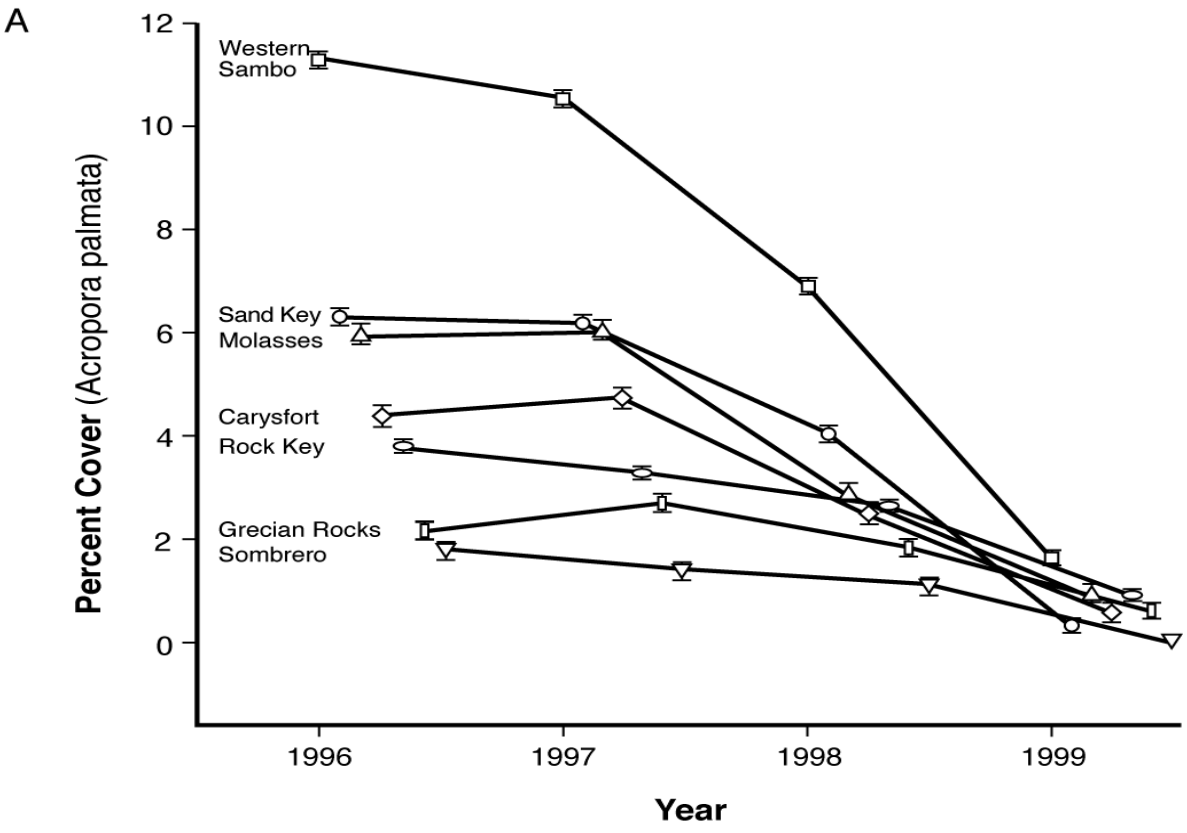
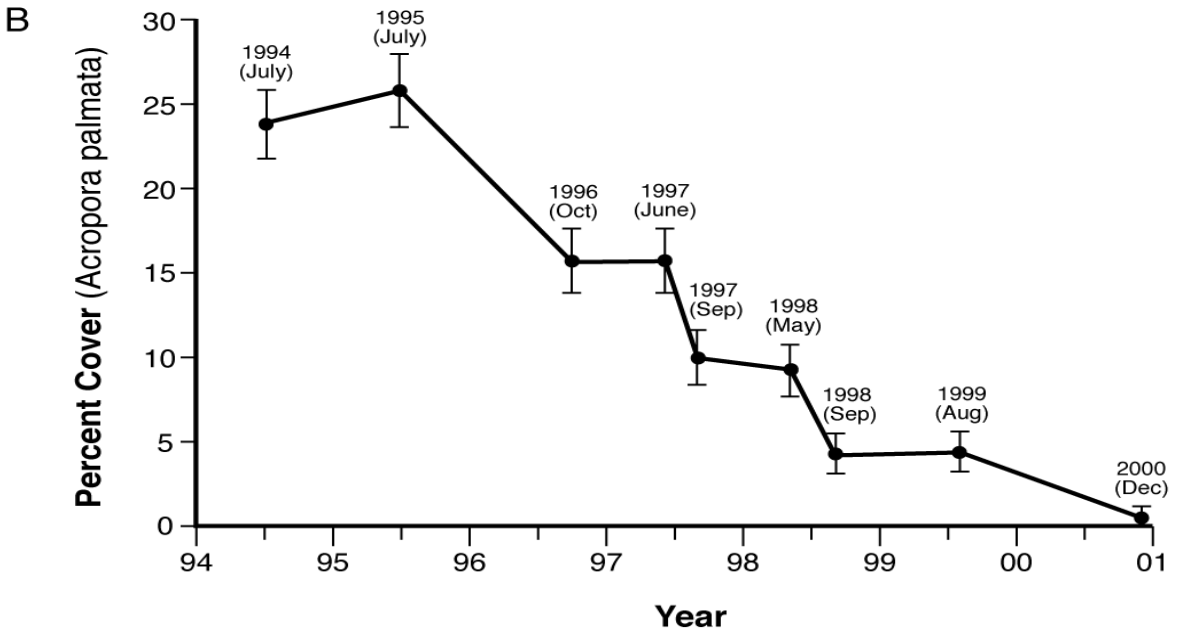

2 µm

Fig. 3.4. (A) The percent cover of *A. palmata* at seven reef sites in the Florida Keys National Marine Sanctuary, 1996-1999. By 1999, percent cover of this species had decreased at each of the sites: Carysfort Reef (diamond), 85%; Grecian Rocks Reef (rectangle), 71%; Molasses Reef (upward triangle), 84%; Rock Key Reef (oval), 77%; Sand Key Reef (circle), 95%; Sombrero Reef (downward triangle), 100%; Western Sambo Reef (square), 84%. Data are presented as mean ± SD. (B) The percent cover of *A. palmata* at Eastern Dry Rocks Reef, Key West, FL, 1994-2000. The effects of seasonal seawater temperatures on rate of tissue loss are evidenced by the stair-step pattern of the graph. Between July 1994 and December 2000, 98% of the *A. palmata* cover on this reef was lost. Data are presented as mean ± SD.


A
Percent Cover (Acropora palmata)
12
10
8
6
4
2
0
Western
Sambo
Sand Key
Molasses
Carysfort
Rock Key
Grecian Rocks
Sombrero
1996
1997
1998
1999
Year


B
Percent Cover (Acropora palmata)
30
25
20
15
10
5
0
1994
(July)
1995
(July)
1996
(Oct)
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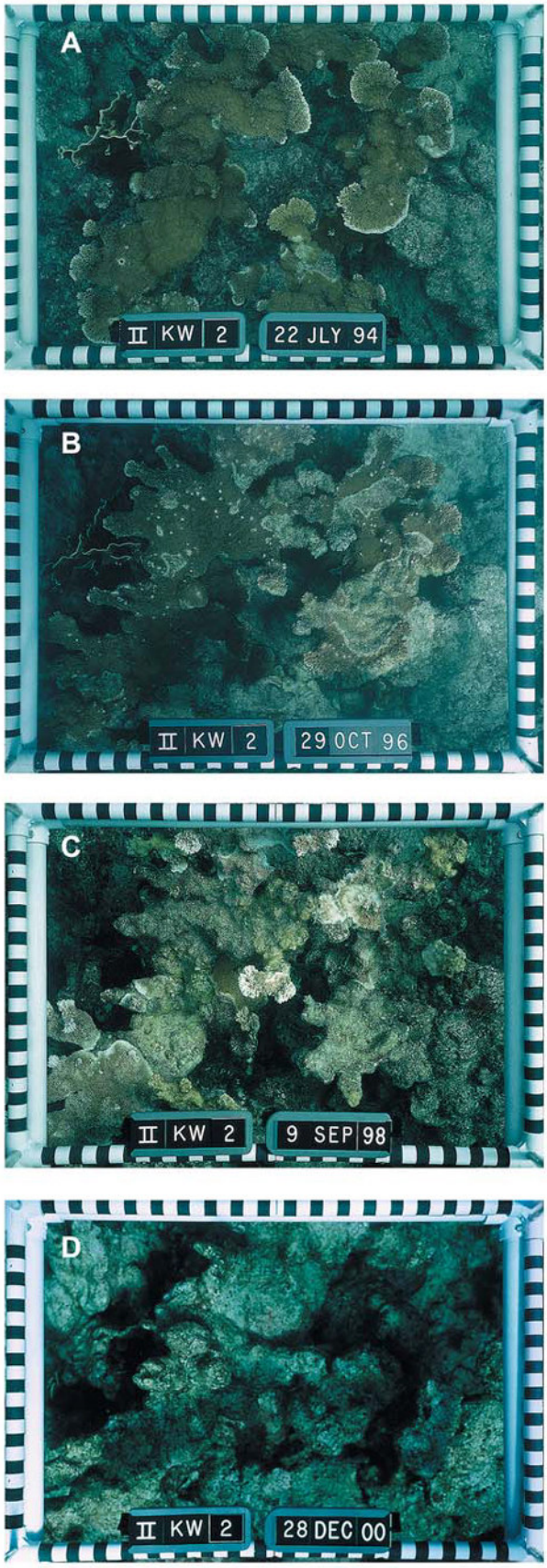
(June)
1997
(Sep)
1998
(May)
1998
(Sep)
1999
(Aug)
2000
(Dec)
94
95
96
97
98
99
00
01
Year

Fig. 3.5. Photographic time series of damage caused by white pox disease on *A. palmata* at Eastern Dry Rocks Reef, Key West, FL: (A) July 22, 1994, (B) October 29, 1996, (C) September 9, 1998, and (D) December 28, 2000. Disease signs were first recognized in 1996. By 2000, no living *A. palmata* remained within this photostation frame (photographs by J.W.P. and K.L.P.).


A
II KW 2
22 JLY 94
B
II KW 2
29 OCT 96
C
II KW 2
9 SEP 98
D
II KW 2
28 DEC 00

CHAPTER 4

WHITE POX DISEASE OF THE CARIBBEAN ELKHORN CORAL *ACROPORA PALMATA*[1]

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[1]Sutherland, K.P. and K.B. Ritchie. Accepted by Springer-Verlag Berlin Heidelberg. In: Rosenberg, E. and Y. Loya (eds.) *Coral Health and Disease*
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INTRODUCTION

Populations of the most common Caribbean reef-building coral, *Acropora palmata,* are being decimated by white pox disease, with losses of living cover in the Florida Keys National Marine Sanctuary (FKNMS) averaging 88%. Elkhorn coral plays a significant role in the structural and functional integrity of Caribbean coral reef ecosystems. *A. palmata* is an important shallow water species, providing elevated rates of calcium carbonate deposition (Adey 1978) and a highly complex three-dimensional structure of the shallow water fore reef. This keystone species provides shelter and food for reef organisms and aids in the protection of coastal regions by serving as a buffer between land and sea. Severe population declines of *A. palmata* in the FKNMS and elsewhere in the Caribbean have led to the identification of this species as a candidate for inclusion on the Endangered Species List (Diaz-Soltero 1999).

White pox disease, also termed acroporid serratiosis (Patterson et al. 2002) and patchy necrosis (Bruckner and Bruckner 1997), exclusively affects *Acropora palmata* and was first documented in 1996 on reefs off Key West, Florida (Holden 1996). White pox has since been observed throughout the Caribbean (Porter et al. 2001, Rodríquez-Martínez et al. 2001, Santavy et al. 2001, Patterson et al. 2002). White pox disease is characterized by irregularly shaped distinct white patches devoid of coral tissue (Figure 4.1). Lesions can vary from a few to greater than 80 square centimeters and can develop simultaneously on all surfaces of the coral colony. Coral tissue loss occurs radially along the perimeter of the lesion at an average rate of 2.5 cm^2 day^{-1} and is greatest during periods of seasonally elevated temperature. In heavily affected *A. palmata,* lesions can merge, resulting in tissue loss that spans the entire colony. White pox is highly contagious with nearest neighbors most susceptible to infection (Patterson et al. 2002).

The disease spreads rapidly within and between reefs in the FKNMS during the mid-1990s (Porter et al. 2001, Patterson et al. 2002).

The distinct white patches and the potential for tissue loss throughout the coral colony distinguishes white pox disease from white band disease, which also affects *Acropora palmata,* developing at the base of a coral branch and progressing upward toward the branch tip in a concentric ring. White band disease is characterized by a distinct white band of recently denuded coral skeleton (type I; Gladfelter 1982) or bleached tissue (type II; Ritchie and Smith, 1998) followed by a line of necrosis. White pox disease signs also clearly differ from coral bleaching and predation scars produced by the corallivorous snail, *Coralliophila abbreviata* (Knowlton et al 1990; Miller 2001).

THE CORAL PATHOGEN, *SERRATIA MARCESCENS*

White pox disease is caused by *Serratia marcescens* (Patterson et al. 2002), a common Gram negative bacterium classified as a coliform and a member of the Enterobacteriaceae family. It is common in feces of humans and other animals as well as other types of environments (Grimont and Grimont 1994). *S. marcescens* is an opportunistic pathogen of humans associated with both waterborne infections in tropical waters (Hazen 1988) and hospital-acquired infections, including urinary tract infections, wound infections, pneumonia, and bacteremia (Grimont and Grimont 1994, Miranda et al. 1996, Shi et al. 1997).

The pathogenic mechanism of *Serratia marcescens* for elkhorn coral are currently unknown, thus our best available model for pathogenesis is derived from human clinical studies. *S. marcescens* is known to produce a number of virulence factors including serralysin (a peptidase), proteases, chitinases, lipases and nucleases (Hertle 2002). *S. marcescens* cell wall

includes a lipopolysaccharide (LPS) that is responsible for endotoxin production (Hejazi and Falkiner 1997). A hemophore secreted by *S. marcescens* was shown to be cytotoxic to cells other than red blood cells (Arnoux et al. 1999, Marty et al. 2002). Polson (2002, MS Clemson U.) showed that the white pox isolate also produces lipases and nucleases, but their role in pathogenesis is presently unknown. There is an inverse relationship between pigment production (prodigiosin) and toxicity of *S. marcescens* strains against nematodes (Carbonelli 2000). Likewise, human clinical isolates are rarely pigmented (Hejazi and Falkiner 1997). The white pox isolate is non-pigmented and, therefore, does not produce prodigiosin, but the role of the pigment in pathogenesis is also unclear. Hejazi and Falkiner (1997) described adherence and pathogenicity differences between *S. marcescens* strains exhibiting mannose-resistant and mannose-sensitive surface pili. Mannose sensitive pili are important in bacterial colonization. In *Vibrio cholerae* MSHA mediates the adherence to commensal zooplankton carapaces (Chiavelli et al. 2001).

Quorum sensing is a signaling process by which a number of marine (and other) bacteria turn on genes (including those for virulence factors) as a result of the production of the signal compound by other bacteria. Most known quorum sensing systems involve the secretion of N-acyl-homoserine lactone or similar lactones and/or quinolones. Swarming and the production of protease (behaviors found in other quorum sensors) have been reported for *Serratia* species (Lindum et al. 1998; Givskov et al. 1997).

The prevalence of *Serratia marcescens* in the marine environment is unknown. However, this bacterium has been found in the marine environment in sewage–polluted estuaries. For example, *S. marcescens* has been linked to disease of white perch (*Morone americanus*) in the sewage-polluted Back River, Maryland (Baya et al. 1992). Other Serratia species are known

to cause disease in marine fishes (Austin and Austin 1999) and to pose a serious threat as opportunistic pathogens of marine organisms (Ingles et al. 1993).

White pox is one of 18 coral diseases documented worldwide, but *Serratia marcescens* is only the fifth pathogen to be confirmed as a coral disease agent through the fulfillment of Koch's postulates (Kushmaro et al. 1996; Smith et al. 1996; Richardson et al. 1998a; Richardson et al. 1998b; Ben-Haim and Rosenberg 2002; Patterson et al. 2002) and the first agent with a possible link to human sewage pollution (Patterson et al. 2002). Identification of *S. marcescens* as a coral pathogen marked the first time that a common member of the human gut microbiota was shown to be a marine invertebrate pathogen. While *S. marcescens* is ubiquitous, its noted association with human hosts prompts speculation that improperly treated sewage from the Florida Keys may be to blame for white pox disease. Human sewage markers (e.g. human enteric bacteria and viruses) are prevalent on coral surfaces and in nearshore, offshore, and canal waters of the FKNMS (Paul et al. 1995a, Paul et al. 1995b, Paul et al. 1997, Griffin et al. 1999, Lipp et al. 2002), suggesting land-based activities may affect reef coral health and coral reef survival.

LOSS OF *ACROPORA PALMATA* IN THE FKNMS: 1994-2002

Annual monitoring of Eastern Dry Rocks Reef (24º 27.715' N, 81º 50.801' W) in the FKNMS began in 1994 (Patterson et al. 2002). Seven additional reef sites in the FKNMS have been monitored on an annual basis since 1996: Carysfort Reef (25° 13.205' N; 80° 12.628' W), Grecian Rocks Reef (25° 06.450' N; 80° 18.410' W), Molasses Reef (25° 00.525' N; 80° 22.589' W), Rock Key Reef (24° 27.285' N; 81° 51.589' W), Sand Key Reef (24° 27.119' N; 81° 52.650' W), Sombrero Reef (24° 37.531' N; 81° 06.624' W), and Western Sambo Reef (24° 28.771' N; 81° 42.970' W) (Patterson et al. 2002, Porter et al. 2002).

Between 1994 and 2002, percent cover of *Acropora palmata* at Eastern Dry Rocks declined by 97%, from 23.9% in 1994 to 0.82% in 2002 (Figure 4.2). Between 1996 and 2001, percent cover of *A. palmata* dramatically declined at each of the seven additional reef sites: Carysfort, 90%; Grecian Rocks, 89%; Molasses, 90%; Rock Key, 79%; Sand Key, 100%; Sombrero, 100%; Western Sambo, 90% (Figure 4.3). The complete loss of *A. palmata* at Sombrero Reef and Sand Key occurred by 1999 and 2001, respectively. Loss of *A. palmata* at all eight surveyed reefs in the FKNMS averaged 92% between 1996 and 2001 (Figure 4.3). Between 2001 and 2002, percent cover of *A. palmata* increased at Carysfort, Molasses, and Rock Key and continued to decline at Grecian Rocks and Western Sambo. The continued loss was especially pronounced at Grecian Rocks where an additional 53% of *A. palmata* cover was lost, with a total loss at this reef of 95% between 1996 and 2002 (Figure 4.3).

Populations of *Acropora palmata* in the FKNMS sustained losses averaging 88% between 1996 and 2002. These losses are approximately double the loss caused by white plague type II disease on *Dichocoenia stokesii* colonies in the FKNMS (Richardson et al. 1998a, Richardson et al. 1998b) and are comparable to *A. palmata* losses at other locations throughout the Caribbean (Bythell and Sheppard 1993, Aronson and Precht 2001, Miller et al. 2002). While population declines elsewhere in the Caribbean were attributed to hurricanes, bleaching, and white band disease (Gladfelter 1982, Porter and Meier 1992, Bythell and Sheppard 1993, Aronson and Precht 2001, Miller 2002), losses in the FKNMS were primarily caused by white pox disease. By 1997, one year after the first documentation of the disease (Holden 1996), white pox was found at all surveyed reefs in the FKNMS (Patterson et al. 2002, Porter et al. 2002). Signs of active white pox disease were observed on *A. palmata* colonies at Eastern Dry Rocks every year between 1996 and 2000 (Patterson et al. 2002) and at each of the other reefs with living cover of

A. palmata every year between 1997 and 2002. One exception was Carysfort Reef, where white pox disease signs were not observed at the time of the 2000 survey. Observations of white band disease, on the other hand, were rare at monitored reefs in the FKNMS between 1996 and 2002.

In late September 1998, reefs in the FKNMS were impacted by both Hurricane Georges and mass-bleaching (Wilkinson et al. 1999). These events may have contributed to the coral decline observed between 1998 and 1999 (Figure 4.3), though the 82% loss of *A. palmata* recorded at Eastern Dry Rocks between 1994 and 1998 (Figure 4.2) occurred prior to both the hurricane and the bleaching event. Hurricane or bleaching damage may have contributed to the further decline of *A. palmata* on this reef after 1998. However, it is important to note that the first post-hurricane/post-bleaching survey (August 1999) showed a 3% increase in percent live cover of *A. palmata* (Figure 4.2).

Between 2000 and 2001, living cover of *Acropora palmata* at Eastern Dry Rocks increased 66.7% from 0.49% to 0.82% (Figure 4.2). This increase in living cover was due to growth of remaining fragments of living tissue (Figure 4.4A and 4B). Coral recruitment was not observed at Eastern Dry Rocks at any time during the nine-year survey. All seven elkhorn colonies that exhibited tissue gain in 2001 showed either complete or partial tissue loss in 2002 (Figure 4.4C). This loss may be attributed to disease or predation. While signs of active white pox disease were not observed on this reef at the time of the 2001 and 2002 surveys, percent cover of *A. palmata* was only 0.82% during these two survey years, and therefore minimal surface area of living tissue was available for the disease to affect. Declining population numbers may make *A. palmata* especially vulnerable to white pox disease and predation by *Coralliophila abbreviata*, which preferentially feeds on this coral species (Knowlton et al. 1990, Miller 2001). Decimation of *A. palmata* populations may also limit the reproductive capacity of this species which

reproduces almost exclusively by fragmentation (Aronson and Precht 2001). While vegetative reproduction may be well adapted to recolonization following mechanical disturbances such as hurricanes, colony fragmentation is ineffectual following severe population declines due to disease, which frequently kills the entire coral colony.

Acropora palmata colonies at Eastern Dry Rocks exhibited reduced tissue loss in the winter followed by accelerated tissue loss in the summer. This seasonal trend in percent change of *A. palmata* is evidenced by the stair-step pattern of tissue loss (Figure 4.2) and may be attributable to effects of seasonal seawater temperatures and precipitation on white pox disease pathogenicity. Elevated seawater temperature is a stressor in corals, causing thermally induced breakdown in the coral-zooxanthellae host-symbiont relationship (Brown 1997), promoting growth and virulence of pathogens (Kushmaro et al. 1996, Kushmaro et al. 1998, Toren et al. 1998, Alker et al. 2001, Banin et al. 2001, Israely et al. 2001), and reducing immune response in host corals (Toren et al. 1998, Alker et al. 2001). Increased rainfall may increase seepage of sewage from septic tanks (Rose et al. 2001) and seed the marine environment with *Serratia marcescens* and other human fecal enteric bacteria and viruses.

The white pox disease epizootic has caused catastrophic losses of *Acropora palmata* in the FKNMS in just nine years. These losses illustrate the impact that disease can have on coral communities. The susceptibility of *A. palmata* to disease and predation, combined with the reproductive strategies of this species, exacerbate the impact of declining populations on the stability of Caribbean coral reef ecosystems. The monumental losses of *A. palmata* in the FKNMS and elsewhere in the Caribbean signify an urgent need for the protection of this keystone species under the Endangered Species Act.

POTENTIAL SOURCES OF THE WHITE POX PATHOGEN

Potential sources of the white pox disease pathogen include wastewater influent, septic tank effluent, feces of reef fishes, canal water, reef water column, and white pox diseased and apparently healthy *A. palmata*. Accumulating evidence suggests that health of reef organisms in the FKNMS and elsewhere in the Caribbean is affected by pollution of fecal origin. White pox disease is caused by a fecal enteric bacterium of possible human origin (Patterson et al. 2002) and human sewage markers are concentrated on coral surfaces in near shore waters of the FKNMS (Lipp et al. 2002). Bacteria common in human fecal contamination have been found within the microbial mat that causes black band disease of corals (Frias-Lopez et al. 2002). Since 1996, populations of the sewage consuming reef sponge *Cliona delitrix* have increased by a factor of ten on reefs in the FKNMS (Ward-Paige and Risk 2003) while, concurrently, corals have declined by 37% (Porter et al. 2002).

It is tempting to speculate that poor waste disposal practices may be to blame for the declining health of tropical marine invertebrates in the FKNMS. Full-scale sewage treatment plants service the communities of Key West, Key Colony Beach on Marathon, and Ocean Reef Resort on Key Largo. The remainder of the Florida Keys utilizes on-site waste disposal practices including septic systems, injection wells, and illegal cesspools. Keys-wide, there are at least 24,000 septic tanks, 600-700 injection wells, and 5,000-10,000 cesspools (Shinn et al.1994, Lapointe et al. 1990). On-site waste disposal contaminates the waters of the FKNMS with nutrients and microorganisms of human fecal origin (Lapointe et al. 1990, Paul et al. 1995a, Paul et al. 1995 b, Paul et al. 1997, Griffin et al. 1999). Fecal bacteria and viruses migrate quickly (0.57-140.9 m/h) from the site of disposal through the porous limestone bedrock of the Florida Keys and finally to the marine environment (Paul et al. 2000). The general direction of

contaminant flow from land is toward the reef tract (Paul et al. 1995a, Paul et al. 1997, Paul et al. 2000).

UNRESOLVED QUESTIONS AND FUTURE RESEARCH

Although *Serratia marcescens* occurs in human sewage, the source of the *S. marcescens* strain that causes white pox disease is uncertain (Patterson et al. 2002). Given the magnitude of *Acropora palmata* loss in the FKNMS, research is needed to identify the source of this pathogen. A variety of potential sources are presently being screened for biotypes similar to the *Serratia* pathogen. These potential sources include wastewater influent, septic tank effluent, feces of reef fishes, canal water, reef water column, and white pox diseased and apparently healthy *A. palmata*. Coral reef managers and wastewater treatment engineers require certainty of the human origin of the white pox pathogen in order to recommend expensive sewage treatment upgrades in Florida and around the Caribbean.

Research is continuing to determine mechanisms of pathogenesis of *Serratia marcescens* against *Acropora palmata.* Future research will identify virulence genes in the coral pathogen and prevalence of these genes in environmental isolates of *S. marcescens*. In addition, future studies will reveal the host range of the *Serratia* pathogen. It is possible that this *Serratia* isolate does not exclusively affect *A. palmata*, but instead affects other coral species on which disease signs are manifested differently. Since the etiologies of the majority of the coral disease conditions described to date are unknown (Richardson 1998), the extent of the pathogenicity of the white pox pathogen warrants examination.

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Figure 4.1. (A) *Acropora palmata* colony affected with white pox disease (Photograph by JW Porter) (B) White pox disease lesions on *A. palmata* (Photograph by KP Sutherland).

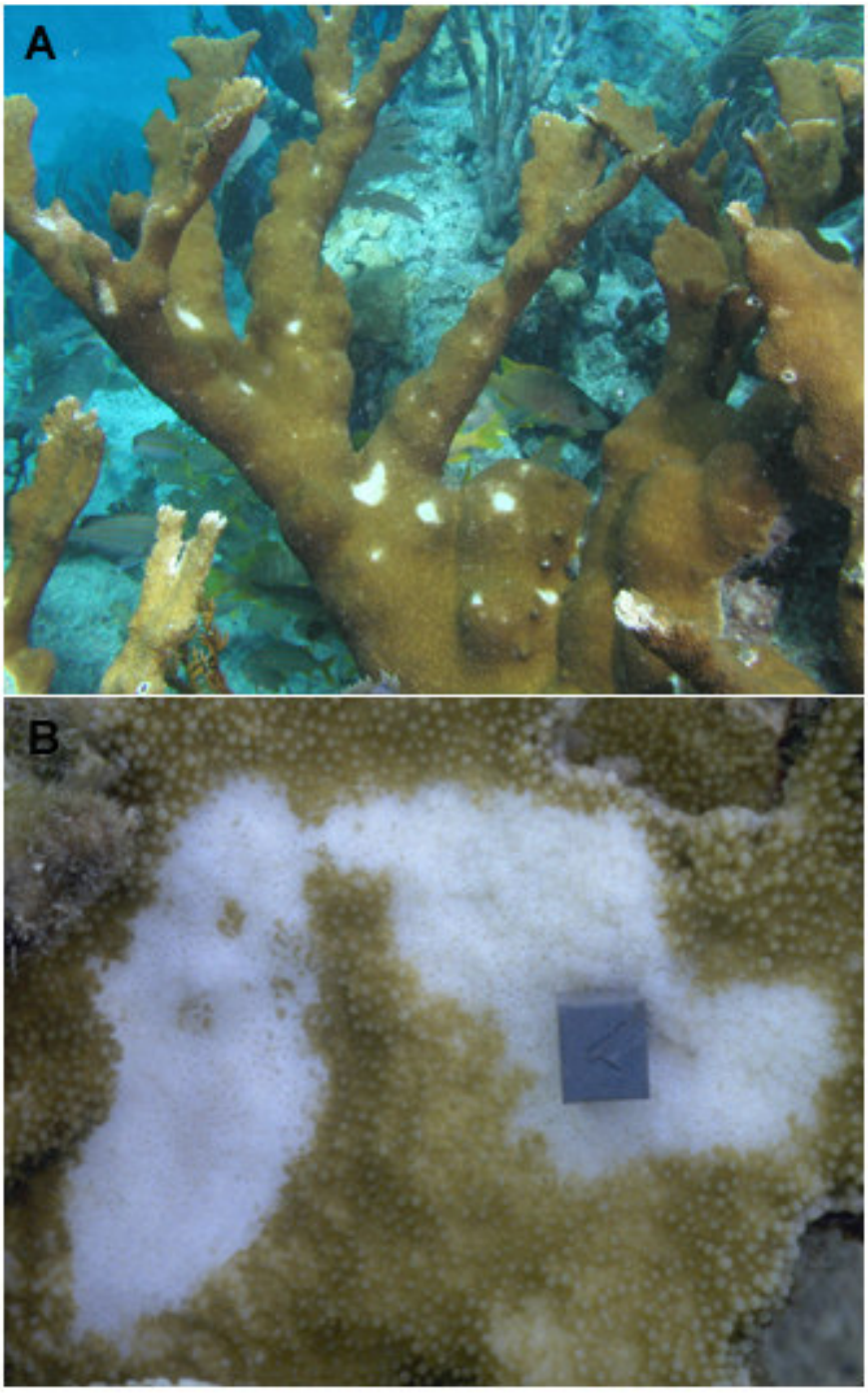

A
B

Figure 4.2. Percent cover of *Acropora palmata* at seven reef sites in the Florida Keys National Marine Sanctuary, 1996-2002: Western Sambo Reef (square), Sand Key Reef (circle), Molasses Reef (upward triangle), Carysfort Reef (diamond), Rock Key Reef (oval), Grecian Rocks Reef (rectangle), Sombrero Reef (downward triangle). Data are presented as mean ± SD.

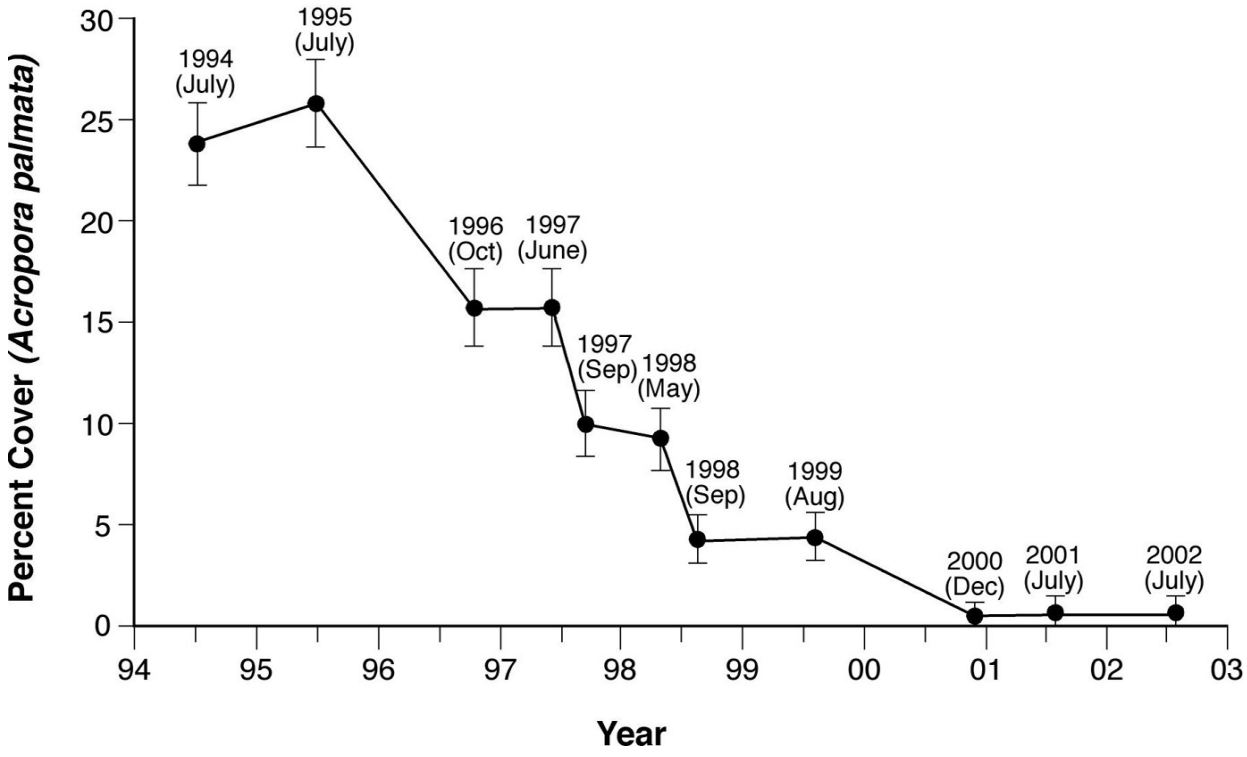

Percent Cover (Acropora palmata)
30
25
20
15
10
5
0
1994 (July)
1995 (July)
1996 (Oct)
1997 (June)
1997 (Sep)
1998 (May)
1998 (Sep)
1999 (Aug)
2000 (Dec)
2001 (July)
2002 (July)
94
95
96
97
98
99
00
01
02
03
Year

Figure 4.3. Percent cover of *Acropora palmata* at Eastern Dry Rocks Reef, Key West, FL, 1994-2002. Data are presented as mean ± SD.

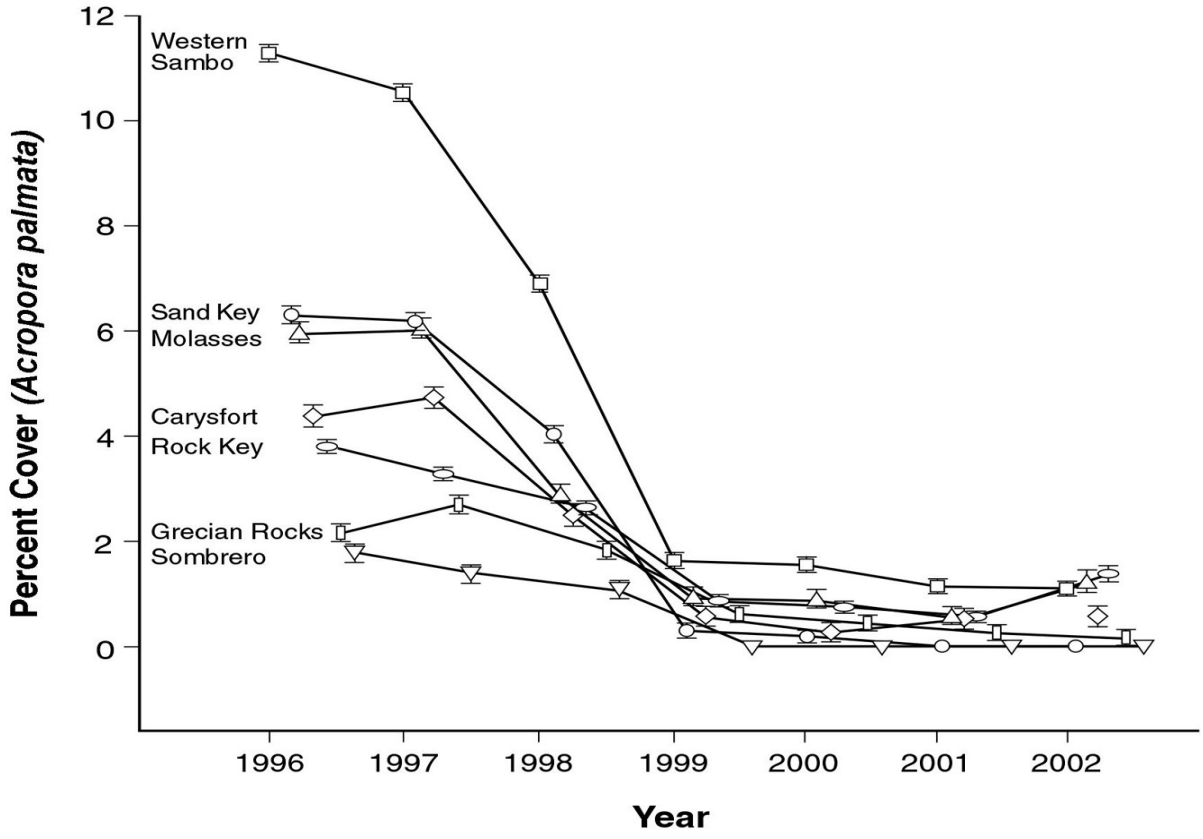
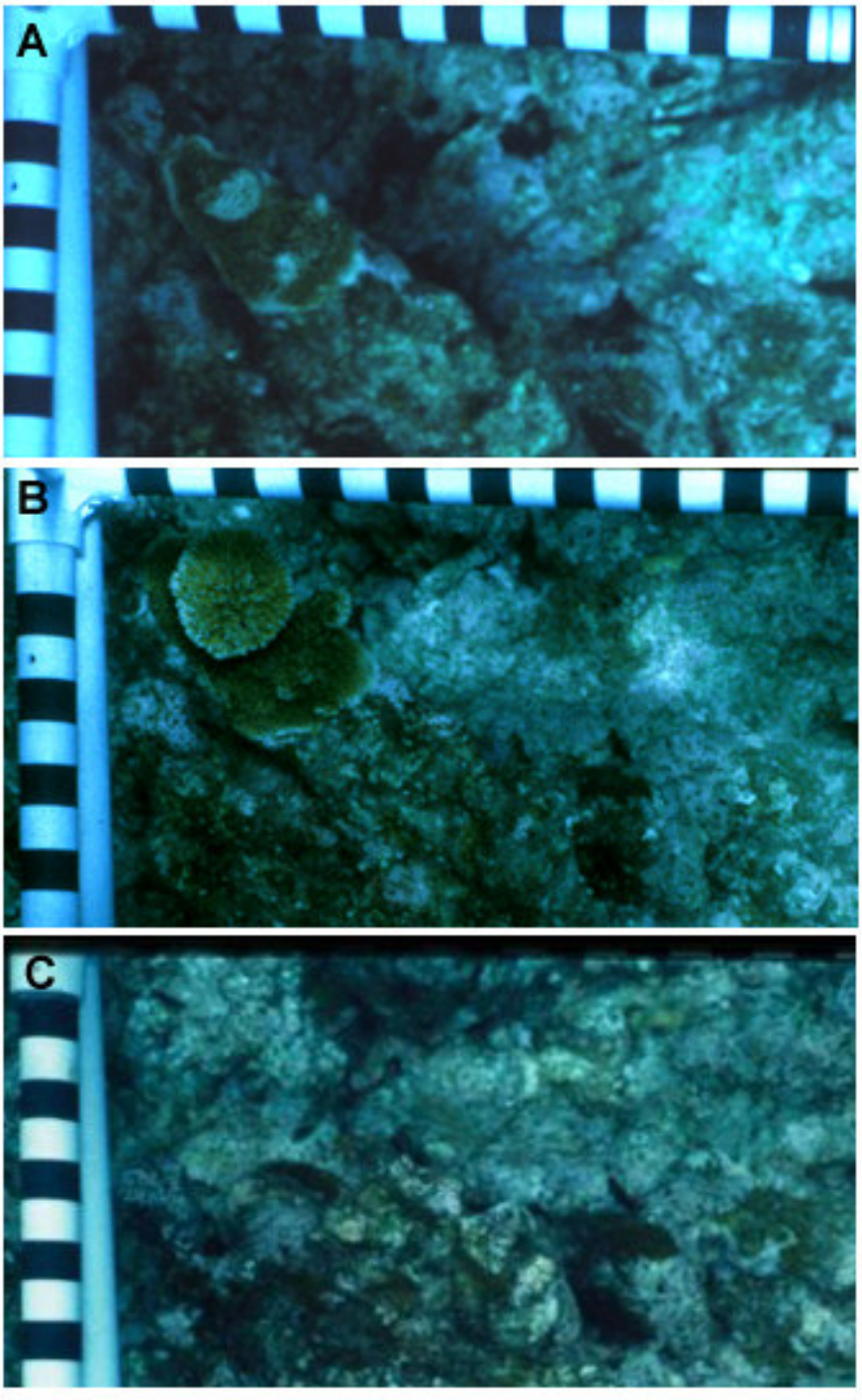

Percent Cover (Acropora palmata)
12
10
8
6
4
2
0
Western
Sambo
Sand Key
Molasses
Carysfort
Rock Key
Grecian Rocks
Sombrero
1996
1997
1998
1999
2000
2001
2002
Year

Figure 4.4. Photographic time series of gain and loss of percent cover *Acropora palmata* at Eastern Dry Rocks Reef, Key West, FL, 2000-2002. The *A. palmata* fragment was photographed on (A) December 28, 2000 and (B) July 30, 2001. Despite the growth observed between 2000 and 2001, the fragment had disappeared by (C) July 29, 2002. This same pattern of tissue gain between 2000 and 2001, followed by tissue loss between 2001 and 2002 was observed on seven *A. palmata* colonies at this reef (photographs by JW Porter and KP Sutherland).


A
B
C

CHAPTER 5

SCREENING FOR THE CORAL PATHOGEN *SERRATIA MARCESCENS* IN THE WATERS OF THE FLORIDA KEYS NATIONAL MARINE SANCTUARY

ABSTRACT

Populations of the Caribbean elkhorn coral, *Acropora palmata,* are being decimated by white pox disease, with losses in the Florida Keys averaging 88%. White pox disease is caused by the enterobacterium *Serratia marcescens. S. marcescens* is common in the intestines of humans and other animals and in other environments. However, the source of the *S. marcescens* strain (PDL100) that causes white pox is uncertain. This study begins the search for the origin of the white pox disease pathogen through the examination of seawater samples collected within the Florida Keys National Marine Sanctuary (FKNMS). Seawater samples were filtered and bacteria from the filters were grown to pure culture. Bacterial isolates were characterized biochemically to identify presumptive *Serratia* to genus. Genomic DNA from presumptive *Serratia* and seven *Serratia* controls, including *S. marcescens* PDL100, was extracted, amplified, and subjected to restriction analysis. The number and size of bands obtained with restriction fragment length polymorphism (RFLP) were used to place isolates into categories. RFLP identified six bacterial isolates as presumptively belonging to the genus *Serratia*. Further analyses are required to assign the bacteria identified in this study as belonging to the genus *Serratia* to species level

taxa. This further step is also necessary to determine if any of these *Serratia* are *S. marcescens* PDL100.

INTRODUCTION

Populations of the most common Caribbean reef-building coral, *Acropora palmata,* are being decimated in the Florida Keys National Marine Sanctuary (FKNMS) with losses of living cover averaging 88% between 1996 and 2002 (Patterson et al. 2002, Sutherland and Ritchie, *in press*). Although hurricanes, bleaching, predation, and white band disease have contributed to declines in elkhorn populations throughout the Caribbean (Gladfelter 1982, Porter and Meier 1992, Hughes 1994, Aronson and Precht 2001), the majority of recent losses in the FKNMS are associated with white pox disease (Patterson et al. 2002, Sutherland and Ritchie, *in press*). Elkhorn coral plays a significant role in the structural and functional integrity of Caribbean coral reef ecosystems. *A. palmata* is an important shallow water species, providing elevated rates of calcium carbonate deposition (Adey 1978) and a highly complex three-dimensional structure of the shallow water fore reef. This keystone species provides shelter and food for reef organisms and aids in the protection of coastal regions by serving as a buffer between land and sea. Severe population declines of *A. palmata* in the FKNMS and elsewhere in the Caribbean have led to the identification of this species as a candidate for inclusion on the Endangered Species List (Diaz-Soltero 1999).

White pox disease is characterized by irregularly shaped distinct white patches of recently dead coral skeleton surrounded by a necrotic front of normally pigmented living coral tissue. Lesions range in area from a few square centimeters to greater than 80 cm^2 and can develop simultaneously on all surfaces of the coral colony. Rate of tissue loss is rapid, averaging

2.5 cm^2 day^{-1} (Patterson et al. 2002), and is greatest during periods of seasonally elevated temperature and rainfall (Patterson et al. 2002, Sutherland and Ritchie, *in press*). White pox is highly contagious with nearest neighbors most susceptible to infection (Patterson et al. 2002). The disease spread rapidly within and between reefs in the FKNMS during the mid-1990s (Porter et al. 2001, Patterson et al. 2002).

White pox disease is caused by *Serratia marcescens* (Patterson et al. 2002), a common Gram-negative bacterium classified as a coliform and a member of the Enterobacteriaceae family. *S. marcescens* is found in the intestines of humans, insects, and other animals, and in freshwater, soil, and plants (Grimont and Grimont 1994). *S. marcescens* is pathogenic to humans, cows, goats, chickens, fishes, insects, and plants (Baya et al. 1992, Grimont and Grimont 1994). The prevalence of *S. marcescens* in the marine environment is unknown. However, this bacterium has been associated with disease in the white perch (*Morone americanus*) in a sewage-polluted estuary in Maryland (Baya et al. 1992). *S. marcescens* is an opportunistic pathogen of humans associated with both waterborne infections in tropical waters (Hazen 1988) and hospital-acquired infections, including urinary tract infections, wound infections, meningitis, endocarditis, pneumonia, and septicemia (Grimont and Grimont 1994, Miranda et al. 1996, Hejazi and Falkiner 1997, Shi et al. 1997).

The *Serratia marcescens* strain that causes white pox disease may originate from an environmental source, e.g from water (freshwater or seawater), soil, plants, or animals. A number of organisms, including marine fishes and birds, may serve as its source. Examples of coral reef organisms that are potential reservoirs and vectors of the pathogen include the gastropod, *Coralliophila abbreviata*, and the polychaete, *Hermodice carunculata*. *C. abbreviata* preferentially feeds on *Acropora palmata* (Knowlton et al. 1990, Miller 2001). *H. carunculata*

is a predator of *A. palmata* and has been shown to be a reservoir and vector of *Vibrio shiloi,* the coral pathogen that induces bleaching in the Mediterranean coral *Oculina patagonica* (Sussman et al. 2003).

Human sewage is another potential source of the white pox pathogen. In the Florida Keys, full scale sewage treatment plants service the communities of Key West (lower keys), Key Colony Beach on Marathon (middle keys), and Ocean Reef Resort on Key Largo (upper keys). The remainder of the Florida Keys utilizes on-site waste disposal practices including septic systems, injection wells, and illegal cesspools. Keys-wide, there are at least 24,000 septic tanks, 600-700 injection wells, and 5,000-10,000 cesspools (Shinn et al. 1994, Lapointe et al. 1990). These on-site waste disposal practices contaminate the waters of the FKNMS with nutrients and microorganisms of human fecal origin (Lapointe et al. 1990, Paul et al. 1995a, Paul et al. 1995b, Paul et al. 1997, Griffin et al. 1999). Fecal bacteria and viruses migrate quickly (0.57-140.9 m/h) from the site of disposal through the porous limestone bedrock of the Florida Keys and finally to the marine environment (Paul et al. 1995a, Paul et al. 2000). The general direction of contaminant flow from land is toward the reef tract (Paul et al. 1995a, Paul et al. 1997, Paul et al. 2000).

Environmental isolates of *S. marcescens* characteristically produce a red pigment, prodigiosin, while clinical isolates of the bacterium are rarely pigmented (Hejazi & Falkiner 1997). The *S. marcescens* strain that causes white pox disease (PDL100) is not pigmented (Patterson et al. 2002), suggesting that PDL100 may not have originated from an environmental source. The lack of red pigmentation in clinical isolates and PDL100 also suggests that prodigiosin is not a virulence factor of *S. marcescens* (Hejazi & Falkiner 1997). However, *S. marcescens* is a cytotoxic bacterium (Carbonell et al. 1996, Carbonell et al. 1997, Marty et al.

2002, Carbonell et al. 2003) that secretes a number of virulence factors including chitinases, proteases, lipases, a lectinase, a nuclease, and a hemolysin (Hines et al. 1988, Auken et al. 1998). *S. marcescens* PDL100 produces lipases and proteases (Polson 2002), but the role that these enzymes play in pathogenesis is unknown.

The source of the *S. marcescens* strain (PDL100) that causes white pox disease is uncertain (Patterson et al. 2002). Given the magnitude of *Acropora palmata* loss in the FKNMS, identification of the pathogen's source is imperative. The objective of this study is to begin the search for the origin of the white pox disease pathogen through examination of seawater samples collected within the FKNMS.

METHODS

Sample Collection and Bacterial Isolations

The FKNMS is composed of 3,100 km^2 of coastal waters and includes the more than 259 km^2 that encompasses the Dry Tortugas National Park (DTNP). In September 2001, seawater samples were collected within the FKNMS, in the lower Florida Keys in close proximity to Key West and in the DTNP (Figure 5.1).

Surface water samples were collected by hand-filling 1 L sterile plastic bottles in the Florida Keys at Rock Key and Sand Key, and in the DTNP at Bird Key, Loggerhead Reef, Pulaski Shoal, White Shoal, and at the harbor, the moat, and the ruins of the coal docks at Fort Jefferson on Garden Key (Table 5.1). Nisken bottles were used to collect water samples from both surface and bottom of the water column at three stations near Key West in the Florida Keys (KW01, KW02, KW03, Table 5.1) and at four stations in the DTNP (DT01, DT03, DT04, DT05,

Table 5.1). An additional bottom water sample was collected at a fifth station in the DTNP (DT02, Table 5.1).

Water samples were maintained at room temperature for less than 1 hr and filtered through sterile 0.22 μm filters using a sterile Gelman 47 mm filtering column. Water was filtered in 100 ml, 200 ml, and 400 ml volumes. Following filtration, filters from each volume were transferred to 47 mm petri plates containing DTC (DNA, toluidine blue, cephalothin) agar medium (Atlas and Parks 1996). DTC medium selects for growth of *Serratia marcescens* and is based on colorimetric detection of DNase production and resistance to the antibiotic cephalothin (Farmer et al. 1973). DTC plates were incubated at 28 ºC and observed for bacterial growth every 12 hours. Bacterial colonies that grew on DTC medium were picked and streaked for isolation on modified marine 2216 (MM2216) agar medium (Atlas and Parks 1996).

Biochemical Characterizations

Bacterial isolates obtained in pure culture were tested for (1) Gram reaction, (2) oxidase reaction, (3) DNase activity, and (4) fermentation of arabinose and production of ornithine decarboxylase. KOH and tetramethyl-*p*-phenylenediamine dihydrochloride methods were used to determine Gram reaction (Buck 1982) and oxidase reaction (Faller and Schleifer 1981), respectively. DNase activity was determined by colorimetric detection on DTC medium; DTC agar surrounding bacterial growth changed from blue to red in cephalothin resistant and DNase positive bacteria (presumptive *Serratia,* Farmer et al. 1973, Atlas and Parks 1996). Fermentation of arabinose and production of ornithine decarboxylase was determined by inoculating each isolate into a glass tube containing solid *Serratia* differential medium (Atlas and Parks 1996). Tubes were incubated at 28 ºC and observed every 24 hours for a color change (Atlas and Parks

1996). A purple to yellow coloration of the agar indicates presumptive *Serratia* (Atlas and Parks 1996).

For all biochemical tests, (1) *Serratia marcescens* ATCC 8100, (2) a red-pigmented *S. marcescens,* and (3) *S. marcescens* isolated from white pox diseased *Acropora palmata* (isolate PDL100, Patterson et al. 2002) were used as positive controls. *S. marcescens* ATCC 8100 and *S. marcescens* PDL100 do not produce the red pigment prodigiosin (Patterson et al. 2002). *Serratia* are Gram-negative, oxidase negative, DNase positive, and positive for the arabinose/ornithine decarboxylase assay. *Enterococcus faecalis* (Gram-positive, oxidase negative) and *Pseudomonas aeruginosa* (Gram-negative, oxidase positive) served as additional controls.

When biochemical tests were complete, each bacterial isolate was inoculated into 5 ml liquid *Serratia* ATCC medium 181 (Atlas and Parks 1996) and incubated on a shaker at 28 ºC overnight. For each bacterial isolate, 1 ml cultivated medium was transferred to a sterile cryotube containing 0.5 ml glycerol. Cryotubes were vortexed, placed at –20 ºC overnight, and transferred to –70 ºC for storage.

Molecular Methods

Genomic DNA, from the presumptive *Serratia* identified with biochemical characterizations, was extracted, amplified, and subjected to restriction analysis. Samples were removed from –70 ºC and thawed to slush consistency. Small aliquots from each tube were transferred to (1) MM2216 solid medium and (2) 5 ml MM2216 broth. MM2216 agar plates were streaked for isolation. Inoculated solid and liquid media were incubated overnight at 28 ºC. Plates were examined to confirm that bacterial cultures were not contaminated.

Genomic DNA Extraction

A Wizard Genomic DNA Purification Kit (Promega, WI) was used to purify the DNA from each overnight MM2216 broth culture. Approximately 1.5 ml of each overnight culture was transferred to a 1.5 ml microcentrifuge tube. Tubes were centrifuged (Hermle Z 230M centrifuge) (5 min) to pellet cells, and supernatant was removed. Bacterial pellets were washed in 1 ml of sterile Tris-EDTA (TE) (pH 8.0) and centrifuged (5 min). Supernatant was removed. Cells were resuspended in 600 µl Nuclei Lysis Solution (Promega, WI), incubated at 80 ºC (5 min) to lyse the cells, and cooled to room temperature. 200 µl Protein Precipitation Solution (Promega, WI) was added to cell lysate in each tube, mixed by inversion, incubated on ice (5 min), and centrifuged (5 min). Supernatant containing DNA was transferred to a clean 1.5 ml microcentrifuge tube containing 600 µl of room temperature isopropanol. Tubes were gently mixed by inversion until DNA stopped forming a visible mass. Tubes were centrifuged (5 min) and supernatant was removed. DNA pellet was washed with 600 µl of room temperature 70% ethanol. Tubes were centrifuged (2 min) and supernatant was removed. Tubes were allowed to air dry (15 min). DNA was rehydrated overnight at 4 ºC with 100 µl of sterile TE. Absorbance of each purified DNA sample was measured at 260 nm and used to calculate concentration (µg/µl) of purified DNA. DNA concentrations were used to calculate amount of purified DNA to be used for genomic DNA amplification.

Genomic DNA Amplification

The polymerase chain reaction (PCR) was used to amplify DNA from 16S rRNA genes. The amplification primers were the universal eubacterial primers Fd_1 and rp_2 (Weisburg et al. 1991). Conditions for PCR amplification were as follows: 1 ng genomic DNA, 5 µl 10X

reaction buffer (Applied Biosystems), 5 µl 2.5 mM $MgCl_2$, 1 µl 10 mM dNTP, 1 µl 100% DMSO, 1 µl 1000 µg/ml BSA, 0.8 µl Fd_1, 0.8 µl rp_2, and 0.5 µl Taq Polymerase (AmpliTaq, Applied Biosystems) were combined in a total volume of 50 µl. *E. coli* DNA and reactions lacking DNA template served as positive and negative controls, respectively. Reactions were incubated in a thermal cycler (PTC-100^{TM}, MJ Research, Inc.). The cycling program was as follows: initial denaturation at 92 ºC for 2 min; 35 cycles of 92 ºC for 60 s, 55 ºC for 60 s, 72 ºC for 60s; a final extension step at 72 ºC for 15 min. 15 µl of each amplified PCR product was analyzed by Tris-borate-EDTA (TBE)-agarose gel electrophoresis (Sambrook et al. 1989). The remaining 35 µl of PCR product was purified according to the manufacturer's protocol using either Centricon concentrators (Amicon, MA) or Microcon®-PCR filter units (Amicon, MA). Purified DNA was stored at 4 ºC.

PCR products purified with Centricon concentrators were washed for removal of TE prior to restriction analysis. Each DNA sample was mixed with 0.1 volume 3M sodium acetate and 2 volumes 95% ethanol, incubated at room temperature (2 min), and centrifuged (5 min). Supernatant was removed and pellet was washed with several volumes of 70% ethanol and centrifuged (5 min). Supernatant was removed and pellet was washed with several volumes of 100% ethanol and centrifuged (5 min). Supernatant was removed and DNA was resuspended in sterile filtered milliQ water. Washed DNA was stored at 4 ºC.

Absorbance of each purified PCR product was measured at 260 nm and used to calculate concentration (µg/µl) of purified DNA. DNA concentrations were used to calculate amount of purified PCR product to use for restriction analysis.

Restriction Fragment Length Polymorphism (RFLP)

PCR products were digested separately with restriction enzymes *Alu*I, *Hha*I, and *Msp*I (New England Biolabs). Conditions for RFLP with *Alu*I and *Msp*I were as follows: 1 µl enzyme, 12 µl purified PCR product, 5 µl 10X NEBuffer #2 (New England Biolabs), and 32 µl filtered milliQ water. Conditions for RFLP with *Hha*I were as follows: 1 µl enzyme, 12 µl purified PCR product, 5 µl 10X NEBuffer #4 (New England Biolabs), 0.5 µl 100X bovine serum albumin (BSA), and 31.5 µl filtered milliQ water. Restriction digests were carried out at 37 ºC for 2 hours followed by heat deactivation at 65 ºC for 20 min. Restriction enzyme products were analyzed by TBE-agarose gel electrophoresis (Sambrook et al. 1989). The 1X TBE running buffer was chilled to 4 °C and gel electrophoresis was carried out in a cold room (4 °C). Gels were stained with ethidium bromide and photographed under UV transillumination (Sambrook et al. 1989). Fragment sizes were estimated using Kodak 1D Image Analysis Software (V. 3.5.3, New Haven, CT). The number and sizes of bands obtained for each seawater isolate with each restriction enzyme were used to place isolates into RFLP clusters.

Restriction digest patterns of presumptive *Serratia* isolates were compared to patterns produced by (1) *Serratia marcescens* PDL100 isolated from white pox diseased *Acropora palmata* (Patterson et al. 2002), (2) *S. marcescens* ATCC 8100, (3) red-pigmented *S. marcescens,* (4) *S. rubidaea* ATCC 33670, (5) *S. rubidaea* ATCC 27593, (6) *S. rubidaea* ATCC 27614, and (7) *S. liquefaciens* ATCC 27592. ATCC 27593 and ATCC 27614 are the type strain and a marine strain of *S. rubidaea,* respectively. ATCC 27592 is the type stain of *S. liquefaciens*.

RESULTS

Bacterial Isolation

Bacterial isolates were obtained in pure culture from three stations in the Florida Keys and eight stations in the DTNP (Table 5.2). No bacterial growth was observed from seawater samples collected at Rock Key, Bird Key, Loggerhead Reef, White Shoal, the harbor at Fort Jefferson, or Key West station 2. A total of 97 bacterial isolates were obtained in pure culture.

Biochemical Characterizations

Of the 97 bacterial isolates, 15 were identified as presumptive *Serratia* based on biochemical tests. These 15 isolates produced seven different biochemical patterns (Table 5.3) and were conservatively selected as presumptively belonging to the genus *Serratia.* To reduce the possibility that *Serratia* isolates would be prematurely eliminated from the study, the results of the biochemical tests were considered both individually and in combination.

Molecular Methods

Restriction Fragment Length Polymorphism

RFLP results suggest that a total of six presumptive *Serratia* were isolated from the waters of the FKNMS (Table 5.4). The isolates were collected from one station in the DTNP and one station near Key West. The number and size of bands produced with RFLP of the 16S rRNA gene indicated that *Hha*I produced six RFLP patterns and three bacterial isolate clusters (Table 5.5). Clusters are defined as RFLP patterns containing two or more bacterial isolates. RFLP with *Alu*I produced six RFLP patterns and five bacterial isolate clusters (Table 5.5). RFLP with *Msp*I produced nine RFLP patterns and five bacterial isolate clusters (Table 5.5).

RFLP was unsuccessful for isolates KW03-1 and KW03-5 with *Msp*I, for isolates FD01-5 and FM01-2 with *Alu*I and *Hha*I, and for isolate FD01-2 with *Hha*I. The bacterial isolates within two *Hha*I clusters (clusters 1 and 2), three *Alu*I clusters (clusters 2, 3, and 5), and two *Msp*I clusters (clusters 1 and 5) produced the same biochemical patterns (Table 5.5). *Hha*I cluster 3, *Alu*I cluster 1, and *Msp*I clusters 3 and 4 were each composed primarily of a single biochemical pattern (Table 5.5).

RFLP with *Hha*I showed that the white pox disease isolate, *Serratia marcescens* PDL100, produced banding patterns similar to six seawater isolates and five *Serratia* controls (Table 5.5). The seawater isolates did not cluster with *Serratia marcescens* PDL100 or the other *Serratia* controls when digested with *Alu*I or *Msp*I (Table 5.5). The one exception was isolate FM01-1, which clustered with the *S. rubidaea* controls when digested with *Msp*I (Table 5.5).

One pair of presumptive *Serratia* isolates (KW03-4 and KW03-5) showed the same biochemical pattern and clustered together when digested with both *Hha*I and *Alu*I, indicating that the isolates are likely to be the same bacterial strain (Table 5.5). A second pair of presumptive *Serratia* isolates (FM01-3 and KW03-3) consistently clustered together when digested with all three restriction enzymes (Table 5.5), but produced different biochemical patterns (Table 5.3).

DISCUSSION

The six presumptive *Serratia* identified in this study are not likely to be the same bacterium as *S. marcescens* PDL100 that causes white pox disease of *Acropora palmata*. The presumptive *Serratia* clustered with PDL100 and the other *Serratia marcescens* controls only when digested with *Hha*I. Additional studies must be carried out before the identity of the

seawater isolates can be confirmed. These tests may include (1) 16S rDNA sequencing of presumptive *Serratia* isolates or (2) *S. marcescens* species-specific PCR, both of which would facilitate the species identification of the six presumptive *Serratia* isolates identified in this study. It is not known what genetic modifications occur to *S marcescens* strains as they colonize different host species, and therefore it is not known if the DNA characteristics of *S. marcescens* PDL100 could be easily derived from a water column inoculum.

The seawater samples included in this study were collected from a limited number of locations and represent a preliminary sampling of the waters of the FKNMS. Additional studies are currently underway to determine the source of *Serratia marcescens* PDL100. These studies include the screening of seawater samples, white pox diseased and apparently healthy *Acropora palmata,* other reef organisms, wastewater influent and effluent, septic tank effluent, nearshore and canal water, and sea bird guano for biotypes similar to the *Serratia marcescens* PDL100. Elucidating the source of the white pox disease pathogen is essential, as coral reef managers and wastewater treatment engineers appreciate certainty of a human origin of the white pox pathogen before recommending expensive sewage treatment upgrades in Florida and around the Caribbean.

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Figure 5.1. A map showing the locations of the seawater collection stations in the Florida Keys National Marine Sanctuary.

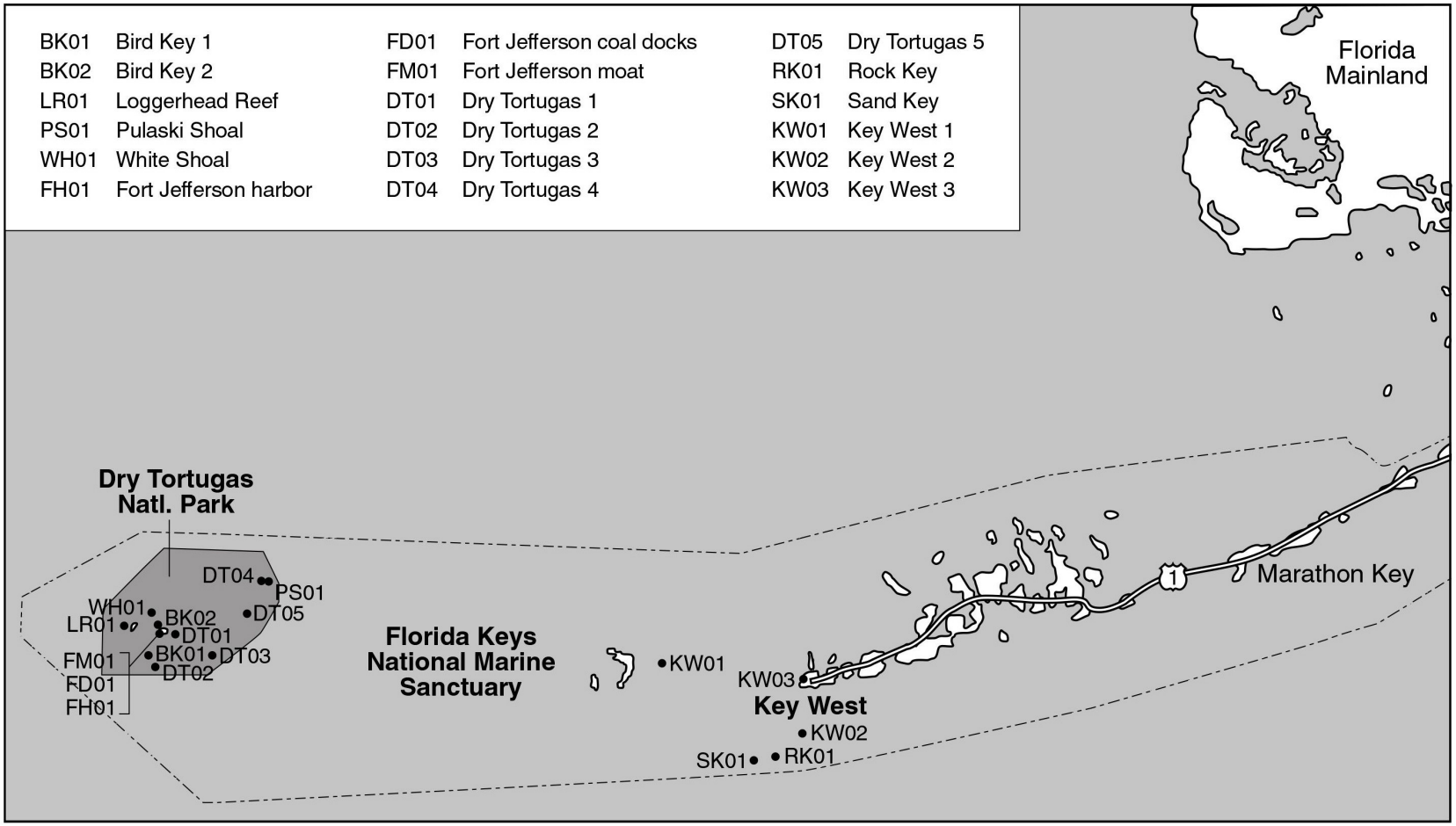
BK01 Bird Key 1
BK02 Bird Key 2
LR01 Loggerhead Reef
PS01 Pulaski Shoal
WH01 White Shoal
FH01 Fort Jefferson harbor
FD01 Fort Jefferson coal docks
FM01 Fort Jefferson moat
DT01 Dry Tortugas 1
DT02 Dry Tortugas 2
DT03 Dry Tortugas 3
DT04 Dry Tortugas 4
DT05 Dry Tortugas 5
RK01 Rock Key
SK01 Sand Key
KW01 Key West 1
KW02 Key West 2
KW03 Key West 3
Florida Mainland
Dry Tortugas Natl. Park
Florida Keys National Marine Sanctuary
Marathon Key
1
Key West

Table 5.1. Stations in the Florida Keys (FK) and the Dry Tortugas National Park (DTNP) from which seawater samples were collected. Type indicates whether the seawater sample was collected from the surface of the water column (S), the bottom of the water column (B), or both (SB).

Region	Station	Code	Type	Latitude & Longitude
DTNP	Bird Key 1	BK01	S	24º 36.703′ N, 82º 52.239′ W
DTNP	Bird Key 2	BK02	S	24º 37.852′ N, 82º 52.705′ W
DTNP	Loggerhead Reef	LR01	S	24º 37.973′ N, 82º 55.911′ W
DTNP	Pulaski Shoal	PS01	S	24º 41.464′ N, 82º 46.301′ W
DTNP	White Shoal	WH01	S	24º 38.532′ N, 82º 53.807′ W
DTNP	Fort Jefferson harbor	FH01	S	24º 37.643′ N, 82º 52.308′ W
DTNP	Fort Jefferson coal docks	FD01	S	24º 37.583′ N, 82º 52.357′ W
DTNP	Fort Jefferson moat	FM01	S	24º 37.663′ N, 82º 52.356′ W
DTNP	Dry Tortugas 1	DT01	SB	24º 37.604′ N, 82º 51.344′ W
DTNP	Dry Tortugas 2	DT02	B	24º 36.606′ N, 82º 52.185′ W
DTNP	Dry Tortugas 3	DT03	SB	24º 37.163′ N, 82º 49.731′ W
DTNP	Dry Tortugas 4	DT04	SB	24º 41.536′ N, 82º 46.094′ W
DTNP	Dry Tortugas 5	DT05	SB	24º 38.626′ N, 82º 47.090′ W
FK	Rock Key	RK01	S	24º 27.358′ N, 81º 50.755′ W
FK	Sand Key	SK01	S	24º 27.140′ N, 81º 52.587′ W
FK	Key West 1	KW01	SB	24º 36.843′ N, 82º 03.627′ W
FK	Key West 2	KW02	SB	24º 29.281′ N, 81º 48.022′ W
FK	Key West 3	KW03	SB	24º 33.549′ N, 81º 48.537′ W

Table 5.2. Stations in the Florida Keys (FK) and the Dry Tortugas National Park (DTNP) from which bacterial isolates were obtained in pure culture. Type indicates whether the isolates were obtained from the surface of the water column (S), the bottom of the water column (B), or both (SB).

Region	**Station**	**Code**	**Type**
DTNP	Pulaski Shoal	PS01	S
DTNP	Fort Jefferson coal docks	FD01	S
DTNP	Fort Jefferson moat	FM01	S
DTNP	Dry Tortugas 1	DT01	SB
DTNP	Dry Tortugas 2	DT02	B
DTNP	Dry Tortugas 3	DT03	SB
DTNP	Dry Tortugas 4	DT04	B
DTNP	Dry Tortugas 5	DT05	SB
FK	Sand Key	SK01	S
FK	Key West 1	KW01	SB
FK	Key West 3	KW03	SB

Table 5.3. Presumptive *Serratia* based on results of biochemical characterization of bacterial isolates from seawater samples collected in the Florida Keys (FK) and the Dry Tortugas National Park (DTNP). Biochemical tests included Gram reaction (G), oxidase reaction (O), DNase activity (D), and fermentation of arabinose and production of ornithine decarboxylase (A/O). For the Gram reaction, (+) = Gram-positive and (-) = Gram-negative. For the remaining microbiological tests, (+) = positive reaction and (-) = negative reaction. Isolates were assigned biochemical patterns (BP) based on the combined results of all four biochemical tests. Type indicates whether the isolate was obtained from the surface (S) or the bottom (B) of the water column.

Isolate	**Region**	**Station**	**Type**	**G**	**O**	**D**	**A/O**	**BP**
Serratia spp. controls				-	-	+	+	a
DT01-2	DTNP	Dry Tortugas 1	S	-	+	-	+	b
DT03-1	DTNP	Dry Tortugas 3	S	-	+	+	+	c
DT03-2	DTNP	Dry Tortugas 3	S	-	+	-	+	b
FD01-1	DTNP	Fort Jefferson coal docks	S	-	+	-	+	b
FD01-2	DTNP	Fort Jefferson coal docks	S	-	+	-	+	b
FD01-3	DTNP	Fort Jefferson coal docks	S	-	+	-	+	b
FM01-1	DTNP	Fort Jefferson moat	S	+	-	+	+	d
FM01-2	DTNP	Fort Jefferson moat	S	-	-	+	+	a
FM01-3	DTNP	Fort Jefferson moat	S	+	-	+	-	e
KW01-1	FK	Key West 1	B	-	+	+	-	f
KW03-1	FK	Key West 3	S	-	+	+	-	f
KW03-2	FK	Key West 3	S	-	-	-	+	g
KW03-3	FK	Key West 3	B	-	-	+	+	a
KW03-4	FK	Key West 3	B	-	+	+	+	c
KW03-5	FK	Key West 3	B	-	+	+	+	c

Table 5.4. Presumptive *Serratia* based on results of RFLP analysis of bacterial isolates from seawater samples collected in the Florida Keys (FK) and the Dry Tortugas National Park (DTNP). Type of sample indicates whether the seawater sample was collected from the surface (S) or the bottom (B) of the water column.

Isolate	**Region**	**Station**	**Type**
FM01-1	DTNP	Fort Jefferson moat	S
FM01-3	DTNP	Fort Jefferson moat	S
KW03-2	FK	Key West 3	S
KW03-3	FK	Key West 3	B
KW03-4	FK	Key West 3	B
KW03-5	FK	Key West 3	B

Table 5.5. Patterns, clusters and fragment sizes (base pairs) obtained by restriction enzyme digestion of 16S rRNA. The biochemical pattern (BP) obtained for each isolate with microbiological testing is listed.

Enzyme	Pattern	Cluster	BP	Isolates	Fragment Size
*Hha*I	A	1	b	DT01-2	400, 280, 260, 160
			b	FD01-1	442, 313, 275, 160
	B	2	f	KW01-1	539, 382, 242, 200, 160
			f	KW03-1	539, 388, 258, 200, 160
	C	3	d	FM01-1	539, 390, 359, 200, 160
			e	FM01-3	569, 390, 359, 200, 160
			g	KW03-2	546, 390, 359, 200, 160
			a	KW03-3	575, 396, 372, 200, 160
			c	KW03-4	531, 400, 359, 200, 160
			c	KW03-5	539, 414, 371, 200, 160
			a	*S. marcescens* PDL100	546, 390, 365, 200, 160
			a	*S. marcescens* ATCC 8100	546, 390, 371, 200, 160
			a	*S. marcescens* red pigment	562, 390, 371, 200, 160
			a	*S. rubidaea* ATCC 27593	546, 390, 377, 200, 160
			a	*S. rubidaea* ATCC 27614	539, 390, 371, 200, 160
			a	*S. rubidaea* ATCC 33670	546, 390, 377, 200, 160
	D		c	DT03-1	790, 368, 283
	E		b	DT03-2	608, 444, 312, 258
	F		a	*S. liquefaciens* ATCC 27592	508, 390, 377, 350, 200
*Alu*I	A	1	b	DT01-2	398, 200, 160
			b	DT03-2	414, 218, 160
			b	FD01-1	414, 218, 160
			b	FD01-2	400, 200, 160
			d	FM01-1	379, 200, 160
			g	KW03-2	379, 200, 160
	B	2	f	KW01-1	600, 471, 379, 200, 180
			f	KW03-1	600, 471, 200, 180
	C	3	a	*S. marcescens* PDL100	395, 350, 200, 160
			a	*S. marcescens* ATCC 8100	395, 350, 200, 160
			a	*S. marcescens* red pigment	395, 350, 200, 160
			a	*S. rubidaea* ATCC 27593	374, 329, 200, 160
			a	*S. rubidaea* ATCC 33670	376, 331, 200, 160
			a	*S. liquefaciens* ATCC 27592	377, 350, 200, 160
	D	4	c	DT03-1	579, 444, 371, 333, 240, 200, 160, 140
			e	FM01-3	594, 463, 413, 372, 240, 200, 170, 160
			a	KW03-3	579, 447, 370, 333, 240, 200, 170, 160
	E	5	c	KW03-4	600, 230, 218, 180, 160
			c	KW03-5	600, 230, 218, 180, 160
	F		a	*S. rubidaea* ATCC 27614	367, 321, 240, 200, 160

Enzyme	Pattern	Cluster	BP	Isolates	Fragment Size
*Msp*I	A	1	b	DT01-2	579, 358, 160
			b	FD01-1	579, 363, 160
			b	FD01-2	571, 363, 160
			b	FD01-5	571, 363, 160
	B	2	f	KW01-1	520, 450, 150, 140
			c	KW03-4	520, 450, 150, 140
	C	3	d	FM01-1	400, 280, 200, 170, 160, 140
			a	*S. rubidaea* ATCC 27593	427, 271, 200, 160, 140
			a	*S. rubidaea* ATCC 27614	427, 281, 200, 160, 140
			a	*S. rubidaea* ATCC 33670	427, 271, 200, 160, 140
	D	4	a	FM01-2	529, 469, 431, 300, 200, 170, 160, 140
			e	FM01-3	528, 475, 438, 300, 200, 170, 160, 140
			a	KW03-3	520, 450, 400, 280, 200, 170, 160, 140
	E	5	a	*S. marcescens* PDL100	521, 271, 160, 140
			a	*S. marcescens* ATCC 8100	528, 270, 160, 140
			a	*S. marcescens* red pigment	514, 262, 160, 140
	F		c	DT03-1	683, 475, 160
	G		b	DT03-2	571, 507, 160, 140
	H		g	KW03-2	324, 242, 200, 160
	I		a	*S. liquefaciens* ATCC 27592	528, 311, 200, 160

CHAPTER 6

HISTOPATHOLOGY OF WHITE POX-AFFECTED *ACROPORA PALMATA*

ABSTRACT

White pox-affected and apparently healthy coral tissues were collected from diseased colonies of the elkhorn coral *Acropora palmata* in August 1998 at Looe Key Reef, Florida. The tissues were processed for histopathological examination with light microscopy. Cellular and tissue degeneration was observed in both apparently healthy and diseased coral tissues. White pox disease is associated with (1) rounding of granular gland cells in the mesenterial filaments, (2) atrophy of the coenosarc, (3) necrosis of the coenosarc, oral disk, gastric cavity, and mesenterial filaments, and (4) disruptions in the gastric cavity. Most lesions were concentrated in the coenosarc tissue. There was no significant difference in the number or type of lesions present in diseased versus apparently healthy samples. While the frequencies of 41% of the abnormalities showed a significant difference between apparently healthy and diseased tissues, half of these abnormalities were more common in diseased tissues. The overall extent and severity of lesions was significantly different between apparently healthy and diseased tissues. However, only seven of the 26 individual lesion types showed a significant difference in extent and severity between apparently healthy and diseased tissues. The similarities between apparently healthy and diseased tissues suggest that colonies affected by white pox sustain a whole-colony reaction to infection.

INTRODUCTION

The coral disease named white pox has been implicated as the principal cause of major population declines of the elkhorn coral *Acropora palmata* in the Florida Keys National Marine Sanctuary (FKNMS), with losses of this species averaging 88% between 1996 and 2002 (Patterson et al. 2002, Sutherland & Ritchie *in press*). The disease, which exclusively affects *A. palmata,* is caused by the enterobacterium *Serratia marcescens* (Patterson et al. 2002). White pox-affected corals are characterized by irregularly shaped distinct white patches of recently denuded skeleton surrounded by a necrotic front of normally pigmented living tissue (Fig. 6.1A, B, Patterson et al. 2002). These patches of tissue loss can occur simultaneously all over the coral colony (Fig. 6.1A). White pox disease, also termed acroporid serratiosis (Patterson et al. 2002) and patchy necrosis (Bruckner & Bruckner 1997), affects elkhorn coral throughout the Caribbean (Bruckner & Bruckner 1997, Porter et al. 2001, Rodríquez-Martínez et al. 2001, Santavy et al. 2001, Patterson et al. 2002).

Elkhorn coral plays a significant role in the structural and functional integrity of Caribbean coral reef ecosystems. *Acropora palmata* is an important shallow water species, providing elevated rates of calcium carbonate deposition (Adey 1978) and a highly complex three-dimensional structure in the shallow water forereef. This species provides shelter and food for reef organisms and aids in the protection of coastal regions by serving as a buffer between land and sea. Severe population declines of *A. palmata* in the FKNMS and elsewhere in the Caribbean (Gladfelter 1982, Porter and Meier 1992, Bythell and Sheppard 1993, Aronson and Precht 2001, Miller 2002, Patterson et al. 2002) have led to the identification of this species as a candidate for inclusion on the Endangered Species List (Diaz-Soltero 1999).

Histopathology of diseased corals is a valuable tool that can be used to identify cell and tissue lesions associated with diseases and to visualize microorganisms that may contribute to disease pathogenesis. Histopathological analyses have been conducted for only 5 of the 18 coral diseases documented to date in the global oceans: black band (Ramos-Flores 1983, Peters 1984a, Bythell et al. 2002), white band type I (Peters et al.1983, Peters 1984a), white plague type I (Peters 1984a, Bythell et al. 2002), white plague type II (Richardson et al. 1998a, Richardson et al. 1998b), and skeletal anomalies (Cheng & Wong 1974, Morse et al. 1977, Morse et al. 1981, Peters et al. 1986, Coles & Seapy 1998,Yamashiro et al. 2000). I report here on the histopathological examination of coral tissues from white pox-diseased *Acropora palmata.*

METHODS

Coral Collection and Histopathology

In August 1998, terminal branches having white pox lesions were collected from five white pox-diseased colonies of *Acropora palmata* at Looe Key Reef (24° 32.7' N, 81° 24.4' W) in the FKNMS. *A. palmata* colonies with no signs of white pox could not be located at Looe Key Reef at the time of sampling. Therefore, each colony was subsampled. Diseased samples were areas of tissue immediately adjacent to and contained the margin of the recently bared skeleton. Apparently healthy samples showed no gross abnormalities, and were removed from the branch approximately 1 to 10 cm away from areas of recent tissue loss. Coral fragments were (1) removed by scuba divers from the colony with a masonry hammer and chisel, (2) placed in plastic Ziploc bags, (3) transferred to a cooler containing seawater, (4) transported to shore by boat, and (5) divided into smaller pieces with a masonry hammer and chisel. Corals were

preserved with modified Helly's solution for 20 hours, washed in fresh water for 24 hours, and stored in 70% undenatured ethanol (Barszcz & Yevich 1975, Peters 2001).

`Preserved corals were cut into small pieces with an electric jeweler's saw and decalcified in a decalcification solution containing 0.5 N HCl and 0.002 M EDTA (Peters 2001). Complete decalcification occurred within 24 to 72 hours. Each piece of decalcified coral tissue was trimmed with a razor blade, placed in processing cassettes, and washed in water for 24 hours to remove the decalcification solution. Coral tissues were processed overnight according to standard procedures for embedding in paraffin (Table 6.1) using a Shandon Hypercenter 2 automated tissue processor (Peters 2001). Embedded corals were sectioned into oral, aboral, and longitudinal polyp surfaces of 6 μm thickness, placed in a 45 °C distilled water bath, and collected on clean glass slides. Sections on slides were placed in a 50 °C oven for at least 24 hours to allow the sections to adhere to the slides. Slides were stained with Harris's hematoxylin and eosin (H & E) and viewed with a light microscope. A total of 154 slides were examined, 78 prepared from apparently healthy tissues and 76 prepared from diseased tissues.

Apparently healthy tissues from six *Acropora palmata* colonies showing no signs of disease were also examined for comparison. These tissues were collected in the FKNMS between 1994 and 1997 for other projects. A total of 77 slides were prepared from these apparently healthy reference tissues. Reference tissues showed no signs of the histopathogy lesions observed in the white pox-diseased and apparently healthy tissues examined in this study.

Data Collection and Analysis

Coding systems, modified from those developed by Reimschuessel et al. (1992) and Peters (2001), were used to classify the lesion abnormalities (Table 6.2) and to record the

severity and extent of each lesion (Table 6.3). Lesion extent was classified as focal, multifocal, or diffuse. Lesion severity was assigned a rank of minimal, mild, moderate, marked, or severe. An extent-severity index was developed based on combinations of extent and severity rankings (Table 6.3). Zooxanthellae abundance was scored as (1) normal or greater than normal numbers, (2) apparently normal numbers, (3) slight loss, (4) moderate loss, or (5) complete loss (Peters 2001).

The number of slides showing a given lesion in apparently healthy versus diseased coral tissues were compared conditionally on nesting each lesion within its composite anatomical structure, system, site, and subsite. Apparently healthy and diseased outcomes are considered a two-category multinomial random variable for a given lesion for each level of nesting. Nesting comparisons were made using a multinomial estimate technique calculating exact, simultaneous 95% confidence intervals for each maximum likelihood category point estimate. The null hypothesis of equality of apparently healthy and diseased coral tissues was rejected if non-overlapping 95% simultaneous confidence intervals were observed within a nesting. All tests were performed using StatXact-4 for Windows (Cytel Software, 1998). To look for overall differences in the number of lesions found within each colony, a paired t-test was used to compare all diseased slides and all apparently healthy slides for each colony. A nested ANOVA with structure and health status as the nested variables was used to determine the difference between apparently healthy and diseased tissues in the number of lesions among all anatomical structures. Paired t-test and ANOVA statistics were performed using Systat computer software.

Extent-severity index data were combined for each lesion type across all structures, systems, and sites. Data were analyzed using a stratified Wilcoxon Rank Sum test to compare healthy versus diseased tissues by their ordinal categorical responses calculated for each extent-

severity index category. The index data were analyzed with 2 x 10 contingency tables using 10 of the 15 extent-severity categories (F:1-M:5, Table 6.3). The more severe categories (D:1-D:5, Table 6.3) were eliminated from the analysis because these categories had minimal to no data. The stratum variable for each table was the type of lesion. To look for an overall difference for all lesion types combined in the extent-severity index, 23 2 x 10 contingency tables were simultaneously compared; each table using a different lesion type as a stratum variable. All tests were performed using StatXact-4 for Windows (Cytel Software, 1998).

RESULTS

Lesions were recorded from six anatomical structures of the coral: coenosarc, oral disk, pharynx, gastric cavity, mesenteries, and mesenterial filaments (Table 6.2, Fig. 6.2A). The coenosarc (Fig. 6.2A, B) covers the entire surface of the coral colony and connects the coral polyps at the surface. The oral disk (Fig. 6.2A, C) is the uppermost surface of the coral polyp and contains the mouth. The mouth is connected to the gastric cavity by a short tube called the pharynx (Fig. 6.2A, D). The gastric cavity contains internal tissue partitions termed mesenteries (Fig. 6.2A, C, D). The mesenterial filaments (Fig. 6.2A, E) are extensions of the mesenteries and are composed of lobes and a cnidoglandular band. The coenosarc, oral disk, pharynx, and gastric cavity consist of three tissue layers: epidermis, mesoglea, and gastrodermis. The mesenteries and mesenterial filaments are each composed of two layers of gastrodermis separated by mesoglea (Peters 2001).

The total number of slides in which each anatomical structure was seen is presented in Table 6.4. Calculations of lesion frequency within each structure were based on the total number of slides in which each structure was present (Table 6.5). Lesions and observations and their

frequencies in apparently healthy and white pox-diseased tissues are presented in Table 6.5. Results of the multinomial estimate technique, indicating whether the frequency of each lesion is significantly different between apparently healthy and diseased tissues, are presented in Table 6.5 and Table 6.6. The extent and severity index for each lesion is presented in Table 6.7. In addition to observations of gonad development, a total of 26 different types of lesions were observed (B-AA, Table 6.5). These lesion types were defined as specific lesions (e.g., necrosis, atrophy, etc.) that may have occurred in multiple structures, systems, or sites. When each individual lesion in each structure, system, site, or subsite was considered separately, and observations of gonad development were excluded, there were a total of 78 different lesions (N = 4-81, Table 6.5).

The apparently healthy and white pox-diseased *Acropora palmata* tissues did not differ significantly in the total number of lesions, even when the data were controlled for coral colony (paired t-test, $t = 0.479$, $df = 4$, $p = 0.657$). The total number of lesions among the different anatomical structures showed no significant difference between apparently healthy and white pox-diseased tissues (nested ANOVA, $n = 208$, $f = 1.091$, $p = 0.371$), and there was no significant difference in the total number of lesions within each of the anatomical structures. The frequency of occurrence of some lesions were significantly different between apparently healthy and diseased tissues (Table 6.5, Table 6.6). Seven of the 26 lesion types showed a significant difference in the extent and severity between apparently healthy and diseased tissues (Table 6.8). When all lesion types were combined, there was a significant difference in overall extent and severity of lesions between apparently healthy and diseased tissues (Table 6.8).

Reproduction

Gonads were present in three of the five *Acropora palmata* colonies, ova in colonies 2, 3, and 4 and spermaries in colony 3 (Fig. 6.2F). Mature ova (Fig. 6.3A), characterized by a vitelline membrane containing a vacuolated cytoplasm of lipid droplets and a large nucleus (Peters 1984b), were present in significanlty more apparently healthy than diseased tissues (Table 6.5). Degenerating ova (Fig. 6.3B), characterized by degeneration of the vitelline membrane and release of the cytoplasm, were observed in diseased tissues of colony 4 and apparently healthy tissues of colony 2. Ova in diseased tissues of colony 4 were released from the mesenteries (Table 6.5).

Microorganism Interactions

Apparently healthy and white pox-diseased tissues exhibited interactions with microorganisms including endolithic fungi and algae, bacteria, protozoa, and crustaceans (Table 6.5). Bacteria, crustaceans, and protozoan ciliates were rare in coral tissues, affecting 6%, 2%, and 15% of coral tissues, respectively (Table 6.5).

Fungal formations (Fig. 6.3C), termed pearls, were observed in all five *Acropora palmata* colonies. Pearls were formed in the skeleton adjacent to coral tissue in both apparently healthy (colonies 1, 4, and 5) and diseased corals (colonies 1, 2, 3, 4, and 5), but were significantly more abundant in diseased than in apparently healthy tissue samples (Table 6.5, Fig. 6.4). The extent and severity of this lesion was significantly different between apparently healthy and diseased tissues (Table 6.8, Fig. 6.5).

Growth of filamentous microorganisms, including algae and fungi, and the infiltration of these filaments into the skeletal-tissue matrix (Fig. 6.3D) were observed in apparently healthy

and diseased tissues from all five coral colonies. The identification of algae and fungi in H & E-stained sections is complicated, so these microorganisms were combined as "filamentous microorganisms" for the purpose of this report. Growth of filamentous microorganisms was observed at the skeletal-tissue interface (Fig. 6.3D). Filamentous microorganisms infiltration describes the invasion of fungal and algal filaments into the skeletal-tissue matrix. The frequency of filamentous microorganism growth was significantly greater in diseased tissues (Table 6.5, Fig. 6.4). The extent and severity of both filamentous microorganism growth and filamentous microorganism inflitration were significantly different between apparently healthy and diseased tissue samples (Table 6.8, Fig. 6.6, Fig. 6.7). Filamentous microorganism infiltration was observed in diseased tissues of colony 2 and in apparently healthy and diseased tissues of colonies 1, 3, 4, and 5.

Cellular and Tissue Degeneration

A variety of lesions that demonstrate cellular and tissue degeneration were observed in both apparently healthy and white pox-diseased tissues. Necrosis, the pathologic death of cells or tissues, was common in both apparently healthy and white pox-diseased samples. Necrosis was observed in the coenosarc (epidermis, mesoglea, gastrodermis), oral disk (epidermis, gastrodermis), pharynx (epidermis, gastrodermis), gastric cavity (epidermis, mesoglea, gastrodermis), and mesenterial filaments (mesoglea, gastrodermis). The frequency of necrosis in the oral disk was significantly greater in diseased tissues, while the frequency of this lesion in the pharynx and gastric cavity were significantly greater in apparently healthy tissues (Table 6.5). Necrosis was most frequently observed in the epidermis (100%) and gastrodermis (93%) of the coenosarc, in the epidermis of the oral disk (98%), and in the mesenterial filaments (98%, Table

6.5, Fig. 6.8). Necrosis of the gastric cavity and pharynx were also common (Table 6.5). Necrosis of the gastrodermis of the gastric cavity and of the epidermis of the pharynx were observed more frequently in apparently healthy than in diseased tissues (Table 6.5). Loss (i.e., absence) of the entire coenosarc was significantly more frequent in apparently healthy tissues (Table 6.5). The extent and severity of necrosis was significantly different between apparently healthy and diseased tissues (Table 6.8, Fig. 6.9).

Necrosis of the gastrodermis of the coenosarc is associated with the rounding up of the cuboidal gastrodermal cells and the detachment of these cells from one another (Table 6.5, Fig. 6.3E). Rounding and loss of adhesion of gastrodermal cells were indicative of sloughing of the gastrodermis in the coenosarc (Fig. 6.3F). Loss of adhesion of gastrodermal cells in the coenosarc was significantly more frequent in diseased tissues, while rounding of these cells in the coenosarc was significantly more frequent in apparently healthy tissues (Table 6.5). While loss of adhesion of gastrodermal cells was not always associated with rounding of these cells, rounding was always associated with loss of adhesion. Although rounding and loss of adhesion of gastrodermal cells were occasionally observed in the oral disk and pharynx, these lesions were most common in the coenosarc (Fig. 6.10), affecting 58% of apparently healthy and 51% of diseased tissue samples (Table 6.5). The extent and severity of rounding of gastrodermal cells was significantly different between apparently healthy and diseased tissues (Table 6.8, Fig. 6.11).

Thickening or swelling of tissues was observed in the coenosarc (epidermis, mesoglea), oral disk (epidermis, mesoglea), and pharynx (mesoglea). The epidermis of the coenosarc is a simple to pseudostratified columnar epithelium composed of ciliated columnar supporting cells and other types of cells (Fig. 6.2A, Peters 1984b, Peters 2001). The columnar cells of the

epidermis appeared to swell, increasing the thickness of the epidermal layer (Fig. 6.12A). Similarly, the mesoglea appeared swollen and thickened in affected tissues (Fig. 6.12B). Swollen tissues were most common in the epidermis of the coenosarc, affecting 97% of the tissues, but was also frequently observed affecting the mesoglea of the coenosarc (Table 6.5, Fig. 6.13). Thickening of the mesoglea was significantly more frequent in apparently healthy tissues (Table 6.5). The epidermis of the coenosarc also exhibited loss of the basophilic mucous secretory cells that are interspersed between the supporting cells (Fig. 6.12C, Peters 1984b, Peters 2001). Loss of mucous secretory cells was significantly more frequent in diseased tissues (Table 6.5, Fig 6.5).

Atrophy, the wasting or shrinking of tissues, was observed in the coenosarc (epidermis, mesoglea, gastrodermis), oral disk (epidermis, gastrodermis), pharynx (epidermis, gastrodermis), and gastric cavity (epidermis, mesoglea, gastrodermis). This lesion was significantly more frequent in diseased tissues of the oral disk and in apparently healthy tissues of the pharynx (Table 6.5, Fig. 6.14). Atrophy was most common in the epidermis and the gastrodermis of the coenosarc (Fig. 6.12C, Fig. 6.14), affecting at least 90% of the apparently healthy and diseased tissue samples (Table 6.5). Atrophy of the gastric cavity (Fig. 6.12D) was significantly more common in diseased tissues (Table 6.5).

Sloughing or loss of adhesion of intact cell layers was apparent in both diseased and apparently healthy tissues, and the extent and severity of this lesion was significantly different between apparently healthy and diseased tissues (Table 6.8, Fig. 6.15). Sloughing is characterized by the separation of an entire cell layer (e.g., epidermis, gastrodermis) from the mesoglea (Fig. 6.12E). Sloughing was observed in the coenosarc, oral disk, and pharynx. The gastrodermis of the coenosarc was most frequently affected by sloughing (Fig. 6.16), and the

lesion in this structure and site was significantly more common in apparently healthy tissues (Table 6.5). However, sloughing in the oral disk and pharynx was significantly more frequent in diseased tissues (Table 6.5).

Disrupted tissues were observed in the coenosarc, oral disk, and pharynx, but were most common in the gastric cavity (Table 6.5, Fig. 6.17). Disruptions were significantly more frequent in diseased tissues of the oral disk and in apparently healthy tissues of the coenosarc and the pharynx (Table 6.5, Fig. 6.17). In the coenosarc and oral disk, disrupted tissue transversed all three tissue layers. Disruptions in the gastric cavity affected all tissue layers and was indicative of tissue degeneration (Fig 6.12F). In the pharynx, disruptions in the epidermis occurred while the gastrodermis and mesoglea remained intact (Table 6.5).

Other Lesions

Acidophilic granular gland cells were present in the epithelium covering the cnidoglandular band of the mesenterial filaments in 98% of all tissues (Table 6.5, Fig. 6.18A). Granular gland cells are normally columnar and approximately the same size and shape as nematocysts. However, unlike nematocysts which contain a pink-stained tightly-coiled thread, the granular membrane-bound gland cells are filled with hydrolytic enzymes (lysosomes) that stain pink. The granular gland cells in the mesenterial filaments were observed to round up (Fig. 6.18B) in 100% of apparently healthy and 95% of white pox-diseased tissue samples (Table 6.5, Fig. 6.4). The rounded granular gland cells have a bumpy surface texture that gives them a "raspberry"-like appearance (Fig. 6.18B). Rounding of granular gland cells was also observed in the coenosarc, oral disk, pharynx, and gastric cavity. The abnormality, which is indicative of necrosis, was frequently encountered in epidermis of the pharynx in both apparently healthy

(48%) and diseased (46%) tissues (Table 6.5), although signs of necrosis of adjacent cells were not yet present.

Basophilic bodies (Fig. 6.18C), characterized by round to oval clusters of purple-stained cellular material, were observed in the coenosarc, oral disk, pharynx, gastric cavity, and mesenterial filaments. This lesion, which is the degeneration of mucus secretory cells, was most often observed in the epidermis of the oral disk and pharynx, and were significantly more common in apparently healthy tissues of the coenosarc, oral disk, and pharynx (Table 6.5, Fig. 6.4, Fig. 6.19).

Zooxanthellae

The majority of the apparently healthy and diseased coral tissues had apparently normal numbers of symbiotic algae (zooxanthellae), however, slight and moderate losses of zooxanthellae were observed in some tissues (Table 6.9). Lesions affecting the zooxanthellae included paling, vacuolation, and release (Table 6.5). Loss of pigment (i.e., paling) in symbiotic algae was most frequent in the coenosarc (Fig. 6.3F, Fig. 6.18D) and was significantly more common in diseased tissues (Table 6.5). Pale symbiotic algae were also observed in the oral disk, gastric cavity, and mesenterial filaments (Table 6.5, Fig. 6.20). Vacuolated symbiotic algae, characterized by a clear space or vacuole separating the outer cell wall from the plasma membrane, were also more frequent in the coenosarc and significantly more frequent in diseased tissues than apparently healthy tissues (Table 6.5). Vacuolated zooxanthellae were less frequently observed in the oral disk, pharynx, and mesenterial filaments (Table 6.5, Fig 6.21). Frequency of this lesion in the oral disk was significantly greater in apparently healthy tissues (Table 6.5)

Zooxanthellae were free-floating in the skeletal spaces adjacent to coral tissues, and likely had been released from necrotic tissues. The gastric cavity and mesenterial filaments exhibited degenerative changes including the loss of normal cellular architecture. These changes were especially pronounced in the lobes of the mesenterial filaments. Mesenterial filaments were released from the mesenteries and were free-floating in the coral tissues. Release of mesenterial filaments was significantly more frequent in diseased versus apparently healthy specimens (Table 6.5, Fig. 6.4). Necrotic tissues of the gastric cavity and mesenterial filaments were observed releasing zooxanthellae, and released symbiotic algae were observed on the epidermal surface of the coenosarc and the pharynx (Table 6.5). Released zooxanthellae in the gastric cavity were observed more frequently in apparently healthy specimens (Table 6.5). Necrosis of mucous secretory cells similar to those previously described from the coenosarc, oral disk, pharynx, gastric cavity, and mesenterial filaments, were observed within the symbiotic algae from one apparently healthy tissue sample (Table 6.5).

DISCUSSION

The lesions observed in the *Acropora palmata* colonies examined in this study are signs of disease. Although tissue appeared grossly normal on all *A. palmata* colonies, except at the margins of the disease lesions, histopathology revealed that both apparently healthy and diseased coral tissues were necrotic. There was no significant difference in the types of lesions or the total number of lesions observed in the white pox-diseased and apparently healthy tissues. The frequencies of 32 of the 78 lesions showed a significant difference between apparently healthy and diseased tissues. However, half (16) of these lesions were more common in diseased tissues (Table 6.5) . The overall extent and severity of lesions was significantly different between

apparently healthy and diseased tissues. However, only seven of the 26 individual lesion types showed a significant difference in extent and severity between apparently healthy and diseased tissues (Table 6.8).

Reproduction

Acropora palmata is a sexually reproducing hermaphroditic broadcast spawner with one reproductive cycle per year (Szmant 1986). Gonads develop from stem cells that migrate to the mesoglea of the mesenteries and differentiate into germ cells (Peters 1984b, Peters 2001). Gametogenesis in *A. palmata* is sequential, with ova production occurring in the months of September to May and spermary production occurring from May to July (Szmant 1986). In the Florida Keys, *Acropora palmata* populations release gonads in a synchronous spawning event that occurs two to four nights after the August full moon (Szmant 1986). The *A. palmata* tissues examined in this study were collected on August 6, 1998 two days prior to the full moon. Since these *A. palmata* samples were collected prior to spawning, tissues were expected to have relatively high numbers of gonads.

The reproductive status of a coral, as determined by histology of coral tissues, may reveal information about the health of the coral. Although both ova and spermaries were observed in the *Acropora palmata* tissues collected for this study, gonads were seen relatively infrequently. This is especially true for the spermaries which were observed in only one (colony 3) of the five coral colonies and in only 1% of the examined tissues. Overall, ova were present in 6% of the coral tissues, with mature ova in colonies 2 and 3 and developing ova in colony 4. Mature ova were more common in apparently healthy tissues. Degenerating ova were present in two of the white pox-diseased colonies, and have been reported from white band-diseased *A. palmata* from

Bonaire (Peters et al. 1983). Histopathology of corals has shown that stressors, including oil pollution (Rinkevich & Loya 1977, Rinkevich & Loya 1979, Peters et al. 1981, Guzmán & Holst 1993), eutrophication (Tomascik & Sander 1987), bleaching (Glynn et al. 1985), and disease (Peters et al. 1983), adversely affect gonad development and reproductive capacity in corals.

An altered reproductive system can affect the status of a coral population (Peters 2001). High fertilization success via sexual reproduction requires that population numbers be high enough to support success. Populations of *Acropora palmata* in the FKNMS have been declining since 1996, when white pox emerged on Floridian reefs, with losses averaging 88% by 2002 (Patterson et al. 2002, Sutherland & Ritchie *in press*). Decimation of *A. palmata* populations may limit the reproductive capacity of this species. Perhaps it is fortunate that *A. palmata* reproduces primarily by colony fragmentation (Lirman 2000). However, while colony fragmentation may be well adapted to recolonization following mechanical disturbances such as hurricanes, asexual reproduction is ineffectual following severe population declines due to disease, which frequently kills the entire coral colony.

Microorganism Interactions

Significant numbers and clustering of bacteria were not visible in the white pox-diseased colonies. Clustering of microorganisms at the disease interface has been observed in histopathology of black-band and white plague type I-affected corals (Peters 1984a, Bythell et el. 2002). Black-band diseased tissues are associated with a dense microbial community (Peters 1984a, Bythell et al. 2002). White plague type I-affected tissues are associated with dense clusters of coccoid bacteria (Bythell et al. 2002). The morphology of these bacteria differs from

the rod-shaped bacterium *Aurantimonas coralicida* (Bythell et al. 2002) that causes white plague type II (Richardson et al. 1998b, Denner et al. 2003).

Although clustering of microorganisms are not associated with the disease boundary in white band type I affected tissues (Peters 1984a, Bythell et al. 2002), this disease is associated with Gram-negative bacterial aggregates (Peters et al. 1983, Peters 1984a). Tissues of some white band type I-diseased and apparently healthy *Acropora palmata* and *A. cervicornis* from the US Virgin Islands, Bonaire, the Bahamas, and the Florida Keys contain these aggregates (Peters et al. 1983, Peters 1984a). The etiological association, if any, between the bacterial aggregates and white band type I has not been established. In fact, similar bacterial aggregates have been observed in the tissues of five additional scleractinian species (*Agaricia agaricites, Porites astreoides, P. compressa, P. lobata,* and *P. porites*) that show no signs of disease (Peters 1984a, Peters, personal communication). Bacterial aggregates were not present in any of the *A. palmata* tissues collected for this study. Other investigators have noted the absence of these aggregates in histopathology sections of white band type I diseased *A. palmata* (Bythell et al. 2002).

Protozoan parasites have been reported from the histological examinations of Caribbean corals (Peters 1984a, Upton & Peters 1986). A sporozoan, *Nematopsis* sp., caused hypertrophy of the calicoblastic epithelium in apparently healthy *Porites porites* (Peters 1984a). The coccidia, *Gemmocystis cylindrus,* were found in the lobes of mesenterial filaments of apparently healthy colonies of *Dendrogyra cylindrus, Diploria strigosa, Porites astreoides,* and *P. porites* and in the gastrodermis of partially bleached and necrotic colonies of *Agaricia agaricites, Montastrea cavernosa* and *Meandrina meandrites* (Peters 1984a, Upton & Peters 1986). Protozoan ciliates were tentatively identified in association with necrotic tissues of *P. astreoides,*

P. porites, and *Acropora palmata* (Peters 1984a). None of these protozoans were present in the samples examined in this study.

The fungal pearls observed in apparently healthy and white pox-affected tissues of *Acropora palmata* may be similar to those described from the Indo-Pacific corals *Porites lobata* and *P. lutea* (Le Champion-Alsumard et al. 1995, Ravindran et al. 2001) and the Caribbean corals *Manicina areolata* and *Montastraea cavernosa* (Peters, personal communication). These corals detect invasion by endolithic fungi and respond by surrounding the site of fungal penetration within a layer of repair calcium carbonate (Le Champion-Alsumard et al. 1995, Ravindran et al. 2001). However, this defense mechanism fails to hinder fungal advancement, and, as hyphae penetrate the layer of calcium carbonate repair, the coral repeats the process, resulting in a calcareous skeletal protuberance composed of a number of carbonate layers (i.e. a fungal pearl, Le Champion-Alsumard et al. 1995). The calcium carbonate skeleton of apparently healthy and white pox-diseased corals was not examined with scanning electron microscopy prior to decalcification and therefore it is not known whether the fungal pearls observed in this study were surrounded by layers of carbonate repair. As described for the fungal pearls associated with other scleractinian corals (Le Champion-Alsumard et al. 1995, Ravindran et al. 2001), skeletal lesions, if present, were not grossly visible on the *A. palmata* examined in this study.

Fungi and algae observed in apparently healthy and white pox-affected coral tissues are likely endolithic organisms or secondary pathogens that opportunistically invaded the white pox-diseased corals. Fungal pearls and filamentous microorganisms were significantly more abundant in diseased tissues. Fungi and algae are common components of apparently healthy corals (Kendrick et al. 1982, Le Champion-Alsumard et al. 1995, Ravindran et al. 2001) and are

associated with a number of coral diseases including black-band (Ramos-Flores 1983), aspergillosis (Smith et al. 1996, Geiser et al. 1998), pink-line syndrome (Ravindran et al. 2001, Ravindran & Raghukumar 2002), fungal-protozoan syndrome (Cerrano et al. 2000) and skeletal anomalies (Morse et al. 1977, Morse et al. 1981, Goldberg et al. 1984).

Cellular and Tissue Degeneration

Degenerative changes of coral cells and tissues have been shown to be associated with oil pollution (Peters et al. 1981), sedimentation (Peters & Pilson 1985), bleaching (Lasker et al. 1984, Glynn et al. 1985), and disease (Peters 1984a, Richardson et al. 1998b, Bythell et al. 2002, Peters et al. 1983, Peters 1984a). Necrosis of coral tissues was observed in the white pox-diseased and apparently healthy tissues, particularly degenerative changes including tissue atrophy, rounding and loss of adhesion of gastrodermal cells, sloughing and disruption of intact tissue layers, and loss of mucous secretory cells.

Atrophy and thickening of tissues are two types of lesions associated with white pox disease that, by definition, represent polar opposites effects on the coral tissue. However, while atrophied tissues of *Acropora palmata* colonies remained intact (Fig. 6.12C), thickened tissues (of the epidermis and gastrodermis) were necrotic (Fig. 6.12A).

Thickening or swelling of the mesoglea of the coenosarc was not associated with necrosis. This lesion was common in white pox-diseased corals and has been reported associated with both coral bleaching (Lasker et al. 1984) and wound repair in the sea anemone (Patterson & Landolt 1979). However, swelling of the mesoglea may be a normal alteration, as the thickness of this tissue layer is capable of change (e.g., atrophy, thickening) in apparently healthy corals (Peters 2001). According to Peters (2001), the mesoglea is relatively resistant to damage, and

indeed, very few lesions were observed in the mesoglea of the *Acropora palmata* tissues examined in this study.

Degradation of the extracellular matrix that holds the gastrodermal cells together was evidenced by the rounding up and loss of adhesion of gastrodermal cells. As the gastrodermis is sloughed, so are the zooxanthellae that provide a means of nutrition and skeletal accretion (Porter et al. 1989, Brown 1997) and are critical to the health status of the coral. Sloughing of the gastrodermis can be a mechanism of coral bleaching (Peters 2001), i.e. the loss of zooxanthellae and/or their pigments from host coral tissues, resulting in pale brown to translucent living tissue. Although some zooxanthellae in the necrotic gastrodermis were pale and vacuolated, the pigmentation of the white pox-diseased *Acropora palmata* colonies appeared grossly normal at the time of collection.

Loss of mucous secretory cells has been reported from histopathology of scleractinian corals affected by bleaching (Glynn et al. 1985), oil contamination (Peters et al. 1981), and sediment accumulation (Peters & Pilson 1985). White pox-diseased tissue samples showed a greater frequency of mucous secretory cell loss than did apparently healthy tissues. Mucous secretory cells contain mucopolysaccharides (Ducklow & Mitchell 1979a) that serve as the first line of defense of corals against invaders (Santavy & Peters 1997). Sloughing of mucus forms a protective layer of mucopolysaccharides on the surface of coral tissues and serves as an important defense mechanism against attachment of potentially pathogenic microorganisms to surface tissues (Ducklow & Mitchell 1979b, Rublee et al. 1980). Four of the five described pathogens affecting scleractinian corals have been isolated from the surface mucopolysaccharide layers of diseased corals. These coral pathogens include the causal agents of white pox (*Serratia marcescens,* Patterson et al. 2002), white plague type II (*Aurantimonas coralicida*, Richardson et

al 1998a, Richardson et al 1998b, Denner et al. 2003), and pathogen-induced bleaching (*Vibrio shiloi* and *V. coralliilyticus*, Kushmaro et al. 1996, Kushmaro et al. 1997, Ben-Haim & Rosenberg 2002, Ben-Haim et al. 2003). The basophilic bodies observed in the apparently healthy and white pox-diseased coral tissues are remnants of necrotic mucous secretory cells. Since the granular bodies were observed more frequently in apparently healthy tissues, their reduced numbers in diseased tissues may be a sign of stress.

Necrosis of white pox-diseased corals occurs throughout the entire coral colony., i.e. in both diseased and apparently healthy tissues. Necrosis associated with other coral diseases, including black band and white plague type I, occurs only at the disease interface (Peters 1984a, Richardson et al. 1998b, Bythell et al. 2002). A whole-colony effect of tissue degeneration and necrosis, similar to that associated with white pox, was observed with histopathology of apparently healthy and diseased tissues from white band type I diseased *Acropora palmata* and *A. cervicornis* (Peters 1984a). Of particular interest is the rounding of granular gland cells, found in high numbers in the mesenterial filaments and the pharynx of apparently healthy *Acropora palmata* (Peters 1984b). The acidophilic granules are lysosomes, membrane-bound vesicles that contain lysosomal enzymes. Lysosomal enzymes contain bactericidal and hydrolytic substances that function in digestion and play a critical role in killing phagocytized microorganisms (Moore 1982, Bayne 1990, Sinderman 1990). Lysosomal enzymes are an important component of humoral immunity in corals (Sinderman 1990). Rounding of granular gland cells was frequently observed in the mesenterial filaments and in the epidermis of the pharynx and indicates the sensitivity of these cells to the *Serratia marcescens* pathogen or a toxin secreted by the bacterium. If these cells that function in defense are the primary target of the pathogen or toxin, then the host coral, once infected, could not kill the pathogen.

Zooxanthellae

The *Acropora palmata* colonies examined in this study showed classic signs of white pox disease and no signs of bleaching. Pigmentation of all corals appeared grossly normal. Coral tissues predominately contained apparently normal numbers of zooxanthellae, although moderate losses of symbiotic algae occurred histologically. Paling and vacuolation of symbiotic algae did not appear to affect the pigmentation of the coral tissues.

Conclusion

White pox disease appears to be associated with (1) rounding of granular gland cells in the mesenterial filaments, (2) atrophy of the coenosarc, (3) necrosis of the coenosarc, oral disk, gastric cavity, and mesenterial filaments, and (4) disruptions in the gastric cavity (Table 6.5). Most lesions were concentrated in the coenosarc tissue. The epidermis of the coenosarc showed high frequency of necrosis, atrophy, and thickening. However, the extracellular matrix of this tissue layer was not degraded and the epidermis remained intact. The gastrodermis of the coenosarc exhibited the greatest number of frequently encountered lesions including necrosis, atrophy, rounding and loss of adhesion of gastrodermal cells, and sloughing of the intact gastrodermis. Zooxanthellae within the gastrodermis of the coenosarc were often pale and vacuolated. The mesoglea of the coenosarc showed relatively high frequencies of thickening or swelling. Diseased tissues were especially vulnerable to interactions with filamentous microorganisms.

Previous studies involving the histopathology of coral tissues have shown a characteristic pattern of tissue necrosis in which the epidermis and gastrodermis undergo liquefactive necrosis while the mesoglea remains intact (E.C. Peters pers. comm.). White pox disease is characterized

by the swelling of the epidermis and mesoglea and by necrosis of the epidermis and gastrodermis. However, the mesoglea and epidermis, though necrotic and swollen, remained intact, while the gastrodermis was sloughed.

Tissue necrosis in white pox-affected corals may be associated with a toxin produced by the coral pathogen, *Serratia marcescens*. *S. marcescens* is a cytotoxic bacterium (Carbonell et al. 1996, Carbonell et al. 1997, Marty et al. 2002, Carbonell et al. 2003) that secretes a number of virulence factors including chitinases, proteases, lipases, a lectinase, a nuclease, and a hemolysin (Hines et al. 1988, Aucken et al. 1998). The *S. marcescens* that causes white pox disease (isolate PDL100, Patterson et al. 2002) produces lipases and proteases (Polson 2002), but the role that these enzymes play in pathogenesis is unknown.

Lesions observed in the white pox-diseased colonies of *Acropora palmata* are signs of disease and are different in this disease than in other coral diseases (E.C. Peters pers. comm.). Tissues that appear grossly normal show the same histopathological signs of disease as those that appear grossly degenerative. Areas of normal cell structure and composition were evident in most of the apparently healthy and white pox-diseased tissues examined in this study, indicating that the changes observed were not artifacts of poor fixative penetration or processing. These samples were collected, handled, and prepared in a manner similar to that done in other coral histopathology studies (E.C. Peters pers. comm) and the lesions were not the result of physical damage during these activities.

The similarity in both the lesions observed and their frequency and severity in white pox-diseased and apparently healthy tissues may indicate that *Acropora palmata* colonies affected by white pox disease sustain a systemic whole-colony reaction to infection. However, the white-pox-diseased and the apparently healthy tissue samples examined in this study were collected

from branch fragments having white pox disease lesions. Apparently healthy tissue samples collected from these fragments were a maximum of 10 cm away from the margin of disease lesions. Future histopathology studies of white pox disease will include the examination of tissues from (1) the disease margin on diseased colonies, (2) less than 10 cm from the disease margin on diseased colonies, (3) greater than 10 cm from the disease margin on diseased colonies, and (4) apparently healthy colonies showing no signs of disease. The patchy distribution of white pox disease lesions, characterized by bare skeleton, on affected colonies may be explained by the apparent systemic effect of this disease on coral tissues. However, the timing and spatial appearance of the gross lesions, could vary according to microhabitat features on the colony surface affecting current speeds, time since infection, or other factors.

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Fig. 6.1. **A**. *Acropora palmata* colony affected with white pox in the Florida Keys National Marine Sanctuary (Photograph by JW Porter). **B**. White pox disease lesions on *A. palmata* (Photograph by KP Sutherland).

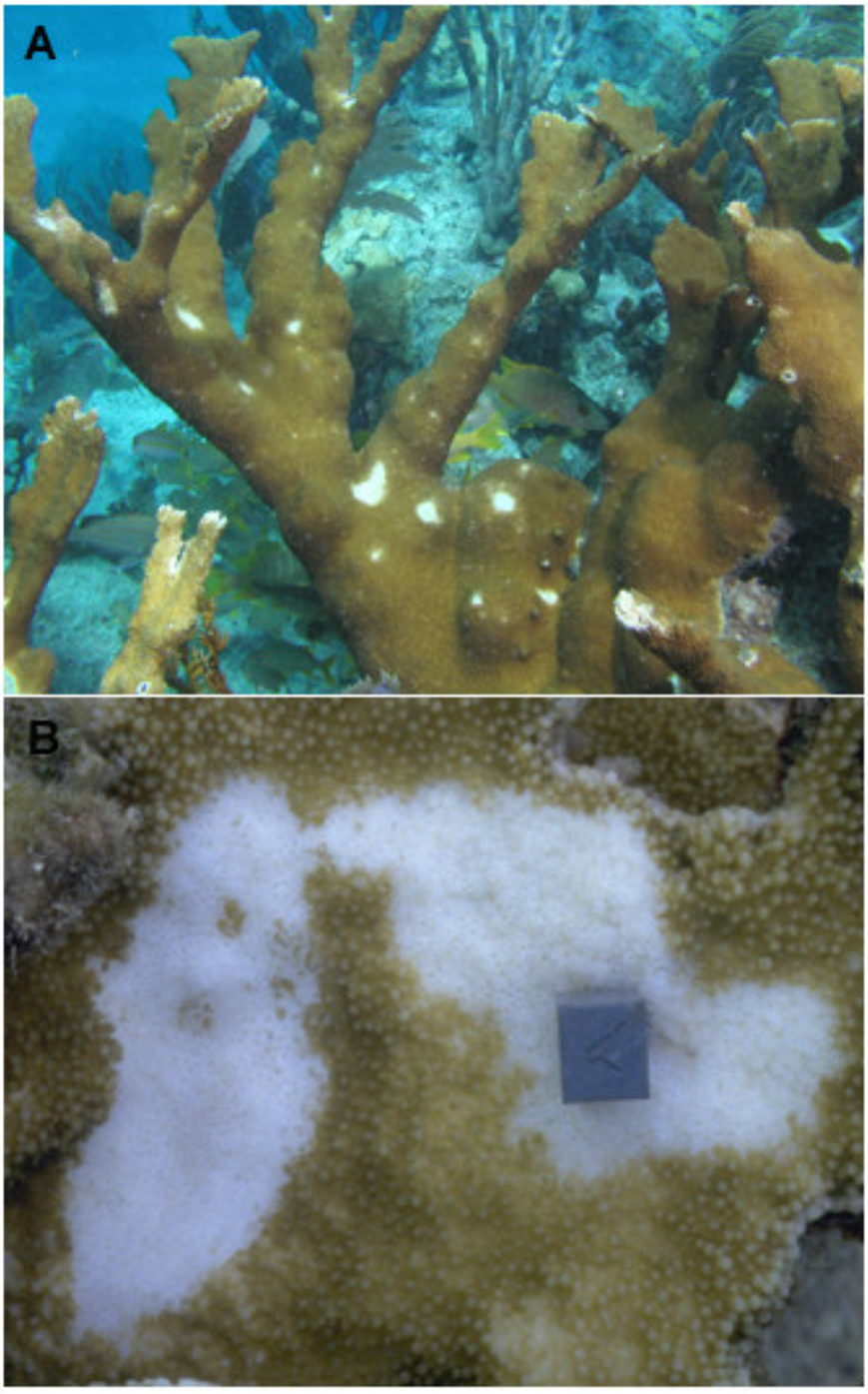

A
B

Table 6.1. Procedure for embedding coral tissue in paraffin with an automated tissue processor.

Step	Reagent	Time
1	70% ethanol	15 hrs
2	80% ethanol	30 min
3	95% ethanol	30 min
4	95% ethanol	30 min
5	100% ethanol	30 min
6	100% ethanol	30 min
7	100% ethanol	30 min
8	xylene	30 min
9	xylene	30 min
10	xylene	30 min
11	paraffin (60 °C)	30 min
12	paraffin (60 °C)	30 min

Table 6.2. Coding system used to classify lesions observed in white pox-diseased and apparently healthy *Acropora palmata.*

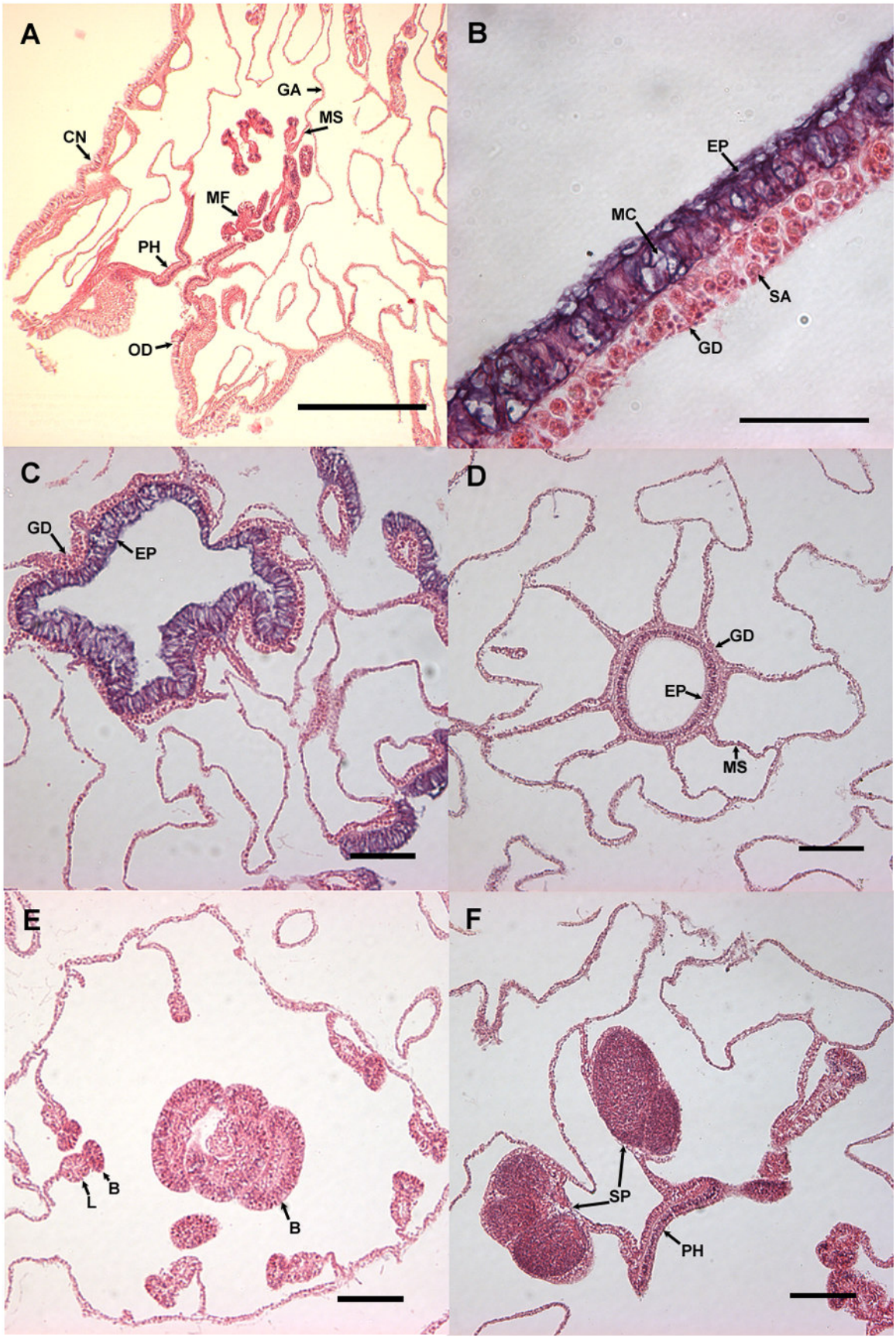
	Description	**Codes**	
Structure	anatomical structure within the coral polyp	**CN**	coenosarc
		OD	oral disk
		PH	pharynx
		GA	gastric cavity
		MS	mesenteries
		MF	mesenterial filaments
System	system within structure; structural or functional role of the tissue	**DS**	digestive system
		IS	integumentary system
		MS	musculoskeletal system
		PS	reproductive system
Site	site within system; tissue layer or gonads	**EP**	epidermis
		GD	gastrodermis
		MG	mesoglea
		GO	gonads
Subsite	subsite within site; cellular or tissue components	**GC**	gastrodermal cells
		MC	mucous secretory cells
		GR	granular gland cells
		SA	symbiotic algae
		OO	ova
		SP	spermaries
Change	type of change	**DC**	degenerative
		GC	growth
		IN	infectious
		PA	parasitism
		OB	observation
Lesion	type of lesion or observation	**GD**	gonad development
		DG	degenerating
		RE	released
		FP	fungal pearl
		MG	endolithic filamentous microorganism growth
		MI	endolithic filamentous microorganism infiltration

	Description	Codes	
Lesion	type of lesion	**BA**	bacteria
		CR	crustacean
		PR	protozoan
		PA	parasite
		LA	loss of adhesion
		SL	sloughing
		RU	rounding up
		NC	necrosis
		TH	thickened
		SW	swollen
		LO	loss of
		AT	atrophy
		DR	disrupted
		GB	granular bodies
		PL	pale
		VA	vacuolated
Additional	additional information	**DE**	developing
		MA	mature
		RE	released
		CI	ciliate

Table 6.3. Extent-severity index of lesions observed in apparently healthy and white pox-diseased tissues of *Acropora palmata.*

Extent Rank	**Extent Code**	**Severity Rank**	**Severity Code**	**Index Category**
Focal	F	minimal	1	F:1
Focal	F	mild	2	F:2
Focal	F	moderate	3	F:3
Focal	F	marked	4	F:4
Focal	F	severe	5	F:5
Multifocal	M	minimal	1	M:1
Multifocal	M	mild	2	M:2
Multifocal	M	moderate	3	M:3
Multifocal	M	marked	4	M:4
Multifocal	M	severe	5	M:5
Diffuse	D	minimal	1	D:1
Diffuse	D	mild	2	D:2
Diffuse	D	moderate	3	D:3
Diffuse	D	marked	4	D:4
Diffuse	D	severe	5	D:5

Fig. 6.2. Micrographs of anatomical structures of *Acropora palmata*. **A**. Cross-section of a coral polyp: coenosarc (CN), oral disk (OD), pharynx (PH), gastric cavity (GA), mesenteries (MS), mesenterial filaments (MF), scale bar = 500 µm. **B**. Coenosarc, including epidermis (EP), gastrodermis (GD), mucous secretory cells (MC), zooxanthellae (SA), scale bar = 50 µm. **C**. Oral disk, including epidermis (EP) and gastrodermis (GD), scale bar = 100 µm. **D**. Pharynx, including epidermis (EP) and gastrodermis (GD), and mesenteries (MS), scale bar = 100 µm. **E**. Mesenterial filaments, including lobes (L) and cnidoglandular band (B), scale bar = 100 µm. **F**. Mature spermaries (SP) and pharynx (PH), scale bar = 100 µm.


A
GA
MS
CN
MF
PH
OD
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GD
C
GD
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D
GD
EP
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E
L
B
B
F
SP
PH

Table 6.4. Number of slides of apparently healthy and white pox diseased tissues in which each anatomical structure was present. Total number of slides examined for each health status is also listed.

Structure	**Healthy**	**Diseased**	**Total**
coenosarc	77	71	148
oral disk	42	40	82
pharynx	60	52	112
gastric cavity	78	75	153
mesenterial filaments	77	74	151
total slides examined	**78**	**76**	**154**

Table 6.5. Lesions and observations in apparently healthy and white pox diseased tissues of *Acropora palmata.* The frequency of each lesion in apparently healthy tissues (H), white pox diseased tissues (D), and apparently healthy and diseased tissues combined (T) are listed. Calculations of lesion frequency within each structure were based on the total number of slides for each health status in which each structure was present. Lesion frequencies for which the multinomial estimate technique indicated a significant difference (S) between H and D are denoted (y). Those with no significant difference are denoted (n), and those for which the multinomial estimate could not be run due to a zero value in one health class are denoted (*). The different types of lesions and observations are designated (L) and each lesion or observation code is assigned a number (N).

				Frequency (%)			
L	**N**	**Code**	**Code Description**	**H**	**D**	**T**	**S**
Reproduction							
A	1	MS.PS.GO.OO-OB.GD. MA	gonad development, ova mature	6	4	5	y
	2	MS.PS.GO.OO-OB.GD.DE	gonad development, ova developing	1	0	1	*
	3	MS.PS.GO.SP-OB.GD.MA	gonad development, spermaries mature	0	1	1	*
B	4	MS.PS.GO.OO-DC.DG.MA	gonads, ova degenerating	3	3	3	n
C	5	MS.PS.GO.OO-DC.RE.MA	gonads, ova released	0	3	1	*
Microorganism Interactions							
D	6	GA-OB.FP	fungal pearl, gastric cavity	18	32	25	y
E	7	GA-IN.MG	filamentous microorganism growth	36	76	56	y
F	8	GA-IN.MI	filamentous microorganism infiltration of coral tissue	38	64	51	n
G	9	GA-IN.BA	bacteria, gastric cavity	6	5	6	n
H	10	GA-PA.CR	crustacean, gastric cavity	1	3	2	n
I	11	CN.IS.EP-PA.PR.CI	protozoan ciliate, coenosarc, epidermis	4	3	3	n
	12	CN.DS.GD-PA.PR.CI	protozoan ciliate, coenosarc, gastrodermis	0	3	1	*

				Frequency (%)			
L	**N**	**Code**	**Code Description**	**H**	**D**	**T**	**S**
Microorganism Interactions							
	13	GA-PA.PR.CI	protozoan ciliate, gastric cavity	9	12	10	n
J	14	GA-PA	unidentified parasite, gastric cavity	0	3	1	*
Cellular and Tissue Degeneration							
K	15	CN.DS.GD-DC.NC	necrosis, coenosarc, gastrodermis	88	99	93	n
	16	CN.IS.EP-DC.NC	necrosis, coenosarc, epidermis	100	100	100	n
	17	CN.MS.MG-DC.NC	necrosis, coenosarc, mesoglea	18	0	9	*
	18	CN-DC.NC	necrosis, coenosarc, all tissues	1	7	4	y
	19	MF-DC.NC	necrosis, mesenterial filaments, all tissues	100	96	98	n
	20	MF.MS.MG-DC.NC	necrosis, mesenterial filaments, mesoglea	13	0	7	*
	21	GA-DC.NC	necrosis, gastric cavity, all tissues	50	29	40	y
	22	OD.DS.GD-DC.NC	necrosis, oral disk, gastrodermis	45	65	55	y
	23	OD.IS.EP-DC.NC	necrosis, oral disk, epidermis	98	98	98	n
	24	PH.DS.GD-DC.NC	necrosis, pharynx, gastrodermis	18	25	21	n
	25	PH.IS.EP-DC.NC	necrosis, pharynx, epidermis	43	8	27	y
L	26	CN-GC.LO	loss of coenosarc, all tissues	16	6	11	y
M	27	CN.DS.GD-GC.DC.LA	loss of adhesion of gastrodermal cells, coenosarc	4	17	10	y
N	28	CN.DS.GD-GC.DC.RU	rounding of gastrodermal cells, coenosarc	58	51	55	y
	29	OD.DS.GD-GC. DC.RU	rounding of gastrodermal cells, oral disk	7	13	10	n
	30	PH.DS.GD-GC.DC.RU	rounding of gastrodermal cells, pharynx	0	10	4	*
O	31	CN.MS.MG-GC.TH	thickened, coenosarc, mesoglea	48	34	41	y
	32	CN.IS.EP-GC.TH	thickened, coenosarc, epidermis	97	97	97	n
	33	OD.MS.MG-GC.TH	thickened, oral disk, mesoglea	10	13	11	n
	34	OD.IS.EP-GC.TH	thickened, oral disk, epidermis	2	5	4	*

				Frequency (%)			
L	**N**	**Code**	**Code Description**	**H**	**D**	**T**	**S**
Cellular and Tissue Degeneration							
	35	PH.MS.MG-GC.TH	thickened, pharynx, mesoglea	13	0	7	*
P	36	CN.IS.EP-MC.GC.LO	loss of mucus secretory cells, coenosarc, epidermis	12	39	25	y
Q	37	CN.DS.GD-GC.AT	atrophy, coenosarc, gastrodermis	94	90	92	n
	38	CN.IS.EP-GC.AT	atrophy, coenosarc, epidermis	88	92	90	n
	39	GA-GC.AT	atrophy, gastric cavity, all tissues	19	36	27	y
	40	OD.DS.GD-GC.AT	atrophy, oral disk, gastrodermis	0	18	9	*
	41	OD.IS.EP-GC.AT	atrophy, oral disk, epidermis	2	18	10	y
	42	PH.DS.GD-GC.AT	atrophy, pharynx, gastrodermis	20	6	13	y
	43	PH.IS.EP-GC.AT	atrophy, pharynx, epidermis	7	0	4	*
R	44	CN.DS.GD-DC.SL	sloughing, coenosarc, gastrodermis	73	61	67	y
	45	CN.IS.EP-DC.SL	sloughing, coenosarc, epidermis	5	4	5	n
	46	OD.DS.GD-DC.SL	sloughing, oral disk, gastrodermis	5	15	10	y
	47	PH.DS.GD-DC.SL	sloughing, pharynx, gastrodermis	8	8	8	n
	48	PH.IS.EP-DC.SL	sloughing, pharynx, epidermis	2	8	4	y
S	49	CN-DC.DR	disrupted, coenosarc, all tissues	18	14	16	y
	50	OD-DC.DR	disrupted, oral disk, all tissues	2	15	9	y
	51	GA-DC.DR	disrupted, gastric cavity, all tissues	50	55	52	n
	52	PH-DC.DR	disrupted, pharynx, all tissues	12	10	11	n
	53	PH.IS.EP-DC.DR	disrupted, pharynx, epidermis	13	10	13	y

				Frequency (%)			
L	**N**	**Code**	**Code Description**	**H**	**D**	**T**	**S**
Other Lesions							
T	54	MF.DS.GD-OB.GR	granular gland cells, mesenterial filaments, gastrodermis	100	96	98	n
U	55	CN.DS.GD-GR.DC.RU	rounding of granular cells, coenosarc, gastodermis	4	0	2	*
	56	MF.DS.GD-GR.DC.RU	rounding of granular cells, mesenterial filaments, gastrodermis	100	95	97	n
	57	GA-GR.DC.RU	rounding of granular cells, gastric cavity	1	0	1	*
	58	OD.IS.EP-GR.DC.RU	rounding of granular cells, oral disk, epidermis	0	5	2	*
	59	PH.DS.GD-GR.DC.RU	rounding of granular cells, pharynx, gastrodermis	0	2	1	*
	60	PH.IS.EP-GR.DC.RU	rounding of granular cells, pharynx, epidermis	48	46	47	n
V	61	CN.IS.EP-GB	basophilic bodies, coenosarc, epidermis	14	1	8	y
	62	MF.DS.GD-GB	basophilic bodies, mesenterial filaments, gastrodermis	10	8	9	n
	63	GA-GB	basophilic bodies, gastric cavity	10	8	9	n
	64	OD.IS.EP-GB	basophilic bodies, oral disk, epidermis	83	55	70	y
	65	PH.DS.GD-GB	basophilic bodies, pharynx, gastrodermis	0	2	1	*
	66	PH.IS.EP-GB	basophilic bodies, pharynx, epidermis	38	23	31	y
W	67	MF-RE	released, mesenterial filaments, all tissues	10	20	15	y
Zooxanthellae							
X	68	CN.DS.GD-SA.DC.PL	pale symbiotic algae, coenosarc	14	35	24	y
	69	MF.DS.GD-SA.DC.PL	pale symbiotic algae, mesenterial filaments	3	5	4	y
	70	GA.DS.GD-SA.DC.PL	pale symbiotic algae, gastric cavity	3	9	6	y
	71	OD.DS.GD-SA.DC.PL	pale symbiotic algae, oral disk	0	5	2	*
Y	72	CN.DS.GD-SA.DC.VA	vacuolated symbiotic algae, coenosarc	38	49	43	y
	73	MF.DS.GD-SA.DC.VA	vacuolated symbiotic algae, mesenterial filaments	4	1	3	y
	74	OD.DS.GD-SA.DC.VA	vacuolated symbiotic algae, oral disk	29	18	23	y

				Frequency (%)			
L	**N**	**Code**	**Code Description**	**H**	**D**	**T**	**S**
Zooxanthellae							
	75	PH.DS.GD-SA.DC.VA	vacuolated symbiotic algae, pharynx	7	0	4	*
Z	76	CN.IS.EP-SA.DC.RE	released symbiotic algae, coenosarc, epidermis (surface)	0	1	1	*
	77	MF.DS.GD-SA.DC.RE	released symbiotic algae, mesenterial filaments	16	0	8	*
	78	GA.DS.GD-SA.DC.RE	released symbiotic algae, gastric cavity	15	9	12	y
	79	PH.IS.EP-SA.DC.RE	released symbiotic algae, pharynx, epidermis (surface)	5	0	3	*
AA	80	CN.DS.GD-SA.DC.GB	basophilic bodies in symbiotic algae, coenosarc	1	0	1	*
	81	GA.DS.GD-SA.DC.GB	basophilic bodies in symbiotic algae, gastric cavity	1	0	1	*

Table 6.6. Results of the multinomial estimate technique for each lesion (N) in apparently healthy and white pox-diseased tissues of *Acropra palmata.* The 95% confidence intervals for apparently healthy and diseased tissues are presented. Non-overlapping 95% simultaneous confidence intervals (boldface type) were used to reject the null hypothesis of equality of apparently healthy and diseased coral tissues. Lesions for which the multinomial estimate could not be run due to either equal observations of the lesion in apparently healthy and diseased tissues or a zero value in one health class are denoted (*).

N	**Healthy**	**Diseased**
4	*	*
5	*	*
6	**0.2977, 0.4091**	**0.5909, 0.7023**
7	**0.2200, 0.4493**	**0.5507, 0.7800**
8	0.2630, 0.5163	0.4837, 0.7370
9	0.4374, 0.6677	0.3323, 0.5626
10	0.1762, 0.5389	0.4611, 0.8238
11	0.4414, 0.7401	0.2599, 0.5586
12	*	*
13	0.3522, 0.5267	0.4733, 0.6478
14	*	*
15	0.4624, 0.5231	0.4769, 0.5376
16	0.4909, 0.5495	0.4505, 0.5091
17	*	*
18	**0.0852, 0.3004**	**0.6996, 0.9148**
19	0.4909, 0.5495	0.4505, 0.5091
20	*	*
21	**0.5944, 0.6819**	**0.3181, 0.4056**
22	**0.3708, 0.4754**	**0.5246, 0.6292**
23	0.4726, 0.5522	0.4478, 0.5274
24	0.3873, 0.5311	0.4689, 0.6127
25	**0.8161, 0.9049**	**0.0951, 0.1839**

N	Healthy	Diseased
26	**0.6658, 0.8188**	**0.1812, 0.3342**
27	**0.1366, 0.2831**	**0.7169, 0.8634**
28	**0.5159, 0.5945**	**0.4055, 0.4841**
29	0.2637, 0.5013	0.4987, 0.7363
30	*	*
31	**0.5612, 0.6502**	**0.3498, 0.4388**
32	0.4911, 0.5504	0.4496, 0.5089
33	0.3323, 0.5626	0.4374, 0.6677
34	*	*
35	*	*
36	**0.1966, 0.2969**	**0.7031, 0.8034**
37	0.4915, 0.5531	0.4469, 0.5085
38	0.4727, 0.5349	0.4651, 0.5273
39	**0.3063, 0.4114**	**0.5886, 0.6937**
40	*	*
41	**0.0634, 0.2315**	**0.7685, 0.9366**
42	**0.7169, 0.8634**	**0.1366, 0.2831**
43	*	*
44	**0.5298, 0.6008**	**0.3992, 0.4702**
45	0.4375, 0.6957	0.3043, 0.5625
46	**0.1579, 0.3721**	**0.6279, 0.8421**
47	0.4374, 0.6677	0.3323, 0.5626
48	**0.1029, 0.3526**	**0.6474, 0.8971**
49	**0.5104, 0.6528**	**0.3472, 0.4896**
50	**0.0727, 0.2615**	**0.7385, 0.9273**
51	0.4478, 0.5274	0.4726, 0.5522
52	0.4803, 0.6796	0.3204, 0.5197
53	**0.5755, 0.7469**	**0.2531, 0.4245**
54	0.4909, 0.5495	0.4505, 0.5091
55	*	*

N	Healthy	Diseased
56	0.4944, 0.5531	0.4469, 0.5056
57	*	*
58	*	*
59	*	*
60	0.4981, 0.5953	0.4047, 0.5019
61	**0.8413, 0.9580**	**0.0420, 0.1587**
62	0.4761, 0.6618	0.3382, 0.5239
63	0.4761, 0.6618	0.3382, 0.5239
64	**0.5672, 0.6589**	**0.3411, 0.4328**
65	*	*
66	**0.5979, 0.7118**	**0.2882, 0.4021**
67	**0.2809, 0.4214**	**0.5786, 0.7191**
68	**0.2537, 0.3628**	**0.6372, 0.7463**
69	**0.2138, 0.4790**	**0.5210, 0.7862**
70	**0.1397, 0.3346**	**0.6654, 0.8603**
71	*	*
72	**0.4092, 0.4978**	**0.5022, 0.5908**
73	**0.5733, 0.8701**	**0.1299, 0.4267**
74	**0.5500, 0.7062**	**0.2938, 0.4500**
75	*	*
76	*	*
77	*	*
78	**0.5500, 0.7062**	**0.2938, 0.4500**
79	*	*
80	*	*
81	*	*

Table 6.7. Extent and severity of lesions in apparently healthy (H) and white pox-diseased (P) tissues of *Acropora palmata.* Presented below are frequencies of each extent:severity category for each lesion (N = 4-82). Frequencies were calculated as percent of apparently healthy or white pox-diseased slides with a specific extent and severity of a given lesion.

		Extent:Severity Category														
N		**F:1**	**F:2**	**F:3**	**F:4**	**F:5**	**M:1**	**M:2**	**M:3**	**M:4**	**M:5**	**D:1**	**D:2**	**D:3**	**D:4**	**D:5**
4	**H**	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	-	50.0	-	-	-	50.0	-	-	-	-	-	-	-	-
5	**H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	50.0	-	-	-	-	50.0	-	-	-	-	-	-	-	-
6	**H**	14.29	28.57	14.29	14.29	-	-	14.29	7.14	7.14	-	-	-	-	-	-
	P	4.17	8.33	-	4.17	8.33	-	25.0	33.33	16.67	-	-	-	-	-	-
7	**H**	-	10.71	14.29	-	-	3.57	14.29	14.29	21.43	3.57	-	-	-	3.57	14.29
	P	3.51	15.79	19.30	-	-	5.26	14.04	10.53	-	1.75	-	-	-	5.26	24.56
8	**H**	-	10.0	3.33	-	-	6.67	13.33	26.67	36.67	-	-	-	-	-	3.33
	P	16.67	18.75	8.33	2.08	-	14.58	10.42	14.58	2.08	-	-	-	2.08	2.08	8.33
9	**H**	60.0	40.0	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	25.0	-	-	-	-	75.0	-	-	-	-	-	-	-	-	-
10	**H**	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	50.0	-	-	-	-	50.0	-	-	-	-	-	-	-	-	-
11	**H**	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	50.0	-	-	-	-	50.0	-	-	-	-	-	-	-	-	-

		Extent:Severity Category														
N		**F:1**	**F:2**	**F:3**	**F:4**	**F:5**	**M:1**	**M:2**	**M:3**	**M:4**	**M:5**	**D:1**	**D:2**	**D:3**	**D:4**	**D:5**
12	**H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	100.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	**H**	14.29	-	14.29	-	-	-	42.86	28.57	-	-	-	-	-	-	-
	P	66.67	11.11	-	-	-	-	11.11	11.11	-	-	-	-	-	-	-
14	**H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	50.0	-	-	-	-	-	-	-	50.0	-	-	-	-	-
15	**H**	-	-	1.47	-	-	-	30.88	30.88	35.29	1.47	-	-	-	-	-
	P	-	7.14	2.86	-	1.43	1.43	38.57	41.43	7.14	-	-	-	-	-	-
16	**H**	-	-	-	-	-	-	2.60	53.25	19.48	-	-	-	-	24.68	-
	P	-	1.41	1.41	2.82	-	-	9.86	52.11	29.58	-	-	-	-	2.82	-
17	**H**	-	-	36.36	-	-	-	9.09	45.45	9.09	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	**H**	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-
	P	-	20.0	20.0	60.0	-	-	-	-	-	-	-	-	-	-	-
19	**H**	2.60	3.90	2.60	1.30	-	2.60	37.66	41.56	6.50	-	-	-	-	1.30	-
	P	1.41	15.49	7.04	5.63	-	1.41	32.39	32.39	4.23	-	-	-	-	-	-
20	**H**	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	**H**	2.56	2.56	5.13	5.13	-	5.13	38.46	35.90	5.13	-	-	-	-	-	-
	P	-	27.27	-	4.55	-	9.09	36.36	22.73	-	-	-	-	-	-	-

		Extent:Severity Category														
N		**F:1**	**F:2**	**F:3**	**F:4**	**F:5**	**M:1**	**M:2**	**M:3**	**M:4**	**M:5**	**D:1**	**D:2**	**D:3**	**D:4**	**D:5**
22	**H**	10.53	10.53	-	-	-	-	63.16	15.79	-	-	-	-	-	-	-
	P	11.54	11.54	15.38	-	-	3.85	34.62	19.23	3.85	-	-	-	-	-	-
23	**H**	-	-	19.51	2.44	-	-	2.44	48.78	26.83	-	-	-	-	-	-
	P	2.56	5.13	15.38	15.38	-	-	25.64	17.95	17.95	-	-	-	-	-	-
24	**H**	-	45.45	9.09	9.09	-	9.09	9.09	18.18	-	-	-	-	-	-	-
	P	-	46.15	7.69	-	-	-	38.46	7.69	-	-	-	-	-	-	-
25	**H**	7.69	38.46	3.85	-	-	-	38.46	11.54	-	-	-	-	-	-	-
	P	-	50.0	-	-	-	-	25.0	25.0	-	-	-	-	-	-	-
26	**H**	-	-	-	-	25.0	-	-	-	-	75.0	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100
27	**H**	-	-	-	-	-	-	33.33	33.33	33.33	-	-	-	-	-	-
	P	-	-	-	-	-	-	41.67	58.33	-	-	-	-	-	-	-
28	**H**	-	-	-	-	-	-	47.73	40.91	11.36	-	-	-	-	-	-
	P	-	2.78	11.11	-	-	2.78	47.22	30.56	5.56	-	-	-	-	-	-
29	**H**	-	-	33.33	-	-	-	33.33	33.33	-	-	-	-	-	-	-
	P	-	20.0	-	20.0	-	-	20.0	40.0	-	-	-	-	-	-	-
30	**H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	20.0	40.0	-	-	-	-	40.0	-	-	-	-	-	-	-
31	**H**	2.70	10.81	-	-	-	8.11	56.76	21.62	-	-	-	-	-	-	-
	P	-	8.33	-	-	-	8.33	58.33	25.0	-	-	-	-	-	-	-

		Extent:Severity Category														
N		**F:1**	**F:2**	**F:3**	**F:4**	**F:5**	**M:1**	**M:2**	**M:3**	**M:4**	**M:5**	**D:1**	**D:2**	**D:3**	**D:4**	**D:5**
32	**H**	-	2.67	-	-	-	-	18.67	62.67	14.67	-	-	-	1.33	-	-
	P	-	5.80	4.35	1.45	-	-	24.64	63.77	-	-	-	-	-	-	-
33	**H**	-	-	-	-	-	-	50.0	50.0	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	20.0	80.0	-	-	-	-	-	-	-
34	**H**	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-
	P	-	50.0	-	-	-	-	-	50.0	-	-	-	-	-	-	-
35	**H**	25.0	-	12.50	-	-	-	37.50	25.0	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36	**H**	-	11.11	33.33	-	-	-	11.11	44.44	-	-	-	-	-	-	-
	P	3.57	3.57	7.14	3.57	-	10.71	39.29	25.0	7.14	-	-	-	-	-	-
37	**H**	-	1.43	2.86	-	-	1.43	11.43	54.29	25.71	-	-	-	1.43	1.43	-
	P	-	3.13	4.69	-	1.56	1.56	3.13	53.13	26.56	-	-	-	-	6.25	-
38	**H**	-	-	3.03	1.52	-	-	16.67	69.70	7.58	-	-	-	1.52	-	-
	P	-	1.54	7.69	3.08	-	1.54	7.69	61.54	16.92	-	-	-	-	-	-
39	**H**	-	-	-	-	-	13.33	73.33	13.33	-	-	-	-	-	-	-
	P	-	7.41	-	-	-	3.70	44.44	18.52	18.52	-	-	-	3.70	3.70	-
40	**H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	-	14.29	28.57	-	-	-	28.57	14.29	-	-	-	-	14.29	-
41	**H**	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	-	57.14	14.29	-	-	-	14.29	14.29	-	-	-	-	-	-

		Extent:Severity Category														
N		**F:1**	**F:2**	**F:3**	**F:4**	**F:5**	**M:1**	**M:2**	**M:3**	**M:4**	**M:5**	**D:1**	**D:2**	**D:3**	**D:4**	**D:5**
42	**H**	-	-	41.67	25.0	-	-	-	25.0	8.33	-	-	-	-	-	-
	P	-	-	66.67	-	-	-	-	33.33	-	-	-	-	-	-	-
43	**H**	-	25.0	0	25.0	-	-	25.0	25.0	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	**H**	1.79	1.79	1.79	-	-	19.64	35.71	25.0	14.29	-	-	-	-	-	-
	P	2.33	18.60	4.65	-	-	13.95	32.56	25.58	2.33	-	-	-	-	-	-
45	**H**	-	-	25.0	50.0	25.0	-	-	-	-	-	-	-	-	-	-
	P	-	33.33	-	-	-	-	33.33	33.33	-	-	-	-	-	-	-
46	**H**	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-
	P	33.33	-	-	-	-	33.33	33.33	-	-	-	-	-	-	-	-
47	**H**	80.0	-	-	20.0	-	-	-	-	-	-	-	-	-	-	-
	P	-	50.0	25.0	25.0	-	-	-	-	-	-	-	-	-	-	-
48	**H**	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-
49	**H**	-	-	28.57	14.29	-	-	14.29	21.43	21.43	-	-	-	-	-	-
	P	-	10.0	30.0	10.0	-	10.0	-	30.0	10.0	-	-	-	-	-	-
50	**H**	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	16.67	16.67	16.67	-	-	-	33.33	16.67	-	-	-	-	-	-

		Extent:Severity Category														
N		**F:1**	**F:2**	**F:3**	**F:4**	**F:5**	**M:1**	**M:2**	**M:3**	**M:4**	**M:5**	**D:1**	**D:2**	**D:3**	**D:4**	**D:5**
51	**H**	2.56	33.33	12.82	-	-	7.69	23.08	15.38	-	5.13	-	-	-	-	-
	P	2.45	17.07	9.76	4.88	-	2.44	24.39	36.59	2.44	-	-	-	-	-	-
52	**H**	-	28.57	42.86	-	-	14.29	-	14.29	-	-	-	-	-	-	-
	P	40.0	20.0	20.0	20.0	-	-	-	-	-	-	-	-	-	-	-
53	**H**	60.0	10.0	10.0	-	-	20.0	-	-	-	-	-	-	-	-	-
	P	60.0	20.0	-	-	-	-	-	20.0	-	-	-	-	-	-	-
54	**H**	1.30	98.7	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	8.45	-	-	-	-	90.14	-	-	-	-	1.41	-	-	-	-
55	**H**	-	-	33.33	-	-	-	-	66.67	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
56	**H**	-	-	-	-	-	-	6.49	85.71	3.90	-	-	-	2.60	1.30	-
	P	1.43	2.86	2.86	-	-	2.86	8.57	75.71	5.71	-	-	-	-	-	-
57	**H**	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	**H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-
59	**H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-
60	**H**	3.45	17.24	17.24	-	-	-	34.48	27.59	-	-	-	-	-	-	-
	P	25.0	37.50	4.17	-	-	-	16.67	16.67	-	-	-	-	-	-	-

		Extent:Severity Category														
N		**F:1**	**F:2**	**F:3**	**F:4**	**F:5**	**M:1**	**M:2**	**M:3**	**M:4**	**M:5**	**D:1**	**D:2**	**D:3**	**D:4**	**D:5**
61	**H**	9.09	45.45	18.18	-	-	-	-	27.27	-	-	-	-	-	-	-
	P	-	100	-	-	-	-	-	-	-	-	-	-	-	-	-
62	**H**	25.0	-	-	-	-	75.0	-	-	-	-	-	-	-	-	-
	P	50.0	-	-	-	-	50.0	-	-	-	-	-	-	-	-	-
63	**H**	-	-	50.0	37.5	-	-	-	12.5	-	-	-	-	-	-	-
	P	-	16.67	50.0	33.33	-	-	-	-	-	-	-	-	-	-	-
64	**H**	8.57	20.0	8.57	-	-	2.86	28.57	28.57	2.86	-	-	-	-	-	-
	P	4.55	13.64	22.73	-	-	-	18.18	27.27	13.64	-	-	-	-	-	-
65	**H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	**H**	21.74	17.39	21.74	4.35	-	8.70	13.04	8.70	4.35	-	-	-	-	-	-
	P	41.67	16.67	33.33	-	-	-	8.33	-	-	-	-	-	-	-	-
67	**H**	75.0	12.5	-	-	-	-	12.5	-	-	-	-	-	-	-	-
	P	40.0	-	6.67	-	-	40.0	6.67	6.67	-	-	-	-	-	-	-
68	**H**	-	-	-	-	-	-	81.82	18.18	-	-	-	-	-	-	-
	P	-	-	-	-	-	4.0	56.0	24.0	16.0	-	-	-	-	-	-
69	**H**	-	-	-	-	50.0	-	-	-	50.0	-	-	-	-	-	-
	P	-	25.0	25.0	25.0	-	-	-	-	25.0	-	-	-	-	-	-
70	**H**	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	14.29	42.86	-	-	-	-	42.86	-

		Extent:Severity Category														
N		**F:1**	**F:2**	**F:3**	**F:4**	**F:5**	**M:1**	**M:2**	**M:3**	**M:4**	**M:5**	**D:1**	**D:2**	**D:3**	**D:4**	**D:5**
71	**H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	100	-	-	-	-	-	-	-	-
72	**H**	-	-	-	-	-	31.03	65.52	3.45	-	-	-	-	-	-	-
	P	-	-	-	-	-	14.29	82.86	2.86	-	-	-	-	-	-	-
73	**H**	-	-	-	-	-	-	-	33.33	66.67	-	-	-	-	-	-
	P	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-
74	**H**	-	33.33	-	-	-	16.67	50.0	-	-	-	-	-	-	-	-
	P	-	14.29	-	-	-	-	85.71	-	-	-	-	-	-	-	-
75	**H**	-	50.0	-	-	-	-	25.0	25.0	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
76	**H**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	100	-	-	-	-	-	-
77	**H**	-	-	-	-	-	25.0	75.0	-	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
78	**H**	16.67	-	-	-	-	41.67	41.67	-	-	-	-	-	-	-	-
	P	28.57	-	-	-	-	14.29	42.86	14.29	-	-	-	-	-	-	-
79	**H**	66.67	-	-	-	-	-	-	33.33	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	**H**	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

		Extent:Severity Category														
N		**F:1**	**F:2**	**F:3**	**F:4**	**F:5**	**M:1**	**M:2**	**M:3**	**M:4**	**M:5**	**D:1**	**D:2**	**D:3**	**D:4**	**D:5**
81	**H**	-	-	-	-	-	-	-	100	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 6.8. Results of the stratified Wilcoxon Rank Sum Test comparing apparently healthy and white pox diseased tissues of *Acropora palmata* for each lesion type (L). Mann-Whitney test statistics, two-sided p-values, and whether exact (EX) or Monte-Carlo (MC) values were used are reported. For each lesion type (B-AA), 100,000 tables were run for each Monte-Carlo. For all lesion types combined (All), 1,000,000 tables were run. P-values of less than 0.05 (in bold typeface) indicate a significant difference in the extent-severity of an lesion type between apparently healthy and diseased tissues. Lesion types for which too few different extent-severity categories were scored to run the Wilcoxon Rank Sum Test are denoted (*).

L	**test statistic**	**values**	**p-value**
B	1	EX	1.0000
C	*	*	*
D	78	MC	**0.0048**
E	642.5	MC	**0.0075**
F	990	MC	**< 0.0001**
G	3.5	EX	0.1429
H	0.5	EX	1.0000
I	87.5	MC	0.1358
J	*	*	*
K	6.70E + 04	MC	**0.0001**
L	*	*	*
M	23	EX	0.5978
N	1365	MC	**0.0193**
O	6705	MC	0.3053
P	114	MC	0.6736
Q	1.32E + 04	MC	0.4865
R	2525	MC	**0.0178**
S	2039	MC	0.1424
T	459	MC	**< 0.0001**
U	5785	MC	0.0914
V	2245	MC	0.3327
W	37.5	MC	0.1065
X	251	MC	0.8084
Y	904	MC	0.1593
Z	122.5	MC	0.5560
AA	*	*	*
All	9.33E + 005	MC	**< 0.0001**

Fig. 6.3. Micrographs of apparently healthy and white pox-diseased tissues of *Acropora palmata*. **A**. Mature ova, characterized by a vitelline membrane (M) containing a vacuolated cytoplasm of lipid droplets (C) and a large nucleus (N), scale bar = 100 µm. **B**. Degenerating ovum, scale bar = 100 µm. **C**. Fungal pearl (FP), scale bar = 500 µm. **D**. Filamentous microorganism growth (MG) and invasion of the skeletal-tissue matrix (MI), scale bar = 500 µm. **E**. Necrotic tissues, including coensarc (CN), gastodermis (GD), and pharynx (PH), scale bar = 100 µm. **F**. Higher magnification of bottom right corner of photo E showing rounding up and loss of adhesion leading to sloughing of gastrodermal cells (GC), zooxanthellae (SA), and pale zooxanthellae (PL), scale bar = 50 µm.

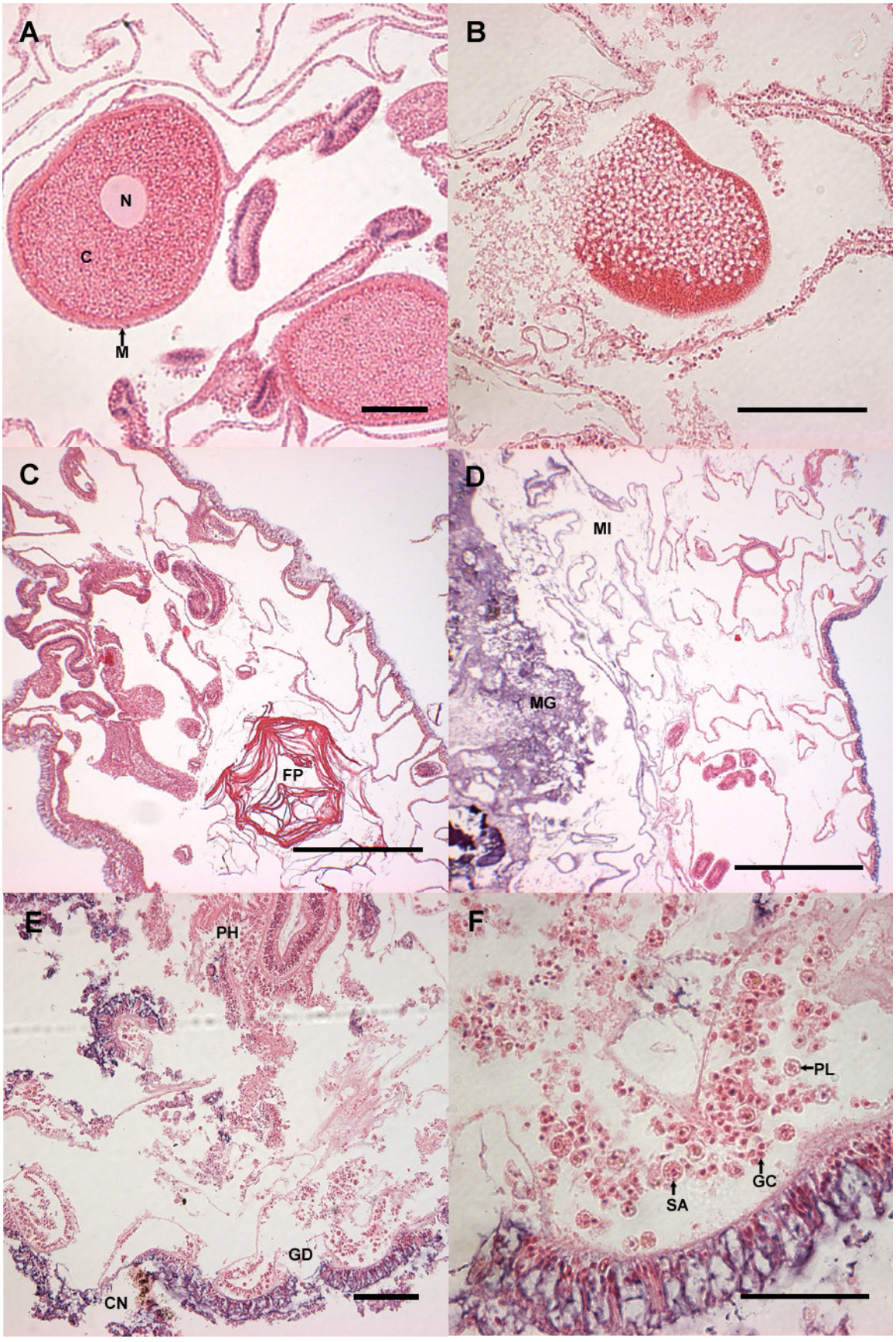

A
N
C
M
B
C
FP
D
MI
MG
E
PH
GD
CN
F
PL
GC
SA

Fig. 6.4. Number of observations of several different lesions in apparently healthy and white pox diseased tissues of *Acropora palmata.* Observations are summed for all structures, systems, and sites.

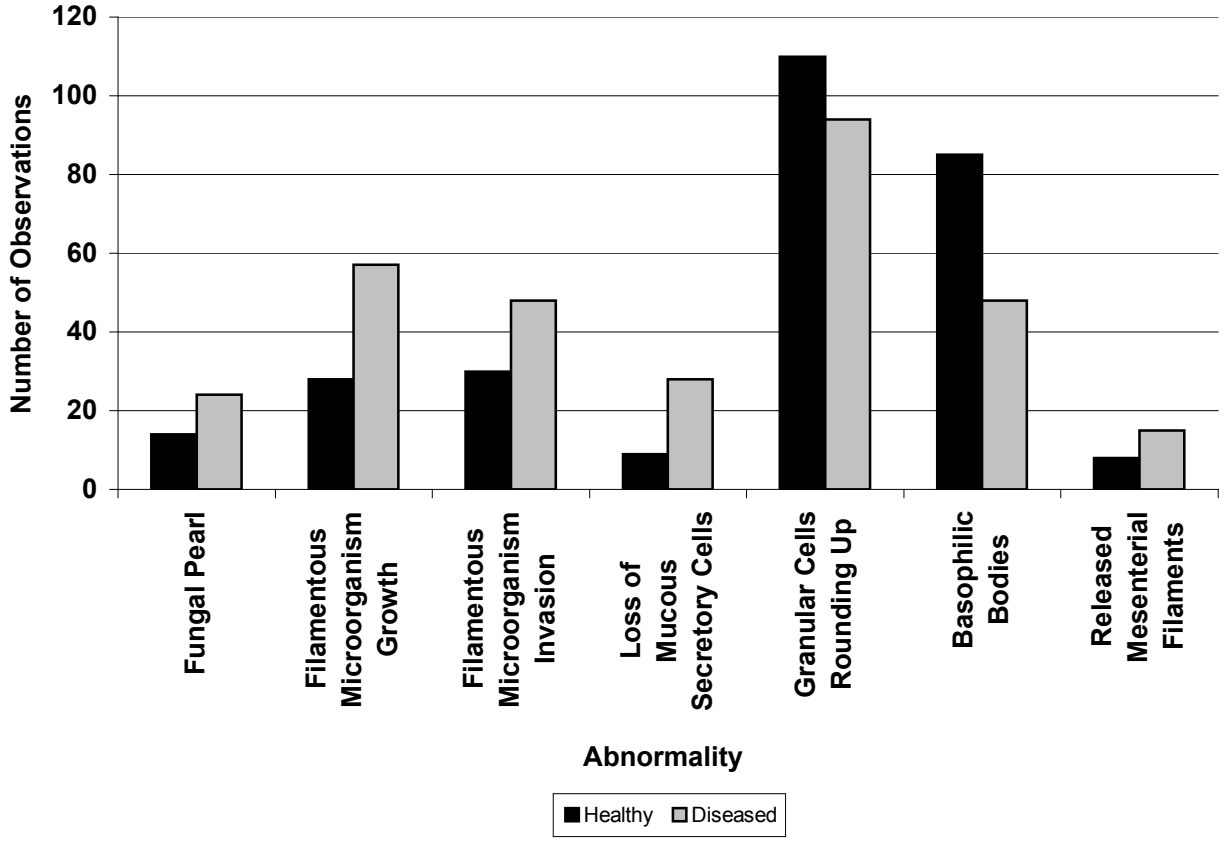


Fig. 6.5. Number of observations of each extent-severity category for fungal pearls in white pox diseased and apparently healthy *Acropora palmata.*

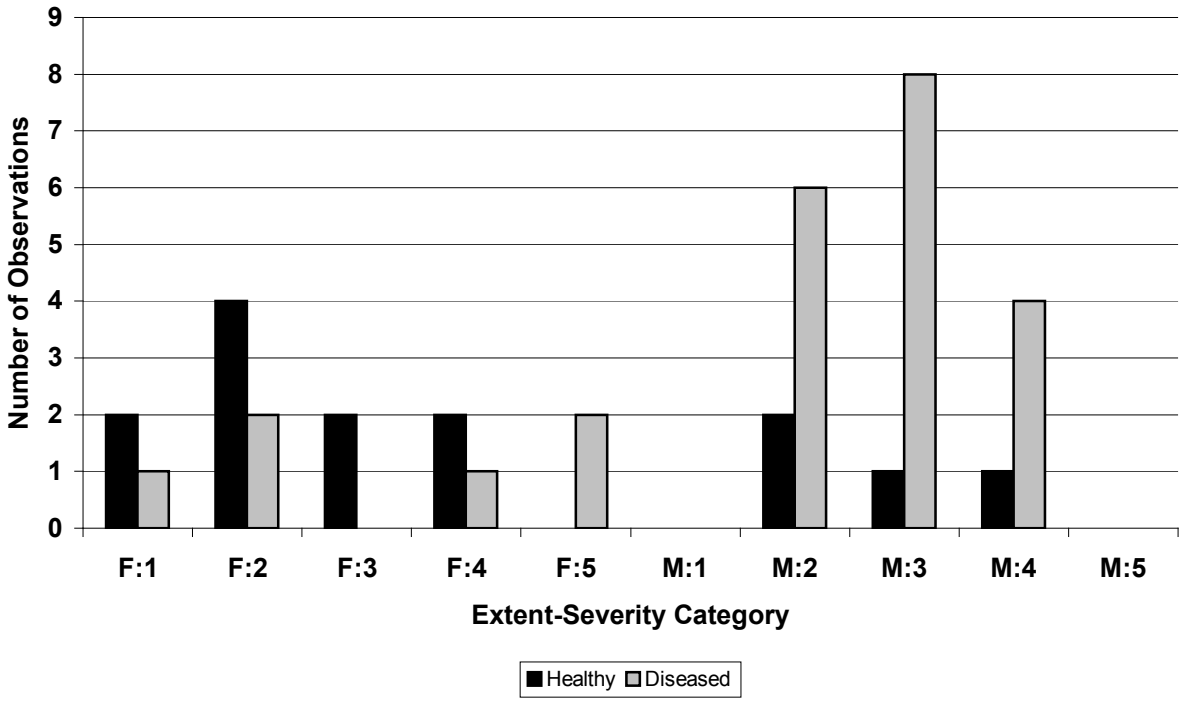


Fig. 6.6. Number of observations of each extent-severity category for microorganism growth in white pox diseased and apparently healthy *Acropora palmata.*

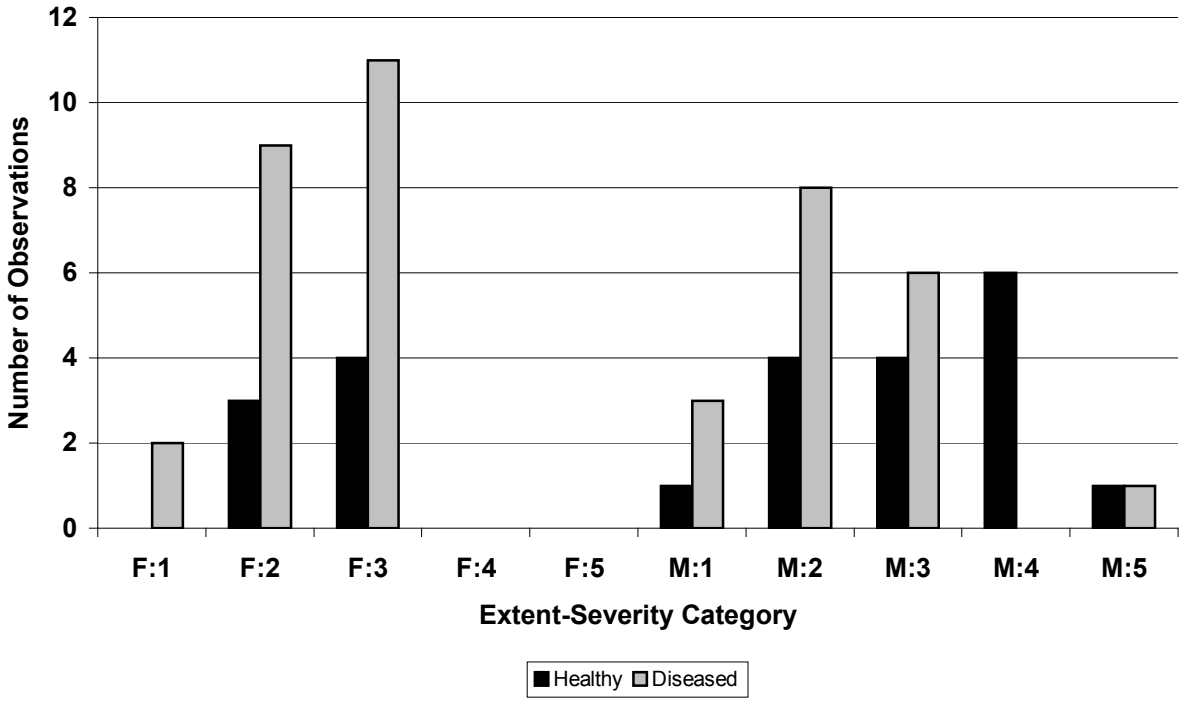


Fig. 6.7. Number of observations of each extent-severity category for microorganism infiltration of the skeletal tissue matrix in white pox diseased and apparently healthy *Acropora palmata.*

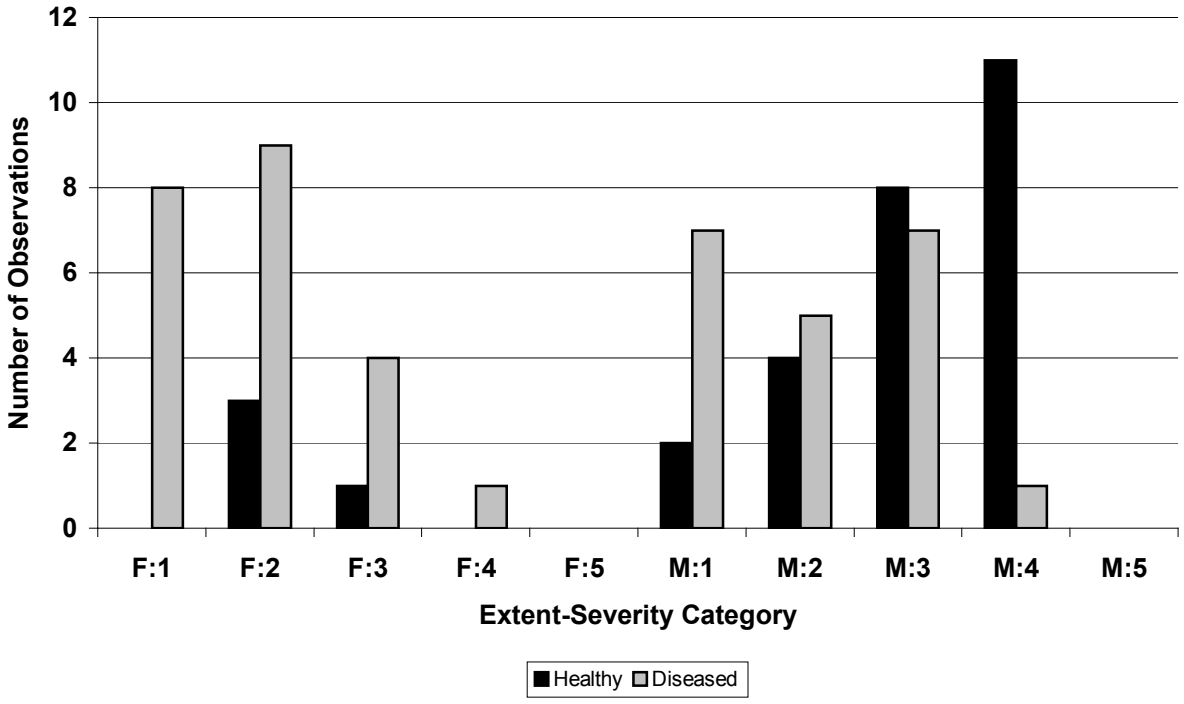


Fig. 6.8. Number of observations of necrosis in different anatomical structures of apparently healthy and white pox diseased *Acropora palmata.* Observations are summed for all systems and sites within each structure. Percent of slides in which each structure is present is as follows: coenosarc (96%), oral disk (53%), pharynx (73%), gastric cavity (99%), mesenterial filaments (98%).

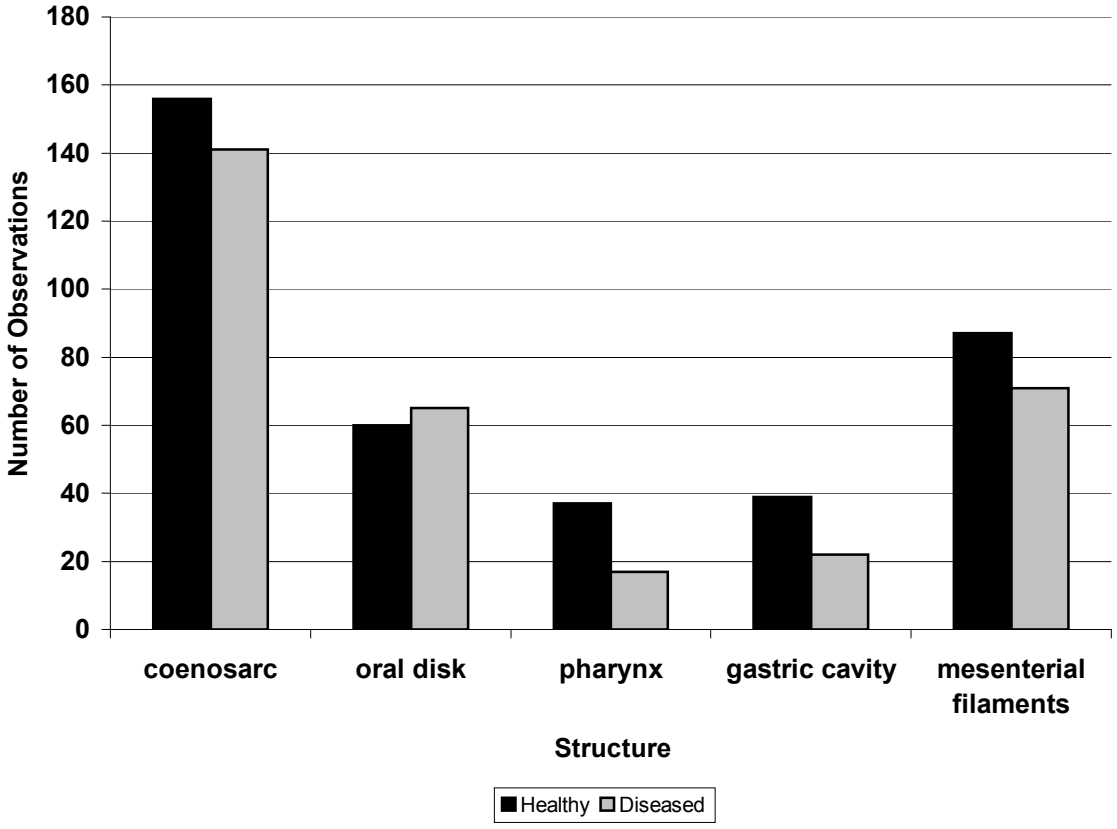


Fig. 6.9. Number of observations of each extent-severity category for necrosis in white pox diseased and apparently healthy *Acropora palmata.*

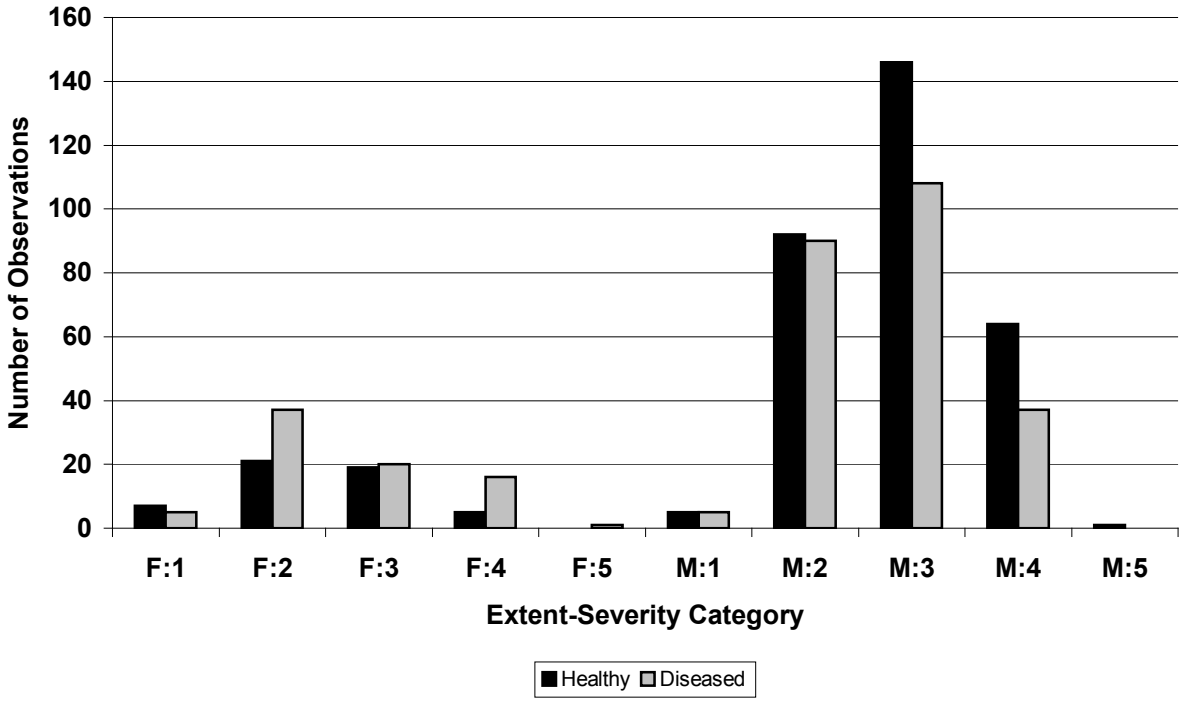


Fig. 6.10. Number of observations of gastrodermal cells rounding up in different anatomical structures of apparently healthy and white pox diseased *Acropora palmata.* Observations are summed for all systems and sites within each structure. Percent of slides in which each structure is present is as follows: coenosarc (96%), oral disk (53%), pharynx (73%).

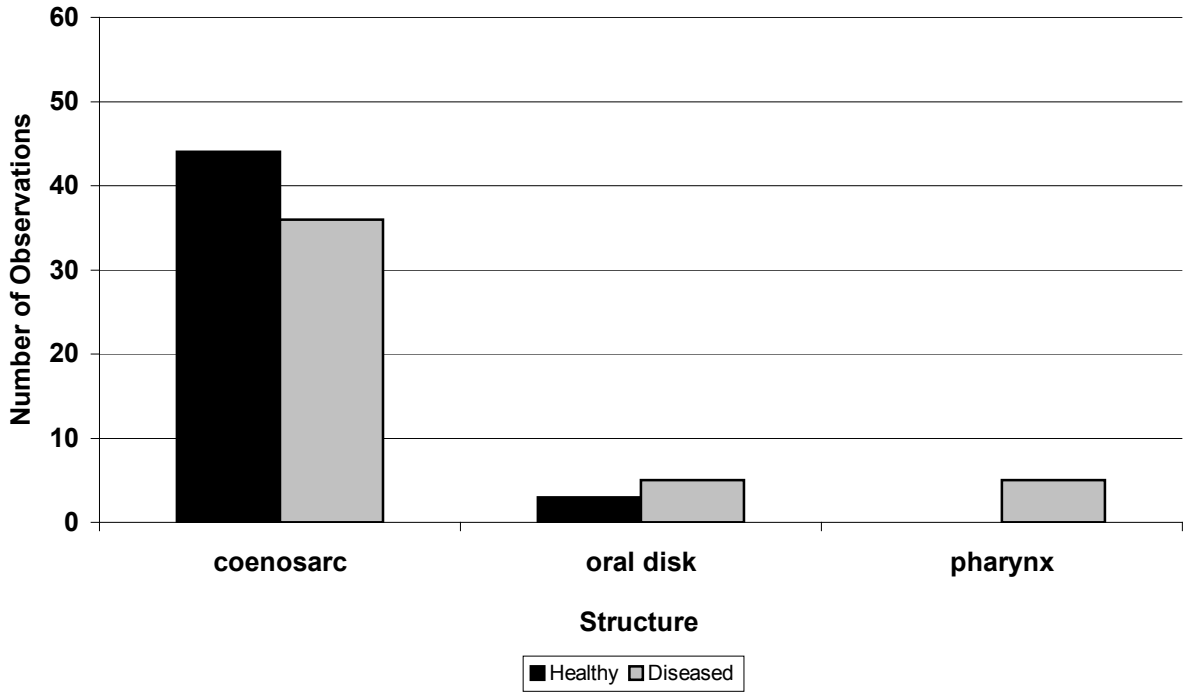


Fig. 6.11. Number of observations of each extent-severity category for rounding of gastrodermal cells in white pox diseased and apparently healthy *Acropora palmata.*

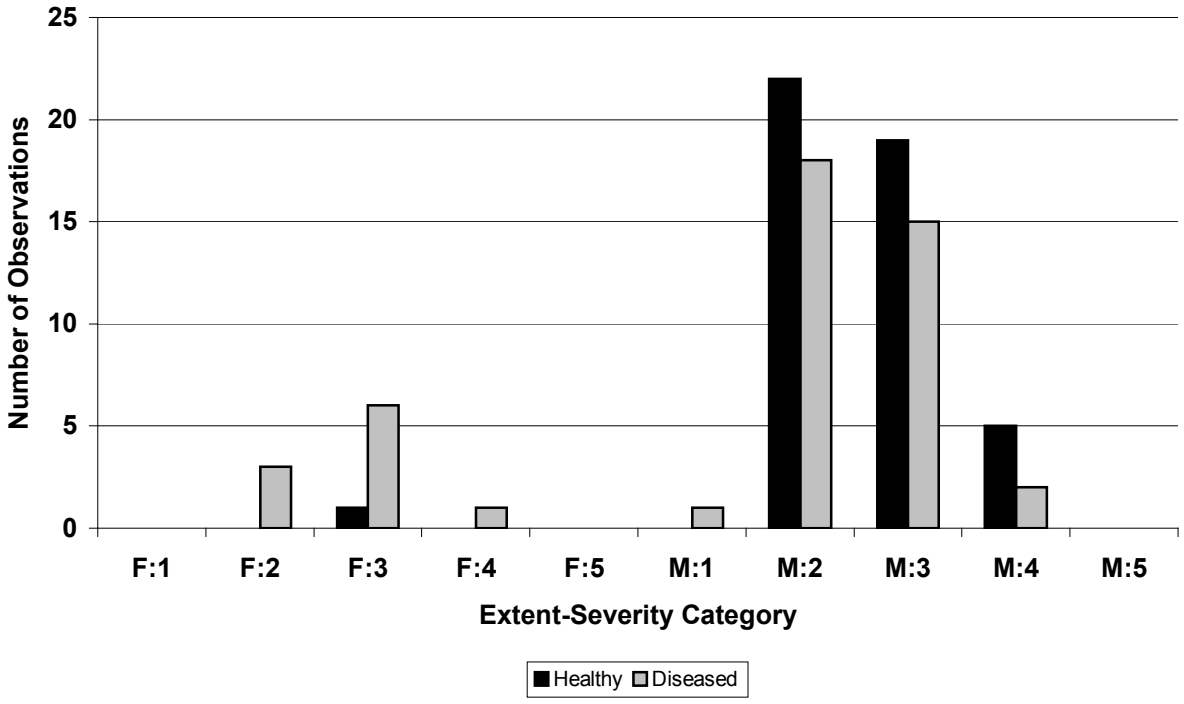


Fig. 6.12. Micrographs of white pox diseased and apparently healthy *Acropora palmata.* **A**. Swelling of the columnar cells in the epidermis (EP) of the coenosarc, resulting in a thickened epidermal layer, scale bar = 100 µm. **B**. Swelling of the mesoglea (MG) of the coenosarc, scale bar = 100 µm. **C**. Loss of mucous secretory cells in the epidermis and atrophy of the epidermis (EP) and gastrodermis of the coenosarc, scale bar = 50 µm. **D**. Atrophy of the gastric cavity (GA) and the epidermis (EP) and gastrodermis (GD) of the coenosarc, scale bar = 100 µm. **E**. Sloughing of the gastrodermis (GD) of the coenosarc, scale bar = 50 µm. **F**. Disrupted gastric cavity (GA); gastrodermis (GD) of the coenosarc (CN), scale bar = 100 µm.

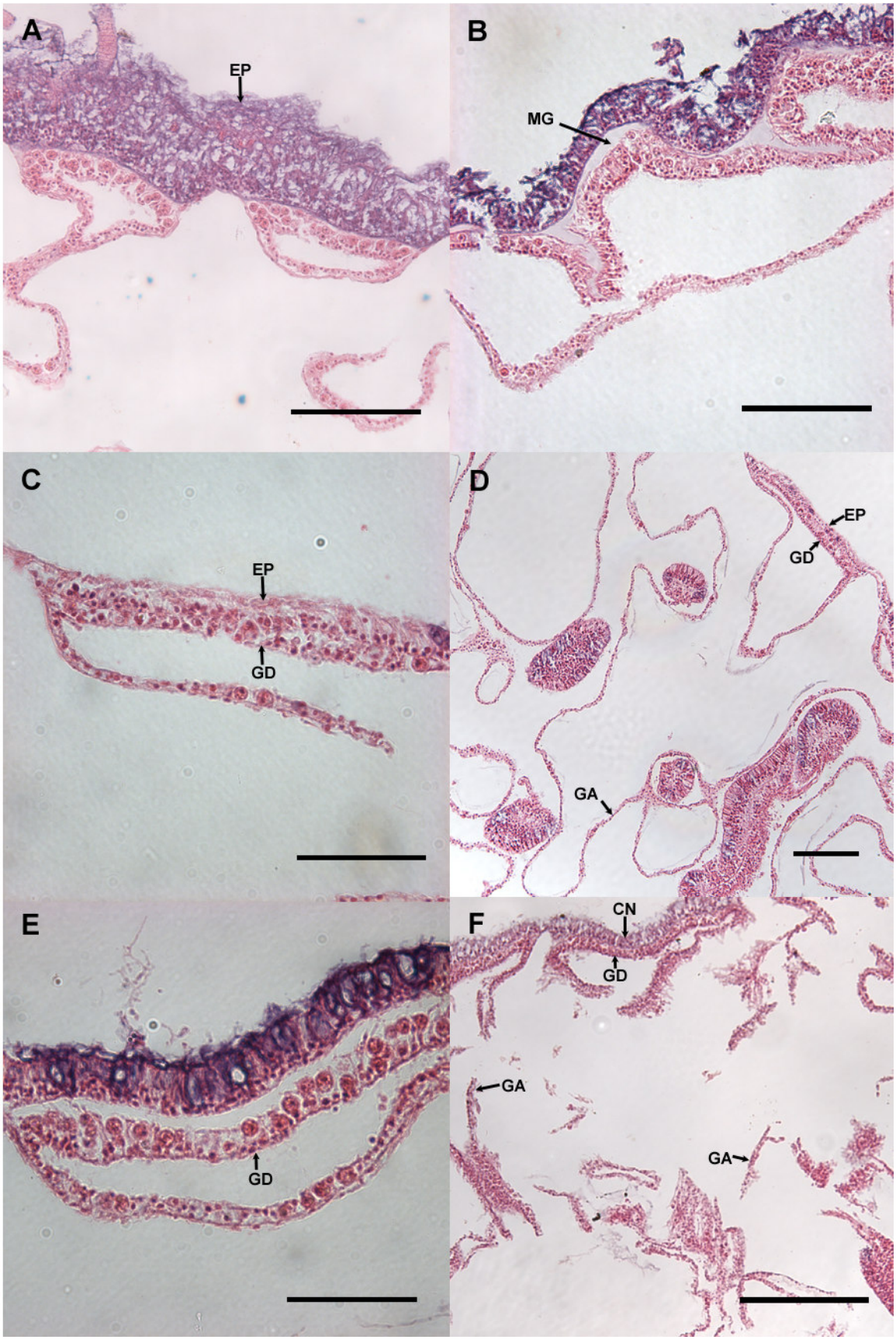

A
EP
B
MG
C
EP
GD
D
EP
GD
GA
E
GD
F
CN
GD
GA
GA

Fig. 6.13. Number of observations of thickening of tissue layers in the epidermis and the mesoglea of the coenosarc of apparently healthy and white pox diseased *Acropora palmata.*

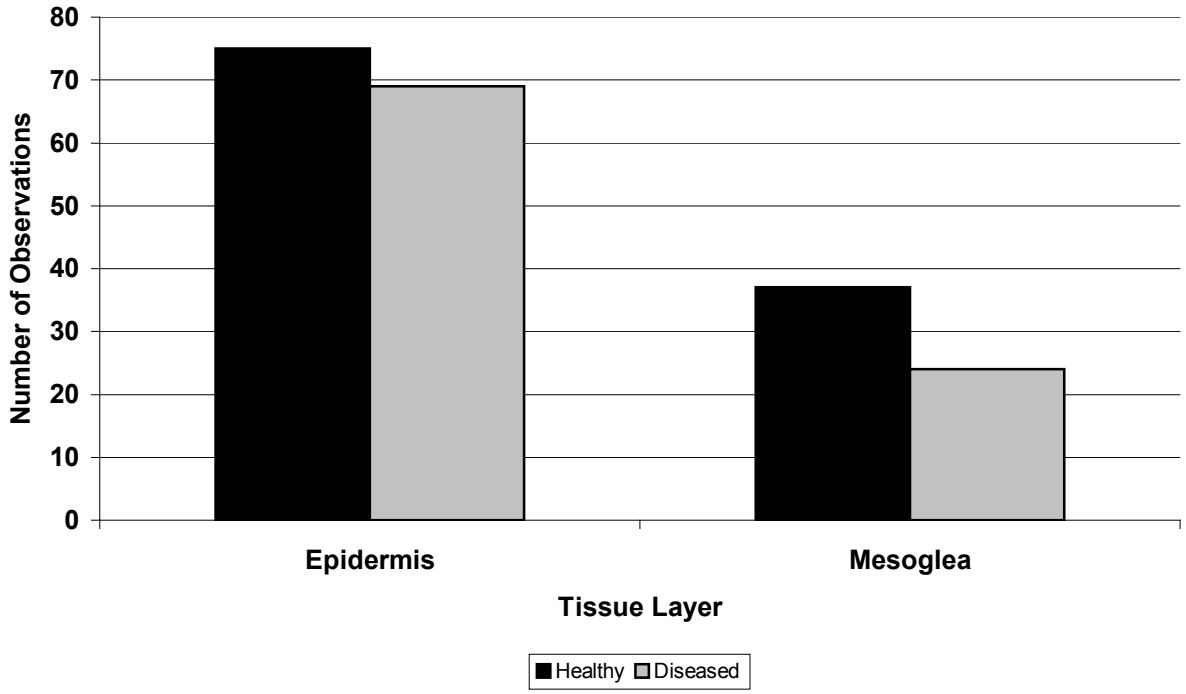


Fig. 6.14. Number of observations of atrophy in different anatomical structures of apparently healthy and white pox diseased *Acropora palmata.* Observations are summed for all systems and sites within each structure. Percent of slides in which each structure is present is as follows: coenosarc (96%), oral disk (53%), pharynx (73%), gastric cavity (99%).

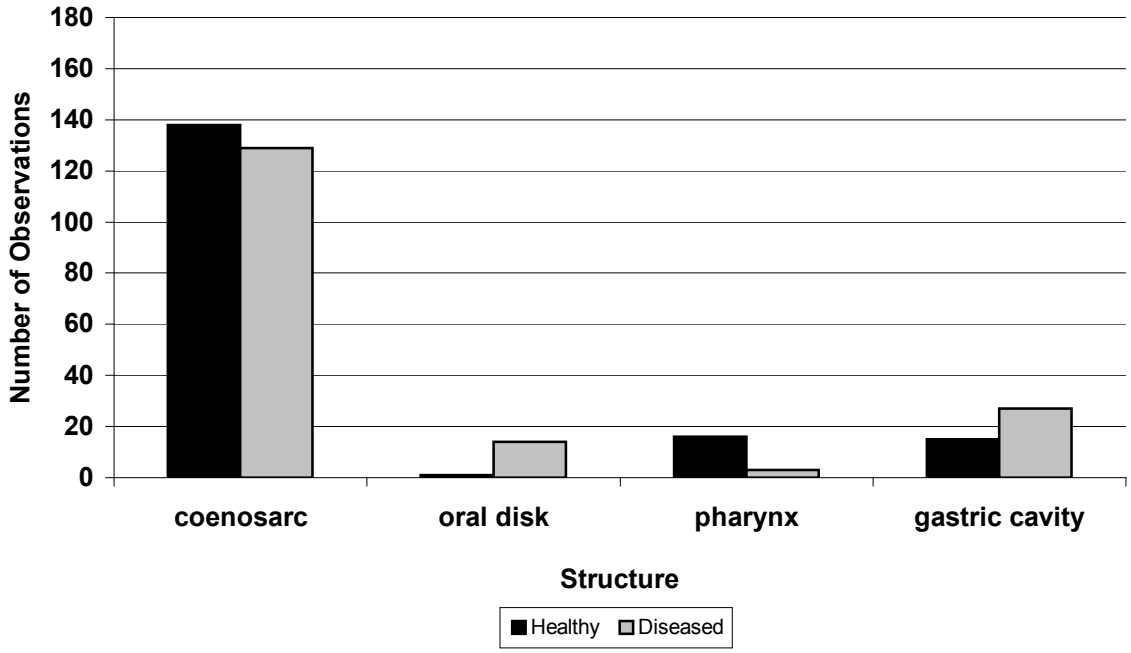


Fig. 6.15. Number of observations of each extent-severity category for sloughing of intact tissue layers in white pox diseased and apparently healthy *Acropora palmata.*

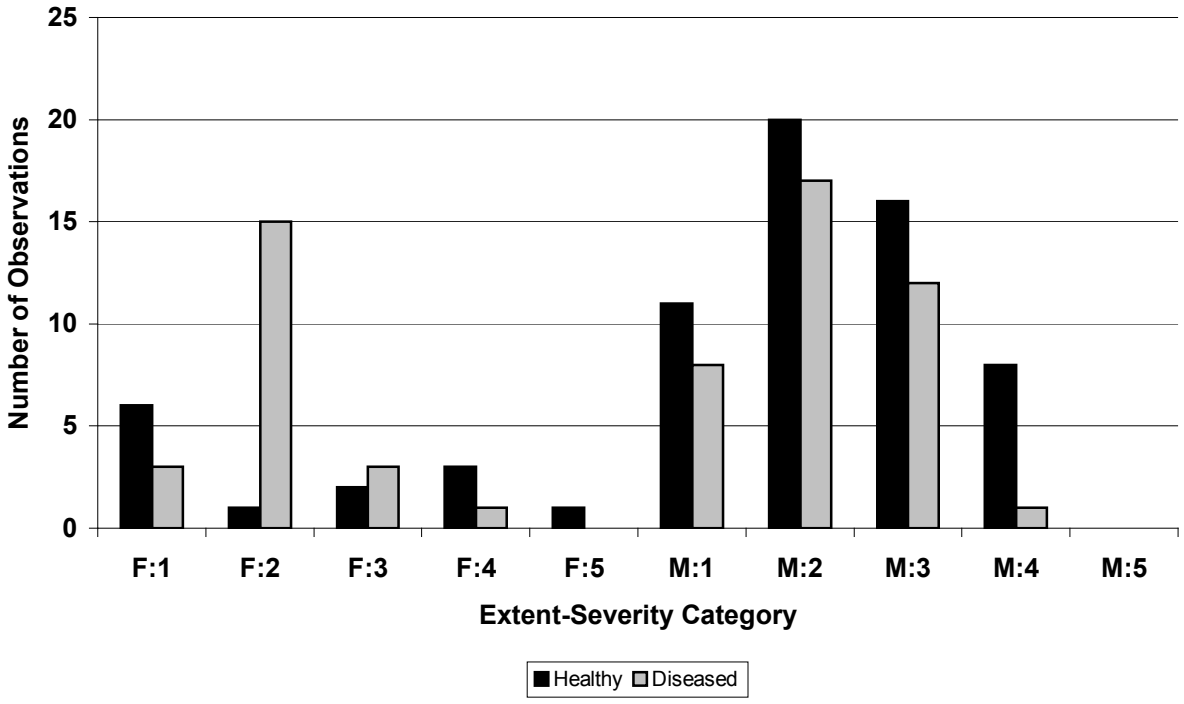


Fig. 6.16. Number of observations of sloughing of intact tissue layers of different anatomical structures of apparently healthy and white pox diseased *Acropora palmata.* Observations are summed for all systems and sites within each structure. Percent of slides in which each structure is present is as follows: coenosarc (96%), oral disk (53%), pharynx (73%).

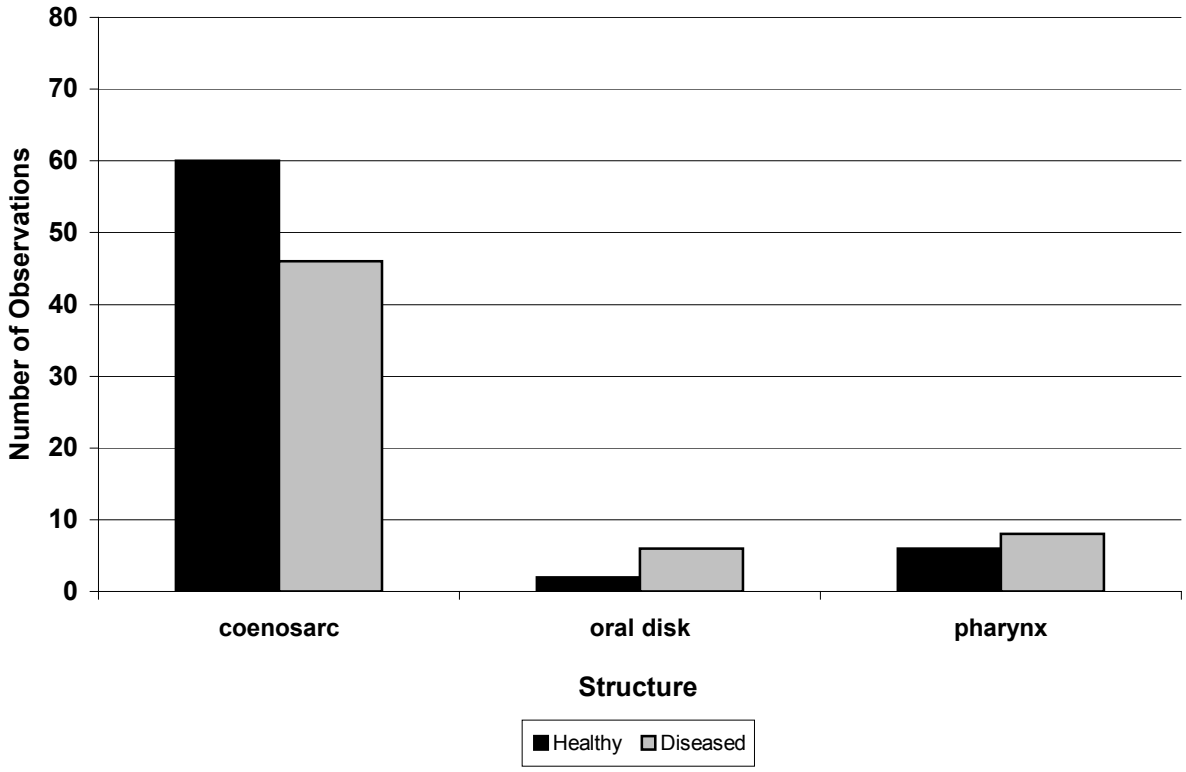


Fig. 6.17. Number of observations of disruptions in the tissue layers of different anatomical structures of apparently healthy and white pox diseased *Acropora palmata.* Observations are summed for all systems and sites within each structure. Percent of slides in which each structure is present is as follows: coenosarc (96%), oral disk (53%), pharynx (73%), gastric cavity (99%).

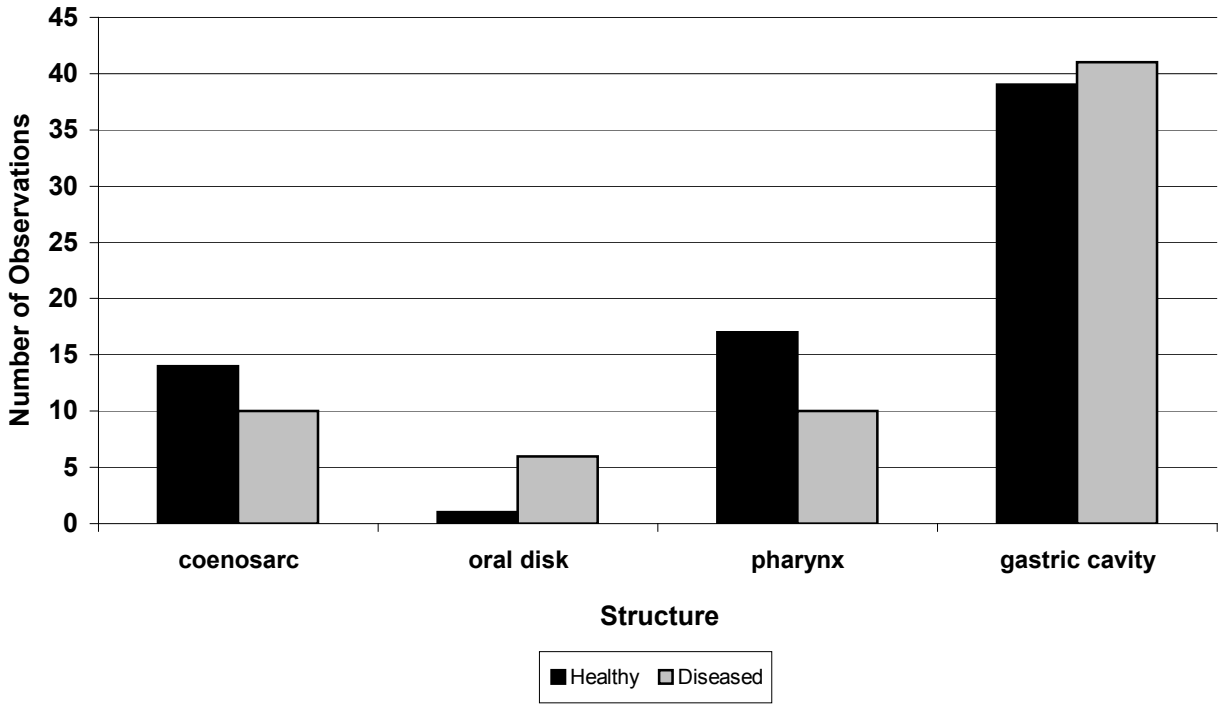


Fig. 6.18. Micrographs of white pox diseased and apparently healthy *Acropora palmata.* **A**. Acidophilic granular gland cells (GR) in the cnidoglandular band of the mesenterial filaments, scale bar = 50 μm. **B**. Rounding up of acidophilic granular gland cells (GR) in the cnidoglandular band of the mesenterial filaments, scale bar = 50 μm. **C**. Basophilic bodies in the epidermis of the oral disk, scale bar = 100 μm. **D**. Pale (PL) and normal (SA) zooxanthellae in the gastrodermis of the coenosarc, scale bar = 25 μm.

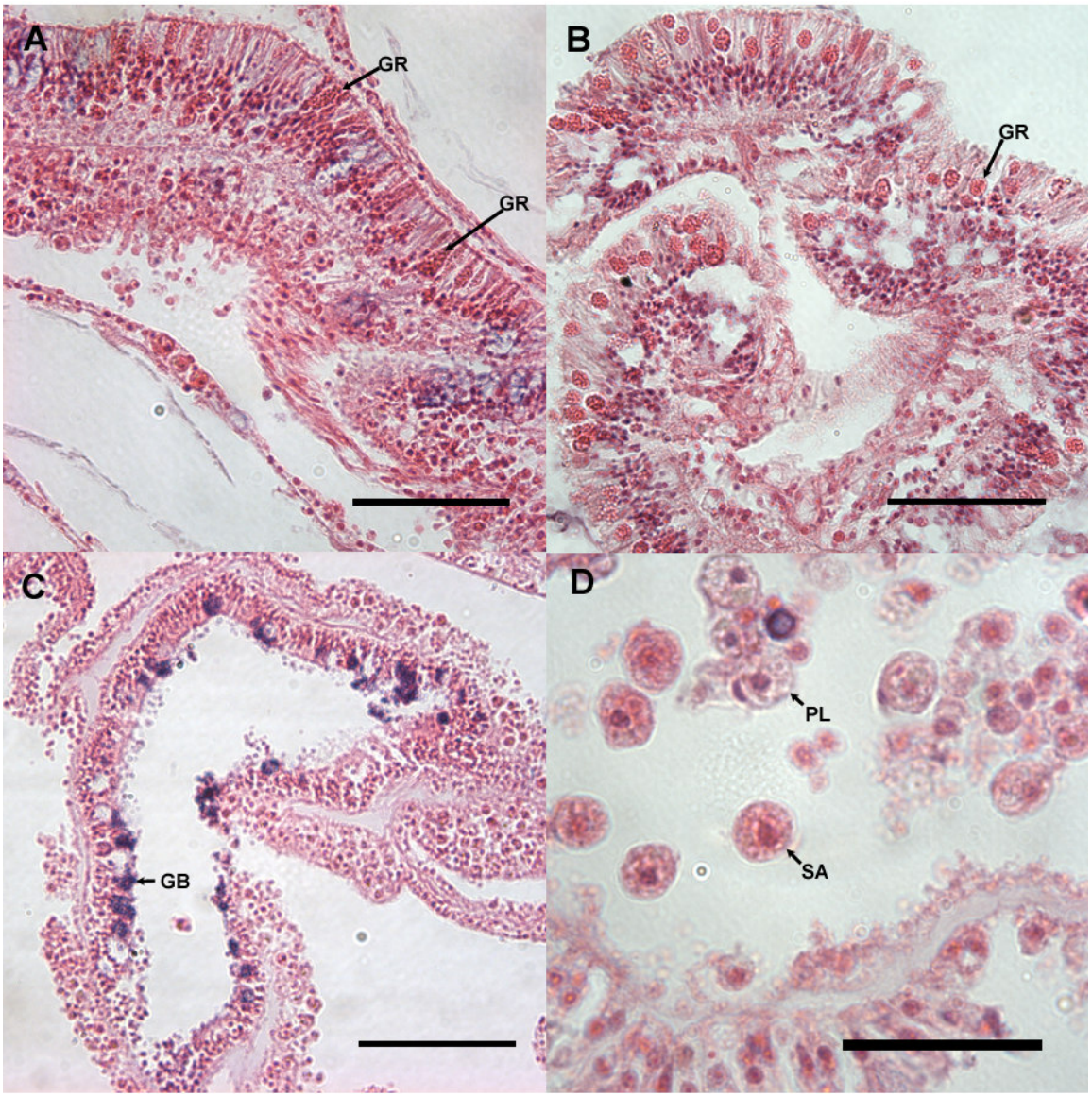

A
GR
GR
B
GR
C
GB
D
PL
SA

Fig. 6.19. Number of observations of basophilic bodies in different anatomical structures of apparently healthy and white pox diseased *Acropora palmata.* Observations are summed for all systems and sites within each structure. Percent of slides in which each structure is present is as follows: coenosarc (96%), oral disk (53%), pharynx (73%), gastric cavity (99%), mesenterial filaments (98%).

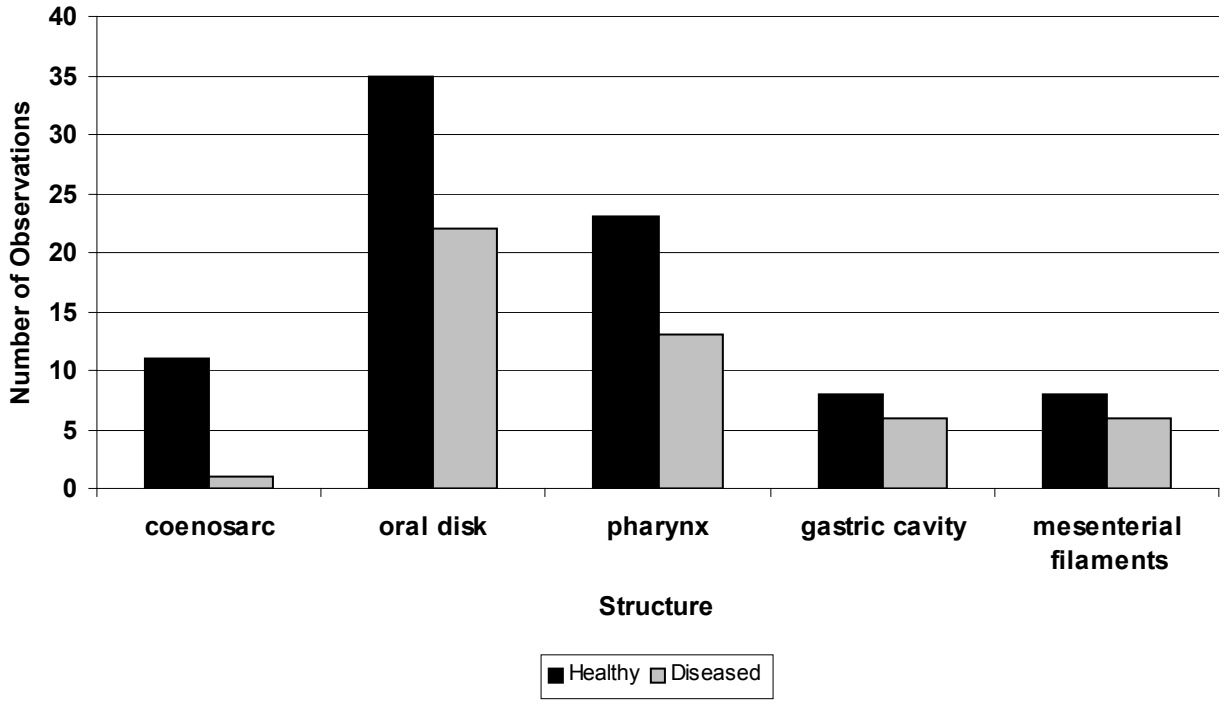


Table 6.9. Number of symbiotic algae in apparently healthy and white pox diseased tissues of *Acropora palmata.* Data are presented as Percent of slides with each lesion code prepared from apparently healthy (H) and white pox diseased (P) tissues. Number of symbiotic algae are ranked as (1) normal or greater than normal numbers, (2) apparently normal numbers, (3) slight loss, (4) moderate loss, or (5) complete loss

		Frequency (%)				
Structure		**1**	**2**	**3**	**4**	**5**
coenosarc	**H**	5	59	27	9	-
	P	3	62	24	5	-
oral disk	**H**	1	50	3	3	-
	P	3	42	8	-	-
pharynx	**H**	-	73	5	-	-
	P	5	57	7	-	-
gastric cavity	**H**	-	74	23	3	-
	P	8	62	20	8	-

Fig. 6.20. Number of observations of pale zooxanthellae in different anatomical structures of apparently healthy and white pox diseased *Acropora palmata.* Observations are summed for all systems and sites within each structure. Percent of slides in which each structure is present is as follows: coenosarc (96%), oral disk (53%), gastric cavity (99%), mesenterial filaments (98%).

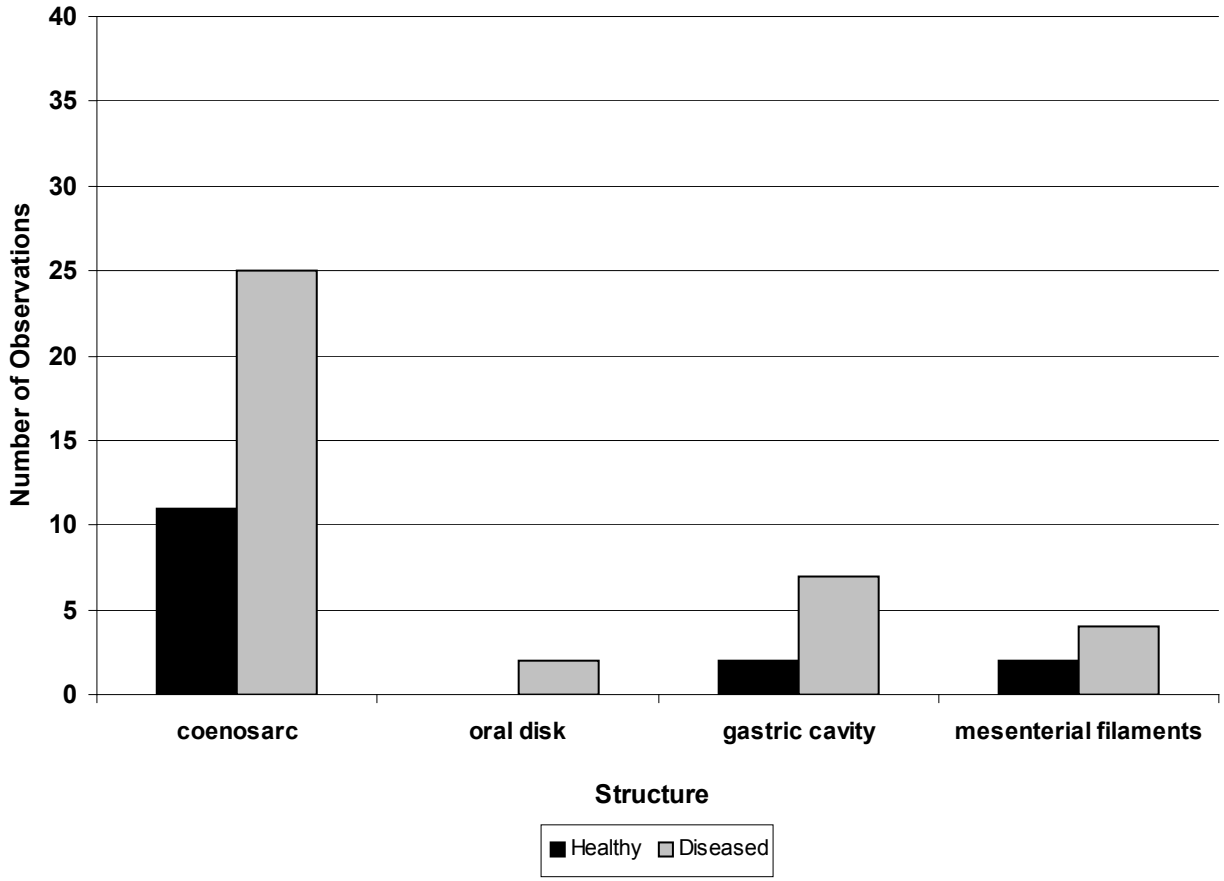
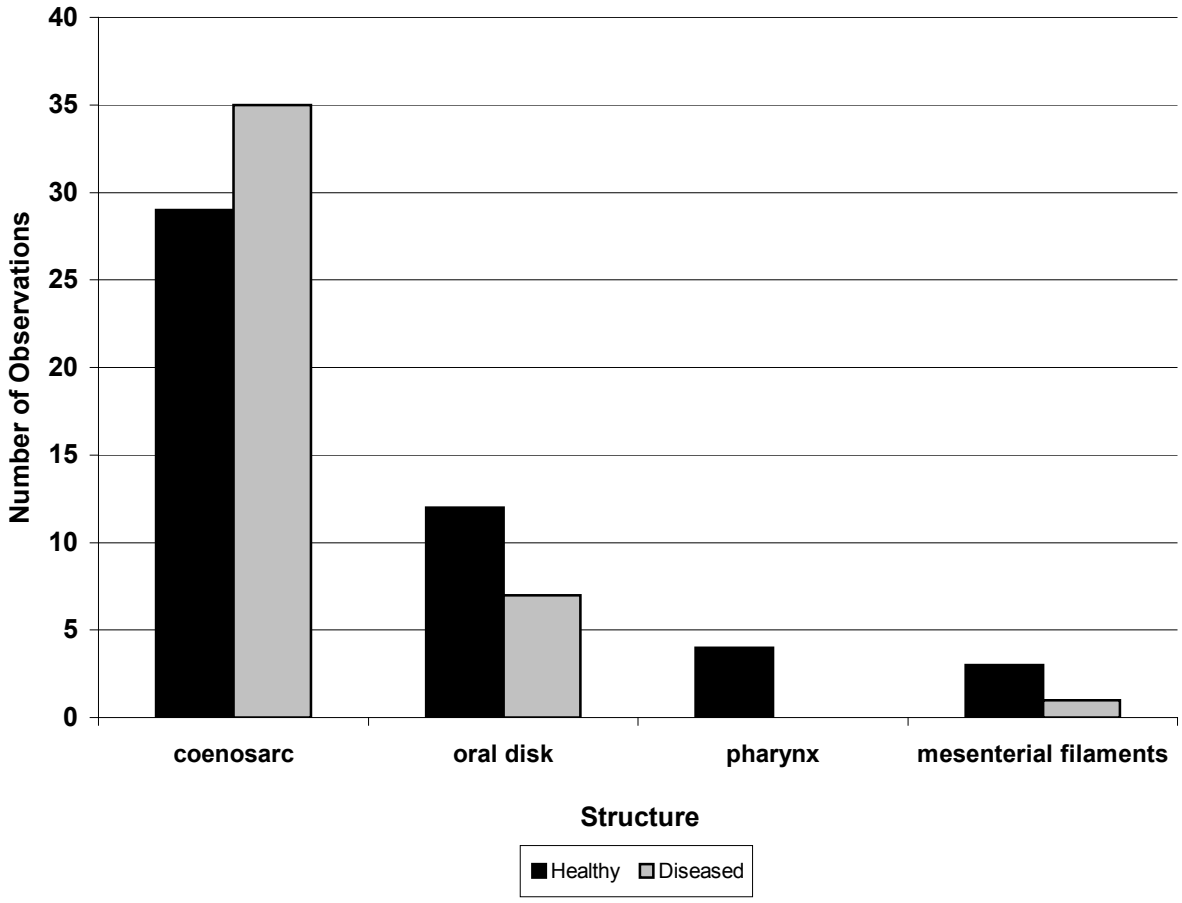


Fig. 6.21. Number of observations of vacuolated zooxanthellae in different anatomical structures of apparently healthy and white pox diseased *Acropora palmata.* Observations are summed for all systems and sites within each structure. Percent of slides in which each structure is present is as follows: coenosarc (96%), oral disk (53%), pharynx (73%), mesenterial filaments (98%).



CHAPTER 7

CONCLUSION

Worldwide, 18 diseases affecting at least 152 scleractinian, gorgonian, and hydrozoan coral species have been described. These diseases are associated with pathogens including bacteria, cyanobacteria, fungi, and protists and with abiotic stressors including elevated seawater temperature, sedimentation, eutrophication, and pollution. The etiologies of only five coral diseases have been determined through fullfillment of Koch's postulates, but several other disease conditions have been linked to specific biotic organisms and abiotic stressors.

Worldwide increases in the number of both new coral diseases and species affected may be directly linked to global climate change issues such as global warming. Elevated seawater temperature may be associated with the etiologies of at least nine coral conditions and is likely the most common abiotic stressor influencing disease pathogenicity. The rate of tissue loss associated with white pox disease correlates with seasonal conditions of elevated temperature, and five coral disease-causing pathogens, including (1) the black band disease cyanobacteria, (2) *Aurantimonas coralicida* (white plague type II), (3) *Aspergillus sydowii* (aspergillosis), (4) *Vibrio shiloi* (bacteria-induced bleaching), and (5) *V. coralliilyticus* (bacteria-induced bleaching and disease), are most virulent at seawater temperature at or above 29 ºC (Kushmaro et al. 1998, Alker et al. 2001, Banin et al. 2001, Israely et al. 2001, Ben-Haim & Rosenberg 2002, Kuta & Richardson 2002, Ben-Haim et al. 2003, Richardson & Kuta 2003). Seawater temperature normally increases during late summer, but all current models of global climate change suggest

that, on average, ocean temperatures will rise over the next century (Kleypas et al. 1999). Elevated temperature is a stressor in corals, causing thermally induced breakdown in the coral-zooxanthellae host-symbiont relationship (Porter et al. 1989, Fitt et al. 1993, Brown 1997). Elevated temperature also promotes growth and virulence of pathogens (Kushmaro et al. 1996, Kushmaro et al. 1998, Toren et al. 1998, Alker et al. 2001, Banin et al. 2001, Israely et al. 2001, Ben-Haim & Rosenberg 2002, Kuta & Richardson 2002, Ben-Haim et al. 2003, Richardson & Kuta 2003) and reduces immune response in host corals (Toren et al. 1998, Alker et al. 2001).

White pox disease is associated with the decimation of *Acropora palmata* populations in the FKNMS, with losses of this species averaging 87% between 1996 and 2002. These and other population declines of this species have occurred throughout the Caribbean (Gladfelter 1982, Porter and Meier 1992, Bythell and Sheppard 1993, Aronson and Precht 2001, Miller 2002, Patterson et al. 2002) and have led to the identification of the once, most common Caribbean coral species as a candidate for protection under the Endangered Species Act.

White pox is caused by the enterobacterium *Serratia marcescens*, however, the source of this coral pathogen remains unknown. Examination of the waters of the FKNMS presumptively identified bacteria of the genus *Serratia* from stations in Key West and the Dry Tortugas National Park. Additional studies are currently underway to determine the source of *Serratia marcescens* strain that causes white pox disease. Potential sources of the coral pathogen include (1) wastewater influent and effluent from the sewage treatment facilities in Key West, Marathon, and Key Largo, (2) septic tank effluent, (3) nearshore and canal water, (4) sea bird guano, (5) the corallivorous gastropod, *Coralliophila abbreviata*, (6) the corallivorous polychaete, *Hermodice carunculata*, and (7) corallivorous parrotfishes. Elucidating the source of the white pox disease pathogen is essential, as coral reef managers and wastewater treatment engineers appreciate

certainty of a human origin of the white pox pathogen before recommending expensive sewage treatment upgrades in Florida and around the Caribbean.

Histopathology of white pox-diseased corals indicate that white pox is associated with lesions of the coenosarc. The epidermis of the coenosarc showed high frequency of necrosis, atrophy, and thickening. However, the extracellular matrix of this tissue layer was not degraded and the epidermis remained intact. The gastrodermis of the coenosarc exhibited the greatest number of frequently encountered lesions including necrosis, atrophy, rounding and loss of adhesion of gastrodermal cells, and sloughing of the intact gastrodermis. Zooxanthellae within the gastrodermis of the coenosarc were often pale and vacuolated. The mesoglea of the coenosarc showed relatively high frequencies of thickening or swelling. Diseased tissues were especially vulnerable to interactions with filamentous microorganisms.

Lesions observed in the white pox-diseased colonies of *Acropora palmata* are signs of disease and are different in this disease than in other coral diseases. There was no significant difference in the types of abnormalities or the total number of abnormalities observed in the white pox-diseased and apparently healthy tissues. The frequencies of 41% of the abnormalities showed a significant difference between apparently healthy and diseased tissues. However, only half of these abnormalities were more common in diseased tissues. The overall extent and severity of abnormalities was significantly different between apparently healthy and diseased tissues. However, only seven of the 26 individual abnormality types showed a significant difference in extent and severity between apparently healthy and diseased tissues. Although tissue appeared grossly normal on all *A. palmata* colonies, except at the margins of the disease lesions, histopathology revealed that both apparently healthy and diseased coral tissues were degenerating and necrotic. The similarity in both the abnormalities observed and their frequency

and severity in white pox-diseased and apparently healthy tissues may indicate that *A. palmata* colonies affected by white pox disease sustain a whole-colony reaction to infection.

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