The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*

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Communicated by Eugene P. Odum, University of Georgia, Athens, GA, May 1, 2002 (received for review December 12, 2001)

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.

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 *A. palmata* in the Florida Keys.

White pox disease was first documented in 1996 on Eastern Dry Rocks Reef (24° 27.715′ North, 81° 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. 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 (24° 32.7′ N; 81° 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, Silver Spring, 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. 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 24° 2.1′ N; 74° 36.1′ W), the U.S. Virgin Islands (St. John 18° 21′ N; 65° 15′ W), and Caribbean Mexico. Surface mucopolysaccharide 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°C for 24–48 h. Individual colonies (differing in colonial or cellular morphology or Gram stain) were then replated to pure culture. Pure cultures of each isolate (221 strains) were exposed to 95 different carbon sources on GN1 microplates (Biolog, 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°C. 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 × *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

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF389108).

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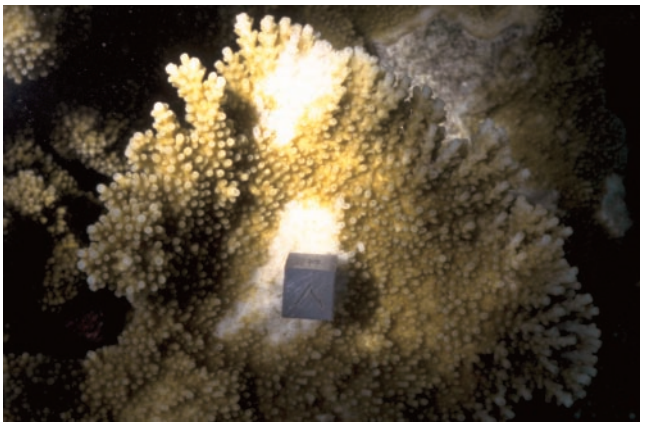


Fig. 1. White pox disease lesion on *A. palmata* at Looe Key Reef, FL, August 1998. The square pin (1.61 cm^2) was used to calibrate the image tracing software (photograph by K.L.P.).

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 by using a $1/4 \times 1/8$-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 Biologsystem.

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, 40 μl of lysozyme (40 mg/ml), and 10 μl of RNase (10 mg/ml), and incubated at 37°C for 10 min. Cell lysis was completed by the addition of 50 μl 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 by 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 *Escherichia 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 of 10× 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 overlaid 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°C for 5 min; 25 cycles of 94°C for 60 s, 40°C for 60 s, and 72°C for 60 s; a final extension step at 72°C 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 by 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% glutaraldehyde 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 × 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) was 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 contain-

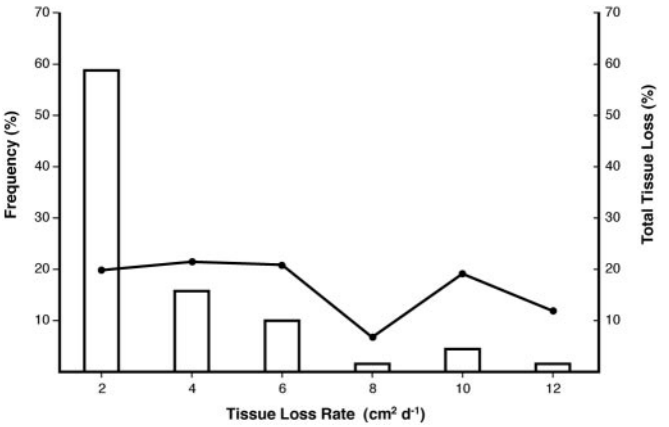


Fig. 2. The size class distribution of white pox disease lesions and percent total tissue loss per size class on *A. palmata* colonies at Looe Key Reef, FL, after 15 days (August 6–20, 1998) *in situ*.

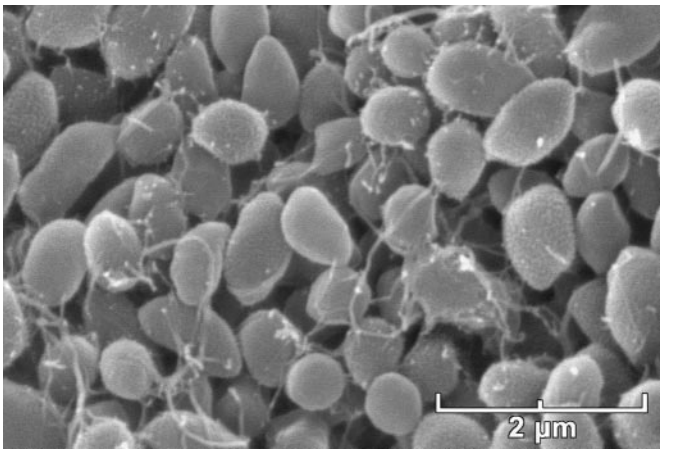


Fig. 3. Scanning electron micrograph of the white pox pathogen (PDL100). The bacterium was identified as *S. marcescens* by 16S rRNA gene sequencing, carbon source utilization patterns, and standard microbiological testing (photograph by S.W.P.).

ing *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 by 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 $cm^2 \cdot day^{-1}$ ($\pm$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 $cm^2 \cdot day^{-1} \pm 0.56$ SD), but this size class represents only 20% of the total tissue loss during the 15-day period (Fig. 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 by 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). 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, NJ). 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. 4*A*). 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 (Figs. 4*B* and 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. 4*B*).

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. 4*B*). 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 (Figs. 4*B* and 5*D*), 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.

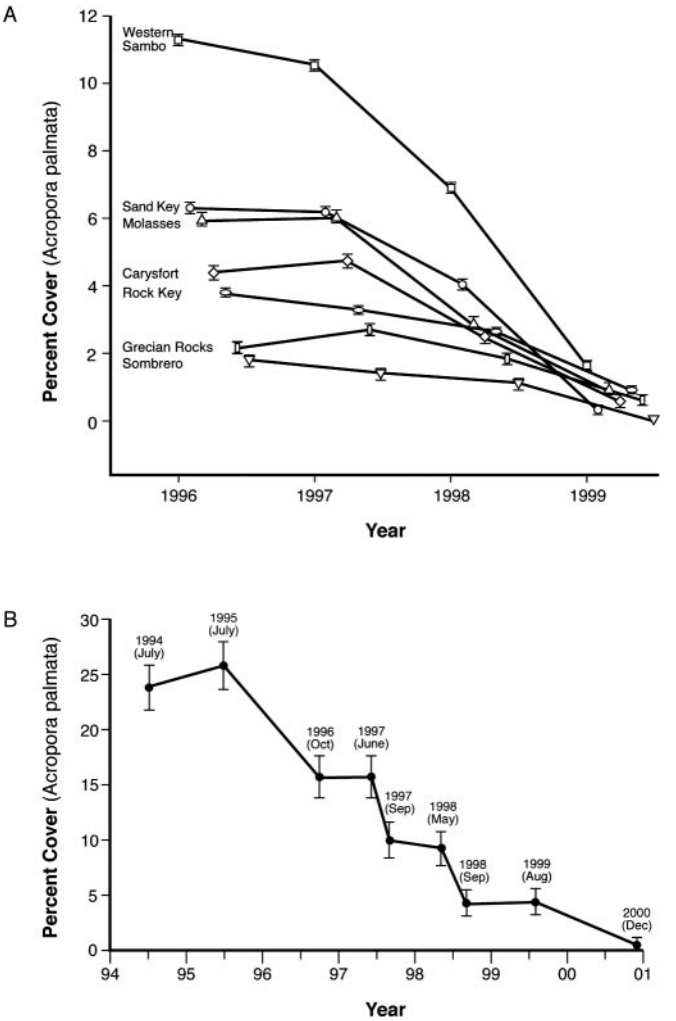
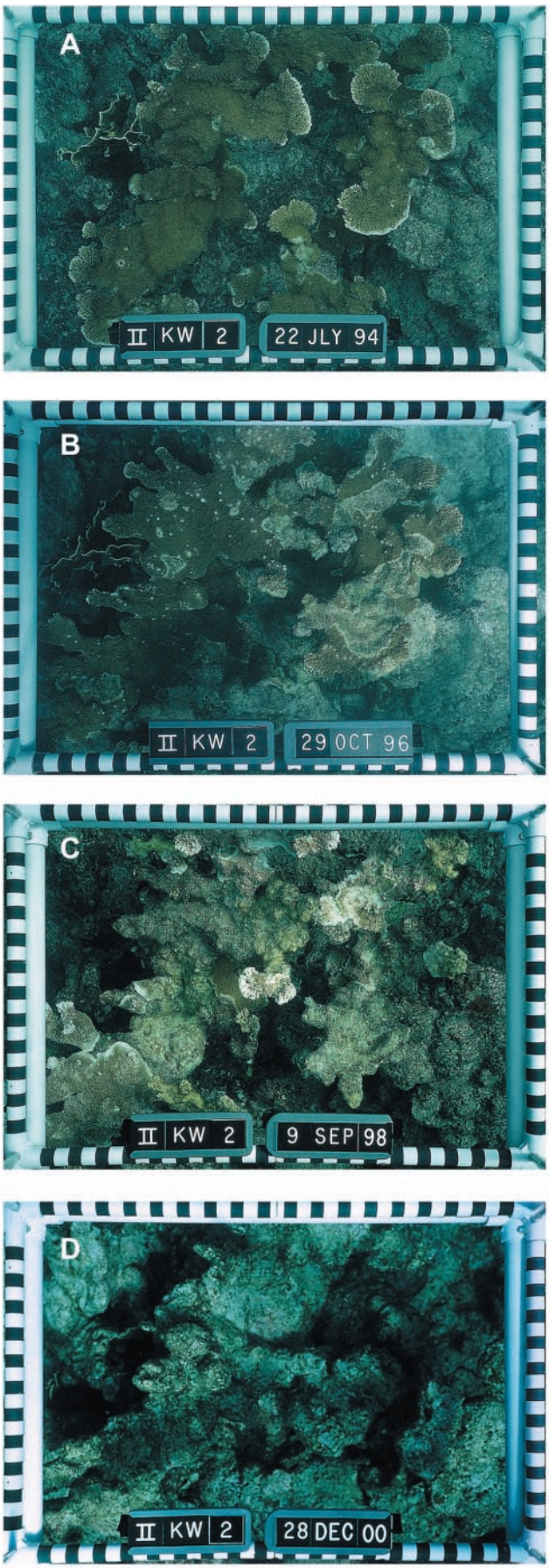


Fig. 4. (*A*) The percent cover of *A. palmata* at seven reef sites in the Florida Keys National Marine Sanctuary, 1996–1999. By 1999, percent cover of this species had decreased at each of the sites: Carysfort Reef (diamond), 85%; Grecian Rocks Reef (rectangle), 71%; Molasses Reef (upward triangle), 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.

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 whether 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°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). Although vegetative reproduction may be well adapted to recolonization after mechanical disturbances such as hurricanes, colony fragmentation is ineffectual after severe population declines due to disease, which frequently kills the entire coral colony (Fig. 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.

Fig. 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.).

We thank P. Dustan, W. Jaap, J. Wheaton, V. Kosmynin, C. Quirolo, R. E. Rodriguez-Martinez, and the crew of the Environmental Protection Agency's research vessel, OSV Peter W. Anderson, for help with field sampling and logistical support. We thank J. Hoch, N. Knowlton, M. A. Moran, and C. D. Harvell for their comments on the manuscript. We thank Dr. Melissa B. Riley, director of the Clemson University Multiuser Analytical Laboratory, for the use of a gas chromatograph and invaluable assistance. Thanks also to Dr. JoAn Hudson and the staff of the Clemson University Electron Microscope Facility for their support, including the use of a scanning electron microscope. This research was supported by an Environmental Protection Agency/University of West Florida Science Training in Ecology Program (STEP) fellowship (to K.L.P.) and by U.S. Environmental Protection Agency Grants EPA/FDEP X-99-34649-94-0 (to J.W.P. and K.L.P.) and EPA X-98-4326.97 (to E.M., E.C.P., J.W.P., and K.L.P.), National Science Foundation Grant OCE-9818830, and U.S. Department of Energy Grant DE-AF26-99FT00782 (to K.B.R. and G.W.S.). Research at Looe Key Reef, FL, was conducted under permit no. FKNMS-046-98 (to E.M., E.C.P., J.W.P., and K.L.P.).

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