

White Death in Blue Water

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No, this time it is not white sharks eating people. This time, and especially in the last eight years or so, it is microorganisms killing, and apparently consuming, coral tissue. This time it is a disease of various reef building corals, and it's called "white plague". I (RBJ) first saw epizootic white plague (WP) on the barrier reef east of Andros Island in The Bahamas in 1998. Over 80% of the elliptical star coral *Dichocoenia stokesii* colonies were stark white, looking like snow balls in the sea. Lack of algal growth on the denuded coral skeletons indicated that they had died in the past few days. While working to develop a funding base to investigate white plague we followed this disease in the Bahamas. On some dives at least twenty white plague affected corals could be seen from a single vantage point about twenty feet above the bottom. Hundreds could be found on a single dive. We tracked the progress of plague on several colonies (Figs. 1 and 2), and found that the disease front, complete loss of all coral tissue leaving only the carbonate skeleton behind, progressed at 1 cm per day or more. *Montastrea faviolata*, the keeled star coral, was one of the most commonly affected species in the Bahamas. One member of our research group (J. Paihe Rothenberger) found that the closely related knobby star coral, *M. annularis*, was commonly affected in the Virgin Islands.

The fact that WP was common in areas distant from human population centers and the report of a new bacterial species, *Aurantimonas coralicida*, as an etiological agent of WP (Denner et al. 2003), led us to hypothesize about the origins of the disease and especially its relationship to the natural microbial communities that are present in the coral reef environment. It is only now, in the era of molecular techniques, that efficient methods are available to evaluate and compare the composition of the "invisible" world of microbes that occupy the water column, sediment and, most importantly, the coral itself. This investigation focuses on characterizing the microbial communities from two distant reef sites, St Croix in the US Virgin Islands and Lee Stocking Island in The Bahamas. Experimentally we collected multiple, small cores of coral tissue from apparently healthy coral colonies (controls), healthy areas on corals with WP and along the disease margin (Fig. 3 and 4). At each location five pairs of healthy and diseased corals were sampled. This repetitive sampling will give us enough discrete analyses to understand the range of variability in microbial community composition within a single coral colony, among corals of the same species within a reef site and between two reef locations hundreds of miles apart.

Samples are being analyzed by three basic methods. On site, we homogenized portions of coral tissue and spread various dilutions on nutrient media to investigate the culturable bacterial community (Fig. 5) abundance and composition. Using the same subsamples we preserved portions in liquid nitrogen for later DNA extraction and analysis. Separate coral cores were preserved on site for histological analysis. Our molecular approach is described as amplicon length heterogeneity. Essentially each bacterial species has a specific length of DNA between two locations on the gene coding

for ribosomal RNA. These portions are amplified by PCR using specific primers. Then the DNA fragments are separated and their lengths determined using a capillary gel electrophoresis method. These DNA length heterogeneity fingerprints are then compared to describe the taxonomic composition and relative abundance of each taxon in the bacterial community.

Initial data shows that the bacterial community on the healthy coral colonies and on the healthy portion of the diseased colonies is very different than the community present at the disease margin. Some taxa are present only in the disease samples, while some are present in all the samples but at very different relative abundances. Principle component analysis (Fig. 6) reveals a clear cluster of disease-associated bacteria (red squares) separated from the taxa in the healthy tissue on diseased colonies and healthy control colonies. One possible outcome of this research is that WP occurs in conjunction with a shift in major species composition of the coral's microflora (polyclonal disease) rather than being associated with a single etiological agent. Molecular analysis of the culturable bacteria does not indicate unique disease associated taxa among those that produced colonies on marine agar. The culturable species seem to be present in all of the samples. Continuing work includes cloning, sequencing and identification of the major bacterial taxa of each sample type, sequencing of the culturable bacteria and comparison of the histological details of the three types of coral tissue samples.



Figure 1: White plague on *Montatrea faviolata* on Andros Barrier Reef (July 29, 2002). Pin marks edge of disease line.



Figure 2: Progression of white plague disease as of Aug. 06, 2002 on *M. faviolata* colony shown in Fig. 1

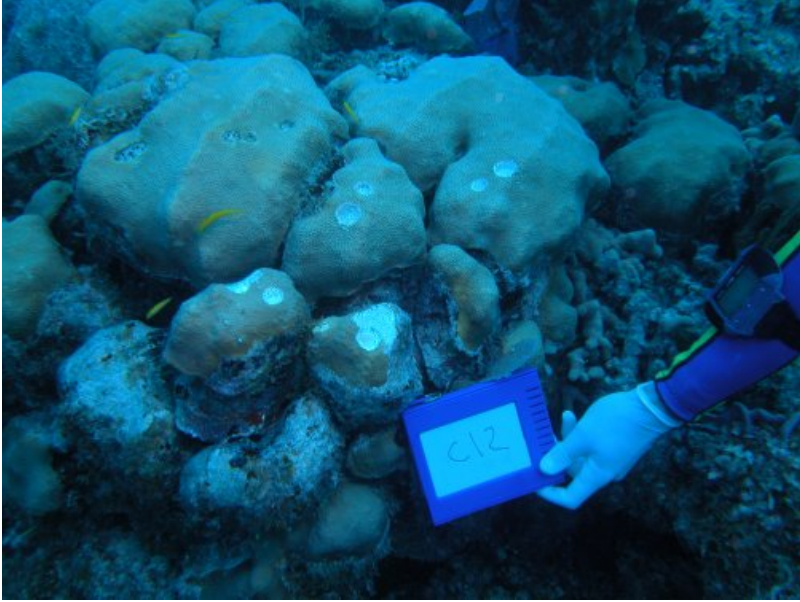


Figure 3: *Montastrea annularis* colony after sampling white plague affected and healthy tissue. Core holes were immediately repaired with epoxy putty.

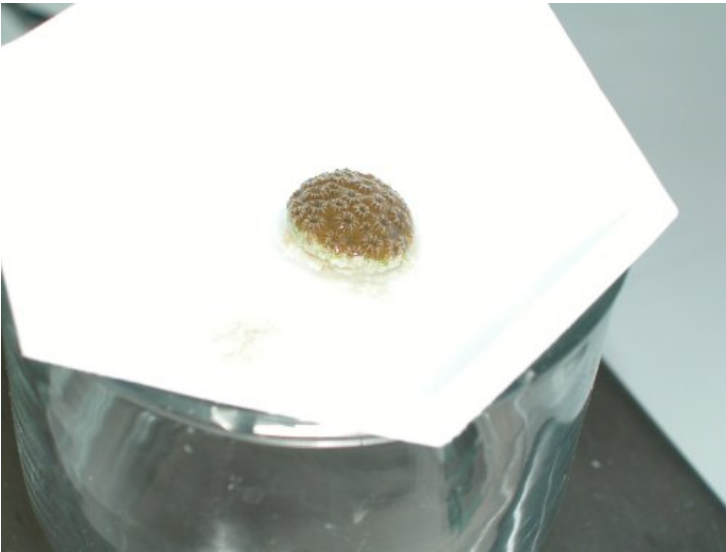


Figure 4: Core of healthy *M. annularis* coral tissue used for microbiological and molecular analyses. Similar cores were preserved for histological examination.

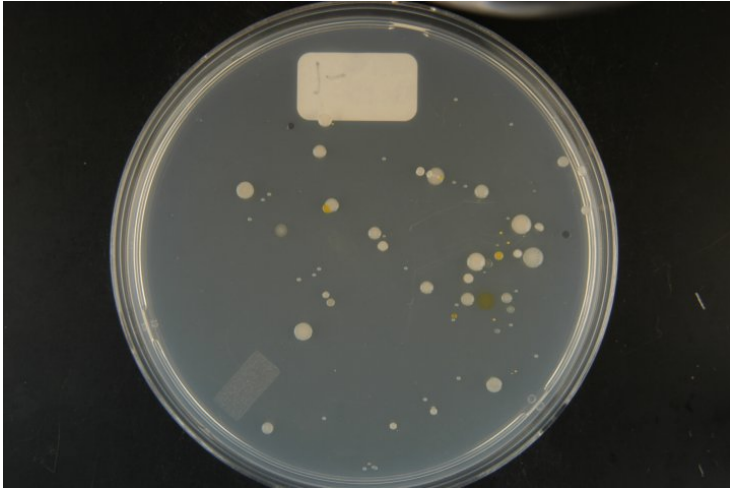


Figure 5: Bacterial colonies on a plate of one half strength marine agar 2216. Colonies were enumerated for abundance estimation and washed in DEPC water for community fingerprinting.

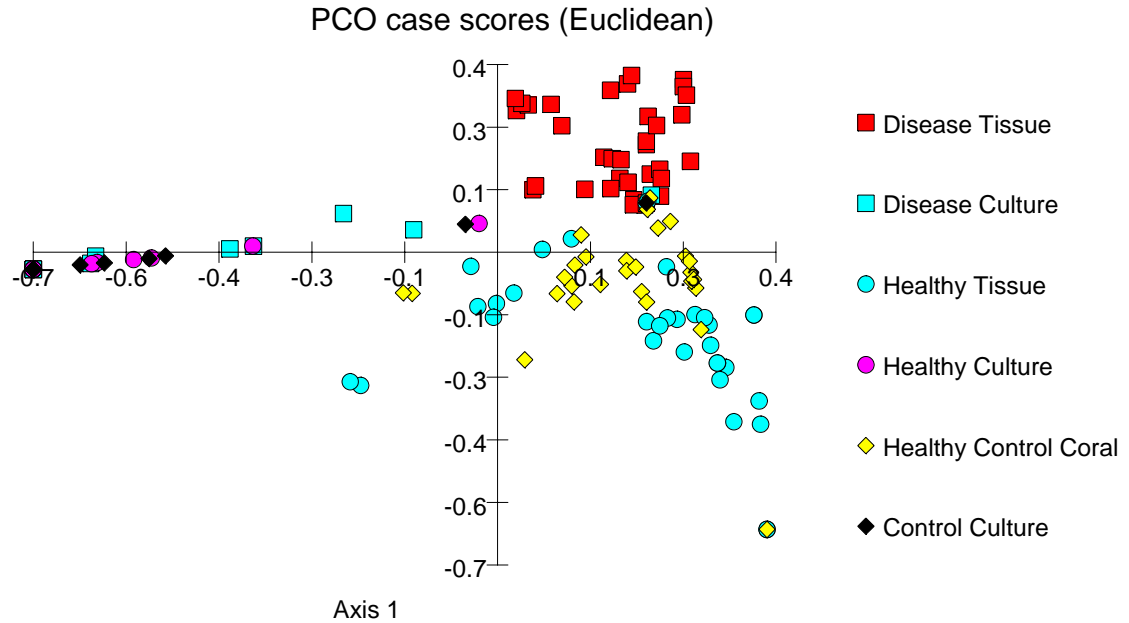


Figure 6: Principle component analysis of data from amplicon length heterogeneity. Diseased tissue components (red squares) cluster separately from healthy tissue (blue circles) and healthy control coral (yellow diamonds). Cultured bacteria from the different samples do not cluster separately.

Literature Cited:

Denner, E.B.M., G.W. Smith, H-J Busse, P. Schumann, T. Narzt, S.W. Polson, W. Lubitz and L.L. Richardson. 2003. *Aurantimonas coralicida* gen. nov., sp.nov., the causative agent of white plague type II on Caribbean scleractinean corals. *Int. J. of Systematic and Evol. Microbiol.* 53:1115-1122.