THE ECOLOGY AND MICROBIOLOGY OF BLACK BAND DISEASE AND BROWN BAND SYNDROME ON THE GREAT BARRIER REEF

Thesis submitted by

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THESIS DEDICATION

This thesis is dedicated to my loving parents, Buddy and Pat Boyett, for their emotional, inspirational, and financial support.

PUBLICATIONS ARISING FROM THIS THESIS

Boyett HV, Bourne DG, Willis BL (2006) Effect of elevated temperatures on the progression and spread of black band disease from the Great Barrier Reef. Marine Biology (to be submitted)

Willis BL *et al.* (2006) The ecology and microbiology of Brown Band Syndrome on the Great Barrier Reef. Science (to be submitted)

ABSTRACT

The overall objective of this study was to investigate the progression and transmission of black band disease (BBD) and brown band syndrome (BrB) on the Great Barrier Reef. Specifically, my aims were to: 1) examine variations in the natural progression and transmission of BBD and BrB between different months of the year and reef sites (Chapter 2); 2) investigate the effect of elevated temperature on the progression of BBD and BrB using experimental aquarium manipulations (Chapter 3); 3) histologically examine the microorganisms associated with BBD (Chapter 4); and 4) investigate the microorganisms associated with BrB using microbiological and molecular techniques (Chapter 5).

The *in situ* rate of progression and transmission of black band disease (BBD) on the coral *Acropora muricata* was measured and compared between seasonal field studies at Lizard Island on the Great Barrier Reef (GBR). BBD progressed along and transmitted between coral branches at a significantly faster rate during the austral summer month of January as opposed to the cooler months of July and May. The 2-3°C increase in seawater temperatures and 650 $\mu E/m^2/s$ rise in light intensities measured between the months of January and July/May suggest that elevated temperatures and light intensities are positively correlated to the progression and transmission of BBD. Temperature manipulations within closed experimental tanks were also performed to determine the effect of elevated temperatures on the rate of BBD progression. Increased rates of BBD progression within the higher temperature treatment (32°C) during summer clearly indicate that elevated temperatures near the upper thermal limits of corals promote the progression of BBD, possibly due to a combination of increased virulence of the pathogen and increased host susceptibility at these higher temperatures. However, the lack of increased progression within elevated temperature treatments (29°C and 31°C) during May trials, raise the question concerning other factors that may also be required to promote the progression of BBD.

The *in situ* rate of spread of brown band syndrome (BrB) on *Acropora muricata* branches was compared between two reefs in the northern and central sectors of the

Great Barrier Reef (GBR). The rate of spread of the syndrome was 2.3 times faster on a lagoon reef at Lizard Island (Horseshoe Reef) than on the reef flat at Davies Reef. Although a combination of parameters is most likely responsible for this variation, the most obvious difference between the two reef sites was the degree of water circulation. Experimentally elevated temperatures in aquarium experiments did not influence the progression of BrB at Lizard Island suggesting that temperature on its own does not enhance the progression of this syndrome. Future research should experimentally investigate whether or not the combination of elevated temperatures and high light intensities, commonly associated with summer months, enhance the progression of this syndrome.

The microbial consortium of BBD isolated from *Acropora elseyi*, *A. florida*, *A. muricata*, *A. nasuta*, *Pocillopora verrucosa*, and *Porites* spp. at Lizard Island consisted of five different taxa of cyanobacteria. Based on morphological characteristics, two of these cyanobacteria taxa appear to belong to the genus *Oscillatoria*, two may be in the Order *Nostocales*, and one of these taxa resembles the morphological features of *Phormidium corallyticum*. However, these predictions are based solely on histological features and further molecular identifications are required before these species can be formally classified. Morphological and molecular studies indicated that the microorganisms associated with BrB on five acroporid colonies from Davis Reef consisted of a newly identified ciliate species and an array of associated bacteria. Analysis of 18S rDNA sequence data confirmed the ciliate as a new species belonging to the Class Oligohymenophora, Subclass Scuticociliatia. Isolation of BrB bacterial species detected a potentially pathogenic strain (HB-8) which was closely affiliated by 16S rDNA comparisons with *Vibrio fortis* strains. Six out of 12 acroporid branches inoculated with this potentially pathogenic strain reached 100% mortality after 48 hours, however the macroscopic signs (brown band) of the syndrome were not observed. This indicates that there are likely two phases of BrB including a tissue necrosis phase, which may be caused by the bacterium strain HB-8, and a ciliate phase which causes the characteristic brown band of the syndrome.

In summary, the rate of progression and spread of BBD and BrB on the Great Barrier Reef appear to be dependent on the response of both the coral host and the disease pathogen to changing environmental conditions. The higher *in situ* rates of BBD progression and transmission during the summer month of January and the higher rates of BBD progression within temperature treatments experimentally elevated to near the upper thermal limits of corals likely reflect that environmental conditions detrimental to the coral host may simultaneously increase the virulence of the coral pathogen while reducing the coral's immunity. However, it is important to note that the mechanisms causing mortality (i.e. ingestion of tissue versus tissue necrosis) may also have an influence on disease progression. Consequently, in order to fully understand the mechanisms and parameters involved in the progression and transmission of coral diseases, coral disease research should continue to investigate these diseases using an ecological and microbiological approach.

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FIGURE 5.6. Phylogenetic tree exhibiting the relationships between the 16S rDNA sequence bacterium strain HB-8 and chosen reference *Vibrio* sequences listed in the GenBank database. Complete sequences were used to construct the tree and did not result in changes in the overall tree topology. The sequenced BrB strain HB-8 is denoted in bold face type while the bar represents 1% estimated sequence divergence. The outgroup used in the construction of this tree was the bacterium *Vibrio cholerae*.

FIGURE 5.7. Average percent tissue loss ± SE in coral branches exposed to a bacterium strain HB-8, control bacterial strain HB-7, and a control with no bacterial addition across time (hours).

FIGURE 5.8. The survival of healthy *Acropora* branch fragments exposed to a bacterium strain HB-8, control bacterial strain HB-7, and a control with no bacterial addition across time (hours).