Ecology and Biology of Coral Disease on the Great Barrier Reef

Thesis submitted by
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THESIS DEDICATION

I dedicate this thesis to my mother, Christine May Page, and to my daughter, Xian Christina Neale. Mum, I agree this thesis should have been completed before now.

ABSTRACT

This study examines the implications of disease for the structure and resilience of Great Barrier Reef (GBR) coral assemblages. Annual disease surveys in the northern and central sections of the GBR between 2004 and 2006 indicate that disease is ubiquitous and persistent throughout much of the GBR. Seven diseases commonly affect a wide range of anthozoan taxa, albeit at relatively low levels of prevalence (~ 3% of scleractinian corals). Nonetheless, values recorded for disease prevalence and for rates of tissue loss and mortality indicate that the acute impacts of diseases such as black band disease (BBD) and skeletal eroding band (SEB) can be similar to those of diseases that have caused significant declines in Caribbean coral populations, highlighting the significant role disease is likely to play in structuring at least some GBR coral assemblages.

Two families, the Pocilloporidae and Acroporidae, consistently ranked highest in a disease susceptibility hierarchy determined for GBR corals. Consistency in spatial patterns in the prevalence of BBD coupled with consistency in disease susceptibility hierarchies over cross- and long-shelf gradients in turbidity, wave action, and water quality, indicate that these environmental factors are not the primary drivers of disease occurrence on the GBR. Instead, concordance in family rankings for susceptibility to disease and susceptibility to other factors that compromise coral health (thermal bleaching, injury from predators, and interactions with macro-algae) suggests that fast growing, branching morphologies of acroporid and pocilloporid corals, and their resultant high abundance in many Indo-Pacific coral assemblages, enhance the vulnerability of these families to a diversity of pathogens, in addition to a range of other biological and physical stressors.

Experimental studies demonstrated the important role that injury is likely to play in the development of SEB. *Halofolliculina corallasia*, the putative pathogen of SEB, rapidly colonised artificial wounds, however, despite initial increases in ciliate densities on wounds, ciliates failed to become pathogenic and cause additional tissue mortality on any of the three coral species tested experimentally. In addition to injury, environmental factors that compromise coral health or the presence of other microbial agents may be required before ciliates become pathogenic. In combination with correlations between family rankings for susceptibility to disease and susceptibility to other factors that compromise coral health, these results highlight the need for management strategies that limit activities and factors that compromise coral health, in order to minimise the spread and transmission of coral diseases.

Partial mortality over three months caused by the three diseases examined in this study ranged from 85.7 ± 1.6% for BBD to 12.9 ± 1.7% for SEB, while rates of whole colony mortality over two years ranged from 84% for BBD to 39% for SEB. Tissue loss was not associated with growth anomalies (GAs) in this study. The combined partial and whole colony mortality caused by BBD was at least two-fold higher than mortality caused by SEB in northern GBR coral populations, however the seven-fold higher prevalence of SEB, combined with further reductions in the growth rates and reproductive output of surviving portions of *A. muricata* colonies, indicate that SEB could have greater fitness consequences for GBR *Acropora* populations than BBD. The potential for failure of gametogenesis in a large proportion of *Acropora* colonies with GAs, indicates that the impact of a chronic outbreak of GAs on the fitness of Indo-Pacific coral populations could rival those of both SEB and BBD.

Sub-lethal impacts of these diseases on growth and reproduction of *Acropora muricata* also varied, suggesting that this species can vary its resource allocation

strategy to maximise contributions to future generations in response to differing levels of disease virulence. Continued investment of resources in colony growth and reproduction in colonies with BBD may represent an attempt to maximise short-term fitness, given rapid rates of tissue loss and the high probability of dying. In contrast, colonies with GAs or slower progressing diseases such as SEB may divert resources from physiological processes not directly linked to colony survival in response to the greater likelihood that some portion of the colony will survive and recover.

Predicted increases in disease with ocean warming and anthropogenic impacts pose the greatest threat to the persistence of the highly susceptible acroporid and pocilloporid corals in this region, taking into consideration both the lethal and sub-lethal impacts of diseases highlighted in this study and the double jeopardy represented by their high vulnerability to other disturbances including cyclones, bleaching and crown-of thorns outbreaks on the GBR.

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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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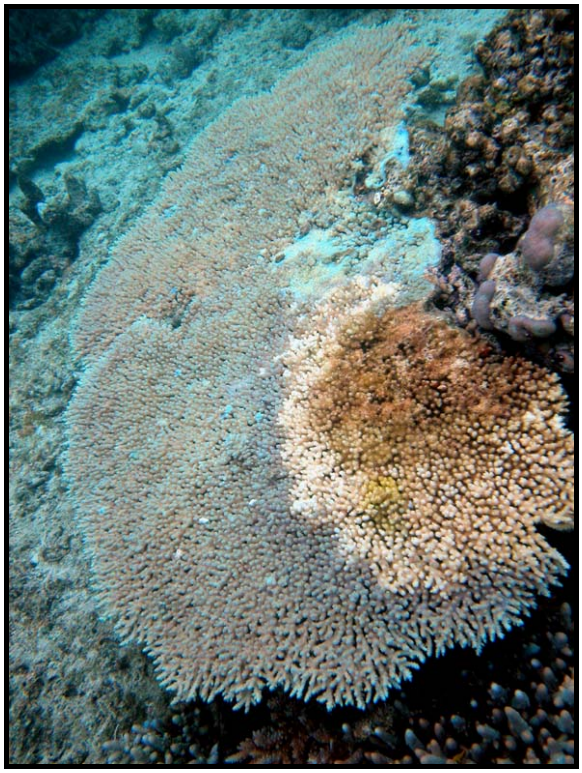
General Introduction



Tabulate *Acropora* sp. affected by white syndrome
(Day Reef, northern Great Barrier Reef)

Disease, defined as an interruption, cessation or disorder of body function, system, or organ (Stedman 2006), is ubiquitous in plant and animal populations and plays an important role in their dynamics (Anderson 1982; Collinge and Ray 2006), generally reducing population sizes of susceptible species and potentially altering community dynamics among interacting species (Park 1948; Lindstrom et al. 1994; Lefcort and Blaustein 1995; Mitchell and Power 2006). Since the 1500's, disease has contributed to the extinction of over 800 species and threatened the continued existence of an additional 2,500 plant and animal species (Smith et al. 2006b). Species extinctions often result in disease impacts being magnified in the remaining species, predisposing further species to losses and even extinction from the compounding impacts of disease outbreaks and other factors, including habitat loss and pollution (Schmidt and Ostfeld 2001; Harvell et al. 2002; Smith et al. 2006b). Disease-driven species losses may trigger trophic cascades that ultimately influence entire food webs, highlighting the important role that disease can play in the dynamics of communities (Schmidt and Ostfeld 2001; Harvell et al. 2002; Altizer et al. 2003; Smith et al. 2006b). The introduction of the *Myxoma* virus in the 1950's provides a dramatic example of the potential catastrophic consequences of disease for the functioning of a community. The virus reduced rabbit numbers throughout England and Wales by an estimated 99% between 1953 and 1955 and led to dramatic changes in grassland communities (Lloyd 1970). Reduced herbivory caused increases in wild flowering plant species, shrubs and tree seedlings and a shift from "smooth grassed lawns to tussocky hay fields" (Ross 1982). Losses of bare areas within grasslands on which wild thyme (*Thymus drucei*) grew, the initial food of the larvae of the large blue butterfly (*Maculina arion*) in Britain, are thought to have led to its extinction (Dobson and May 1986). The increased height of vegetation also reduced areas of bare sand which lowered rates of egg hatching in the sand lizard (*Lacarta*

agilis). Populations of foxes, buzzards and stoates declined in response to reductions in rabbits, which were their main prey. Breeding in tawny owls was reduced as a consequence of competition when these predators switched to preying on small rodents (summarised in Ross 1982). As highlighted by this example, knowledge of the impacts of disease on host populations and the consequence of demographic changes for other members of the community is critical to understanding the role that disease plays in the dynamics of populations and communities and to begin to develop strategies to manage disease outbreaks (Holt and Dobson 2006).

In contrast to the above examples of disease reducing species abundances and causing species losses, disease can also increase species diversity, thus understanding the role of disease in population and community dynamics is complex. If pathogens moderate the abundance of dominant species within communities (Gilbert 2002) by acting as density-dependent regulators of populations, pathogens can change the outcomes of competitive interactions between species, thereby allowing the persistence of otherwise competitively inferior species (Kiesecker and Blaustein 1999; Mitchell and Power 2006). Studies that document how species differ in susceptibility to disease are therefore critical, not only for understanding how the impacts of disease vary among species, but also for understanding the role disease plays in structuring communities (Gilbert 2002; Holt and Dobson 2006; Mitchell and Power 2006).

Increases in the number and magnitude of disease outbreaks in both marine and terrestrial ecosystems worldwide in the past few decades are widely attributed to anthropogenic-driven climate warming, habitat degradation, pollution and introductions of novel pathogens (Epstein 2001; Harvell et al. 2002; Ward and Lafferty 2004; Smith et al. 2006b). Disease outbreaks have been favoured by environmental changes that have undermined host resistance and/or increased pathogen virulence (Harvell et al.

1999; Epstein 2001). In particular, increasing temperatures associated with climate warming have contributed significantly to recent increases in epizootics (an outbreak of disease in an animal population (Stedman 2006)) and the emergence of novel diseases in both terrestrial and marine taxa (Colwell 1996; Epstein 2001; Hofmann et al. 2001; Harvell et al. 2002; Karl and Trenberth 2003; McMichael and Woodruff 2004). Future increases in global temperatures are expected to further increase the frequency and impact of disease epizootics (Epstein 2001; Harvell et al. 2002), highlighting the urgent need for research that documents the consequences of disease, particularly for taxa most vulnerable to disease, as well as the cascading impacts on other species, to help understand how management strategies might best mitigate the impacts of predicted increases in disease.

1.1. Role of Disease in Structuring Coral Reef Communities

The first reports of disease epizootics in coral reef taxa originated almost exclusively from reefs in the Caribbean (Gladfelter 1982; Lessios 1988; Garzón-Ferreira and Zea 1992). In the early 1980's, disease resulted in widespread and catastrophic declines in populations of the sea urchin *Diadema antillarum* throughout the Caribbean; over 93% of *D. antillarum* individuals perished at locations from which quantitative data were available (Lessios 1988). Mass mortalities of gorgonians were also reported from throughout the Caribbean in the 1980's (Guzmán and Cortés 1984; Garzón-Ferreira and Zea 1992), with further mortalities occurring during a subsequent epizootic that spread throughout the Caribbean in the mid-1990's (Nagelkerken et al. 1997; Kim and Harvell 2004). Over 50% of gorgonian tissues were lost from reefs in the Florida Keys during this second epizootic (Kim and Harvell 2004). Numerous novel diseases also emerged to infect Caribbean scleractinian corals (summarised in Weil

2004). Acroporid corals in particular suffered dramatic declines from the cumulative impacts of novel diseases such as white band disease and white pox (Gladfelter 1982; Miller et al. 2002; Patterson et al. 2002). The cover of *Acropora palmata* and *A. cervicornis* declined by over 90% on reefs in the Florida keys between 1983 and 2000 (Miller et al. 2002). Losses of *A. palmata* and *A. cervicornis* have been so dramatic throughout their Caribbean range (Gladfelter 1982; McClanahan and Muthiga 1998; Williams et al. 1999) that these two species are now listed under the United States Endangered Species Act as threatened (Hogarth 2006). Acroporid corals no longer dominate reefs on which they had been the dominant coral taxa for at least 95,000 years (Pandolfi and Jackson 2006) and have been replaced by coral taxa with significantly slower growth rates (Aronson and Precht 2001). Losses of acroporid corals able to rapidly monopolise bare substratum via rapid growth, in combination with low herbivory rates due to low urchin densities, have facilitated increases in macro-algae and a phase-shift from coral dominated to algal dominated reefs in the Caribbean (Hughes 1994; McClanahan and Muthiga 1998). The cumulative impacts of disease have dramatically changed the structure and function of Caribbean coral reef communities (Williams et al. 1999).

Understanding disease impacts in modular colonial organisms that dominant benthic communities on coral reefs is complicated by the fact that disease and other agents of mortality often kill some, but not all, modules, a process which is known as partial colony mortality. Since many demographic processes, including reproduction, growth and mortality are strongly size dependent in these organisms (Hughes and Jackson 1980; Jackson and Hughes 1985), partial colony mortality may cause substantial reductions in colony and population fitness. For example, disease infections that kill coral tissues reduce the number of polyps contributing to the colony's

reproductive output (Hughes and Jackson 1980; Jackson and Hughes 1985). Partial colony mortality also reduces the number of modules contributing to both autotrophic and heterotrophic nutrition of the colony and hence the energetic reserves available for reproduction and growth, processes that determine the fitness of organisms (Jackson and Hughes 1985; Hall 1997). Disease infections that reduce the size of coral colonies may increase the likelihood of whole colony mortality, given that mortality is size dependent and the likelihood of whole colony mortality highest in small colonies (Hughes and Jackson 1980; Jackson and Hughes 1985). Thus the implications of partial mortality for the fitness of modular colonial organisms are enormous, and knowledge of rates of partial and whole colony mortality, as well as the sub-lethal impacts of disease for colony growth and reproduction, are needed to understand the impacts of disease on coral population and community dynamics.

In the comparatively well-studied sea fan - Aspergillosis system, the fungal pathogen, *Aspergillus sydowii,* reduced total tissue area of the gorgonian, *Gorgonia ventalina,* by over 50% on Florida Keys reefs in the Caribbean between 1997 and 2004 (Kim and Harvell 2004). Such high rates of partial and whole colony mortality dramatically reduced the fitness of this gorgonian population. The suppression of reproduction in diseased gorgonians (Petes et al. 2003) and the greater susceptibility of large colonies that contribute disproportionately to the reproductive output of the population, reduced sea fan recruitment and resulted in a shift to smaller colonies more vulnerable to whole colony mortality (Kim and Harvell 2004). As this example clearly shows, partial and whole colony mortality caused by disease infections can have dramatic implications for the fitness of coral populations and yet few studies have documented the fitness consequences of disease for scleractinian coral populations. Three studies have examined the consequences of disease for the growth of coral

colonies (Cheney 1975; Bak 1983; Aeby 1991) and another three their impact on coral reproduction (Hunter 1997; Yamashiro et al. 2000; Petes et al. 2003). All six studies indicate that disease infections have negative consequences for the growth and reproduction of corals, further highlighting the importance of documenting rates of partial and whole colony mortality as well as any sub-lethal reductions in the growth and reproductive output of diseased colonies.

1.2. Coral Disease Impacts on Indo-Pacific Coral Reef Communities

The impact of disease on coral populations and communities in reef regions outside of the Caribbean is poorly understood (Willis et al. 2004; Harvell et al. 2007). Although comparatively fewer studies of disease have been completed in these reef regions, coral diseases have nevertheless been reported from the majority of the worlds reef regions, including: the eastern, central, southern and western-Pacific Ocean (Aeby 1991; Littler and Littler 1996; Santavy et al. 1999; Yamashiro et al. 2000; Raymundo et al. 2003; Willis et al. 2004; Work and Rameyer 2005; Dalton and Smith 2006; Phongpaichit et al. 2006), as well as in the northern, western and central Indian Ocean (Antonius 1988; Coles 1994; Jordan and Samways 2001; Ben-Haim and Rosenberg 2002; Riegl 2002; McClanahan et al. 2004b; Ravindran and Raghukumar 2005; Weil and Jordán-Dahlgren 2005; Barnesh et al. 2007; Zvuloni et al. 2009). Evidence that disease is playing an increasing role in the ecology of Indo-Pacific coral assemblages comes from the number of novel diseases that have emerged in the past decade, including atramentous necrosis (AN), white syndromes (WS), skeletal eroding band (SEB), brown band disease, and *Porites* ulcerative white spot disease, (Antonius 1999; Raymundo et al. 2003; Jones et al. 2004; Willis et al. 2004). In addition, increases in the abundance of disease cases and in the frequency of epizootics in the past two decades in

the few Indo-Pacific studies that have monitored disease (Antonius and Lipscomb 2001; Jones et al. 2004; Willis et al. 2004; Bruno et al. 2007) provide evidence that disease may have a greater role in structuring Indo-Pacific coral communities and populations than previously thought. The frequency of skeletal eroding band (SEB) cases increased from rare to frequent (an increase from 1-3 cases to 13-25 cases per 30 minute scan) on reefs of Mauritius between 1990 and 1998 and in the Red Sea between 1994 and 1997 (Antonius and Lipscomb 2001). On the Great Barrier Reef (GBR), SEB also increased in frequency from rare to moderate at Lizard Island in the northern GBR between 1988 and 1998 (an increase from 1-3 to 4-12 cases per 30 minute scan) (Antonius and Lipscomb 2001). Also on the GBR, large-scale surveys spanning its length and breadth, documented a 20-fold increase in cases of WS between 1998 and 2003 (Willis et al. 2004). Moreover, epizootics affecting corals on both the GBR and along the east-African coast (Jones et al. 2004; McClanahan et al. 2004b) highlight the important role disease is likely to play in structuring Indo-Pacific coral assemblages.

1.3. Coral Disease on the Great Barrier Reef

Monitoring programs such as those described above are providing evidence of increasing abundance of disease on the GBR, but the majority of these diseases are poorly characterised in terms of their etiology and ecology. Seven diseases are currently recognised to affect corals of the GBR (skeletal eroding band (SEB), black band disease (BBD), other cyanobacteria infections (OtCy), white syndrome (WS), brown band disease (BrB), atramentous necrosis (AN) and growth anomalies (GAs); Jones et al. 2004; Willis et al. 2004), three of which have increased in frequency during the last decade in this region (see details above for skeletal eroding band (SEB), atramentous necrosis (AN), and white syndromes (WS)) (Antonius and Lipscomb 2001; Jones et al.

2004; Willis et al. 2004). However, the prevalence and host range of diseases affecting GBR corals are known only from small-scale surveys (surveys completed on a maximum of three reefs) (Loya et al. 1984; Dinsdale 2000; Willis et al. 2004). The applicability of results from such small-scale surveys for the larger GBR is unclear since surveys don't encompass major gradients of anthropogenic pollution, wave action, light and coral assemblage composition, highlighting the need for large-scale surveys of disease prevalence in this region. Large-scale surveys that document the susceptibility of coral hosts and the distribution of diseases are also required to identify GBR corals most susceptible to and hence most vulnerable to losses from disease infections over large spatial scales. Determining whether taxa most susceptible to disease are also those most susceptible to other disturbances (e.g. cyclones, crown-of-thorns starfish outbreaks, bleaching etc) will provide insights into how losses of corals to disease might compound coral losses from other disturbances. Such information is critical for determining how disease, either solely or in combination with other disturbances, influences the structure of coral assemblages on the GBR.

As in other reef regions, little information about the consequences of disease infections for the fitness of GBR coral populations is available. Linear rates at which coral tissues are killed as disease fronts progress across coral colonies are known from short-term (maximum 5 month) studies of AN, WS and black band disease (BBD) from this region (Lonergan 2006; Roff et al. 2006; Boyett et al. 2007). Rates of partial and whole colony mortality, however, are currently not known for any of the seven diseases affecting corals on the GBR. The sub-lethal impacts of six of these diseases on growth and reproduction of corals have not been investigated on the GBR or in any other Indo-Pacific region. The systemic suppression of coral growth and reproduction by tumours found in studies on reefs in Japan, Guam and the Caribbean (Cheney 1975; Hunter

1997; Yamashiro et al. 2000) suggests that growth anomalies may have similar impacts on GBR corals. The concurrence of both dramatic increases in WS in the northern and southern GBR and the AN epizootic on reefs surrounding Magnetic Island with elevated seawater temperatures in 2002 (Jones et al. 2004; Bruno et al. 2007) highlights the likelihood that the prevalence and impacts of disease on the fitness of GBR coral populations will increase as seawater temperatures increase with predicted climate warming (IPCC 2002). The likelihood of future increases in disease on the GBR highlights the urgency with which studies of the pathology and ecology of coral diseases are needed from this region. Most diseases affecting corals on the GBR are widely distributed throughout the Indo-Pacific (Willis et al. 2004; Harvell et al. 2007), therefore knowledge of disease impacts on the GBR will also provide insights into the possible impacts of these diseases in other Indo-Pacific regions.

1.4. Objectives

The research presented in this thesis addresses questions about the ecology, etiology and population impacts of coral disease on the Great Barrier Reef (GBR). The specific objectives of my research were to:

1. Examine the influence of coral community attributes and environmental factors on the prevalence of black band disease over large spatial scales on the GBR

Determining the environmental factors or attributes of coral communities that contribute to the elevated prevalence of black band disease within the GBR will assist with identifying how management strategies might minimise the impact of this disease in coral assemblages in this and other Indo-Pacific regions.

2. Investigate the etiology, impact, and role of injury in the initiation of skeletal eroding band on corals of the GBR

SEB is the most prevalent disease on the GBR, therefore knowledge of its etiology and impact in GBR corals represents a critical step towards understanding the consequences of disease for coral populations in this region. Understanding the role of injury in SEB initiation is important to help develop management strategies that minimise the spread and transmission of this prevalent disease.

3. Determine the susceptibility of scleractinian taxa to disease on the GBR

Disease plays an important role in structuring plant and animal communities and insights into the likely role of disease in structuring scleractinian assemblages on the GBR is gained from an examination of how scleractinian taxa differ in their susceptibility to disease. Comparisons of the taxa most susceptible to disease and other disturbances will help to identify taxa vulnerable to losses from their cumulative impacts.

4. Document population impacts of diseases commonly affecting corals on the GBR

Knowledge of the lethal and sub-lethal impacts of diseases for the fitness of coral populations is crucial for understanding how the resilience of coral populations on the GBR is compromised by disease.

Chapter 2

Distribution, host range and large-scale spatial variability in black band disease prevalence on the Great Barrier Reef, Australia

Tagged *Acropora* colony with numerous branches affected by black band disease (Lizard Island, northern Great Barrier Reef)

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2.1. ABSTRACT

The prevalence and host range of black band disease (BBD) was determined from surveys of 19 reefs within the Great Barrier Reef Marine Park, Australia. Prevalence of BBD was compared among reefs distributed across large-scale cross-shelf and long-shelf gradients of terrestrial or anthropogenic influence. BBD was widespread throughout the Great Barrier Reef (GBR) and was present on 73.7% of the 19 reefs surveyed in three latitudinal sectors and three cross-shelf positions in the summer of 2004. Although BBD occurred on all mid-shelf reefs and all but one outer-shelf reef, overall prevalence was low, infecting on average 0.09% of sessile cnidarians and 0.1% of scleractinian corals surveyed. BBD affected ~7% of scleractinian taxa (25 of approximately 350 GBR hard coral species) and 1 soft coral family, although most cases of BBD were recorded on branching *Acropora* species. Prevalence of BBD did not correlate with distance from terrestrial influences, being highest on mid-shelf reefs and lowest on inshore reefs (absent from 66%, n = 6, of these reefs). BBD prevalence was consistently higher in all shelf positions in the northern (Cooktown/Lizard Island) sector, which is adjacent to relatively pristine catchments compared to the central (Townsville) sector, which is adjacent to a more developed catchment. BBD cases were clustered within reefs and transects, which was consistent with local dispersal of pathogens via currents, although the spread of BBD was not dependent on the density or cover of any of the coral taxa examined. In combination, these results suggest that BBD is part of the natural ecology of coral assemblages of the GBR, and its prevalence is relatively unaffected by terrestrial influences on the scales characteristic of cross-shelf gradients.

2.2. INTRODUCTION

Black band disease (BBD) was first reported from reefs of Belize and the Florida Keys in 1973 (Antonius 1973) but is now known to be widely distributed throughout most reef regions of the world. BBD has been recorded from the Caribbean (Garrett and Ducklow 1975; Rutzler et al. 1983; Edmunds 1991), the Red Sea (Antonius 1988; Al-Moghrabi 2001), and from Indo-Pacific locations including Fiji (Littler and Littler 1996), the Philippines (Antonius 1985b), Papua New Guinea (Frias-Lopez et al. 2003) and the Great Barrier Reef (GBR) (Miller 1996; Dinsdale 2000; Willis et al. 2004). Although generally low in prevalence (< 5% of all corals) in most reef regions (Edmunds 1991; Bruckner et al. 1997; Dinsdale 2000; Weil et al. 2002), BBD infections have been found to persist on the same reef for years and possibly for decades in the Caribbean, contributing to the long-term mortality of susceptible coral species (Bruckner and Bruckner 1997b). Low level chronic infections of BBD over years to decades are predicted to reduce the size of coral populations (Edmunds 1991) and consequently their reproductive output and recruitment, while differential susceptibility of species to BBD may result in long-term changes to coral community structure (Bruckner and Bruckner 1997b).

Despite the potential chronic impact of BBD on reef corals worldwide, most research on this disease has focussed on reefs within the wider Caribbean region (Edmunds 1991; Bruckner and Bruckner 1997b; Porter et al. 2001; Weil et al. 2002). On Indo-Pacific reefs, BBD was first recorded on massive faviid species in the Philippines and the Red Sea between 1981 and 1984 (Antonius 1985b). It was almost another decade before BBD was recorded on reefs of the GBR (Miller 1996), despite its probable presence prior to this. Miller (1996) recorded BBD on 19% of 110 reefs surveyed over a three year period (1993 to 1996) and concluded that BBD “exists at low

levels" throughout the GBR, with cases restricted to the families Acroporidae and Poritidae. The first quantitative study of BBD prevalence on the GBR expanded the number of susceptible taxa to 21 species in 5 scleractinian families (Acroporidae, Pocilloporidae, Faviidae, Poritidae and Mussidae) and found BBD affected 1.3 to 4.9% of all corals at Lizard Island (Dinsdale 2000). More recently, BBD and other cyanobacterial infections were recorded on 28 species from 8 scleractinian families on three GBR reefs selected because of high disease abundance in general. However, the low prevalence of BBD and other cyanobacterial infections (0.01 to 1.5% of all corals) in this study and the very low frequency of BBD cases recorded throughout the previous five year period (1998 to 2003) (Willis et al. 2004) suggest that BBD prevalence has remained low on the GBR since at least 1994. BBD levels on the three GBR reefs where prevalence has been quantified (Willis et al. 2004) are comparable to prevalence on many reefs in the Caribbean (Edmunds 1991; Kuta and Richardson 1996; Bruckner and Bruckner 1997b), but studies over larger spatial scales are required to determine how indicative these reefs are of the GBR in general and to determine the distribution and host range of BBD throughout the Great Barrier Reef.

Environmental conditions experienced by corals are thought to be important in determining the occurrence and prevalence of BBD (Antonius 1981a; Antonius 1985a; Littler and Littler 1996; Bruckner et al. 1997; Al-Moghrabi 2001; Frias-Lopez et al. 2002; Kuta and Richardson 2002), although only one study has quantitatively examined links between environmental parameters and BBD (Kuta and Richardson 2002). Florida Keys patch reefs with BBD-infected corals present were shallower in depth, experienced higher water temperatures, higher nitrite concentrations, lower orthophosphate concentrations and had lower coral diversity than reefs with no BBD infections (Kuta and Richardson 2002). Environmental gradients among GBR reefs

associated with (1) distance across the continental shelf (cross-shelf gradients), and (2) distance from developed catchments (long-shelf or latitudinal gradients) provide a unique tool for identifying factors correlated with the prevalence of BBD in the Indo-Pacific. Cross-shelf gradients between inner- and outer-shelf reefs on the GBR include decreasing exposure to terrestrial influences such as nutrients, suspended particulate matter (including sediments) and pollutants (Furnas et al. 1990; Furnas 2003) coupled with increasing wave exposure (Done 1982). On mid- and outer-shelf reefs of the GBR, the concentrations of suspended matter are typically less than 1 mg l^{-1}, while concentrations on inner-shelf reefs span a much larger range from <1 mg l^{-1} to >200 mg l^{-1} (Furnas 2003). Higher levels of suspended matter on inner-shelf reefs result in a reduction in water transparency and light levels for corals growing on these reefs and sediment smothering in extreme cases (Wolanski et al. 2003). Dissolved and particulate nutrients, especially particulate nitrogen, are also typically higher in inshore waters of the GBR and decrease across the continental shelf (Furnas 2003). Long-shelf gradients between the northern (Cooktown/Lizard Island) and central (Townsville) sectors relate to land use patterns and development levels in adjacent catchments. In particular, catchments adjacent to the Townsville sector are characterised by urban development and heavy land clearing for sugar cane farming and cattle grazing, whereas catchments adjacent to the Lizard Island sector are comparatively pristine (Furnas et al. 1990, Furnas 2003). Within the main river catchments adjacent to the northern Cooktown/Lizard Island sector, between 1 to 7% of land is cleared compared to 17 to 30% of the main river catchments adjacent to the central Townsville sector (Furnas 2003). High levels of clearing in the Townsville sector may contribute to higher output of terrigenous sediment by river systems in adjacent catchments (Furnas 2003). Reefs in the southern (Heron Island) sector are more than 80 km from the coast and relatively

free from coastal terrigenous inputs. Consequently, cross-shelf studies of coral disease in these three sectors of the GBR provide a unique opportunity to examine the influence of environmental parameters related to terrestrial inputs on the prevalence of BBD.

In this study, I quantify the prevalence of BBD on 19 reefs of the GBR located in three latitudinal sectors and spread over three cross-shelf positions that represent a gradient of terrestrial influence with respect to nutrients, suspended sediment and other pollutants. This is the first study of coral disease prevalence on the GBR that encompasses both large-scale cross-shelf and long-shelf gradients. The aims of this study were to: 1) compare variability in BBD prevalence between latitudinal sectors and cross-shelf positions, 2) identify all taxa susceptible to BBD on the GBR, and 3) examine coral community attributes and environmental factors influencing BBD prevalence across a gradient of terrestrial influence.

2.3. METHODS

To examine spatial variability in the prevalence of BBD between reefs adjacent to comparatively pristine versus developed catchments and to determine the taxa affected by BBD on the GBR, disease prevalence was surveyed within 3 latitudinal sectors in January to March 2004 according to methods described in Willis et al. (2004). According to Willis et al. (2004), BBD and other cyanobacterial infection prevalence was significantly higher during summer compared to winter surveys in one year at Lizard Island, consistent with BBD prevalence varying seasonally on the GBR. Therefore, to maximise documentation of host ranges and terrestrial impacts on prevalence of BBD on the GBR, reefs were surveyed during summer when the BBD prevalence is likely to be highest (Willis et al. 2004).

To examine spatial variability in the prevalence of BBD along gradients of terrestrial influences, three haphazardly selected reefs were surveyed within each of three cross-shelf positions (inner-, mid- and outer-shelf) in 2 latitudinal sectors (Cooktown / Lizard Island and Townsville, henceforth referred to respectively as the northern and central sectors)(Fig 2.1). The cross-shelf transects represent a gradient of decreasing terrestrial influence and increasing wave exposure from the inner to outer-shelf positions. On each reef, two sites were haphazardly selected within the north-west sheltered back reef zone. One sheltered and one exposed site at one reef (Heron Island) in the southern-most Capricorn Bunker group (henceforth referred to as the southern sector) was surveyed to examine how BBD prevalence varied between northern and southern sectors of the GBR. Mid- or inner-shelf reefs were not surveyed in the southern sector due to a paucity of coral reefs in these cross-shelf positions in this latitudinal sector (Cheal et al. 2001). Reefs surveyed in each latitudinal sector are shown in Fig 2.1 and listed in Table 2.1.

At each site, I haphazardly placed three 20 m × 2 m belt transects parallel to depth contours on the reef slope at 3 to 6 m depth. Within each 20 m × 2 m belt, all scleractinians, gorgonians, alcyonaceans and hydrocorals were examined for signs of BBD. BBD cases were characterised by diffuse areas of tissue loss revealing bare white coral skeleton separated from live coral tissues by black mats or bands comprised primarily of cyanobacteria. Samples of some, but not all, recorded cases of BBD were collected and examined microscopically to confirm identification. The presence or absence of BBD on each colony was recorded and corals were identified to the lowest taxonomic or morphological group, as appropriate. All other signs of disease on colonies with BBD were also recorded. All corals with signs of BBD observed outside

of the belt-transects were also identified to the lowest taxonomic or morphological group as appropriate and noted separately.

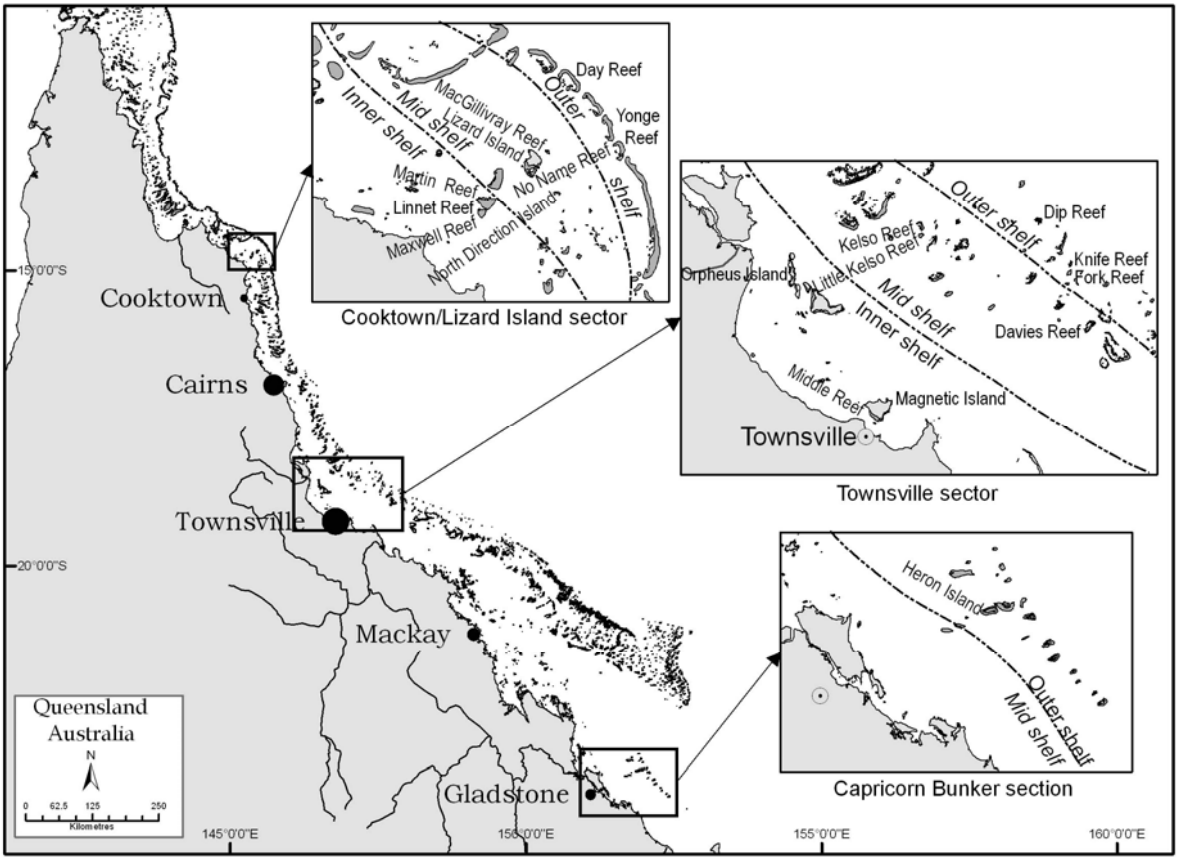
The effects of latitude, cross-shelf position and their interaction were tested using the linear model: BBD = latitude + cross-shelf position + latitude x cross-shelf position. The response variable was the binary classification of coral colonies as either diseased or non-diseased. Binary data are not modelled well by classical analysis of variance because random errors of binary data are unlikely to be normally distributed. Consequently, I chose a categorical data analysis of the linear model using a logistic regression in which the independent factors were defined as classes rather than continuous variables and the logit (the log of the ratio of probability of disease versus non-diseased) was the link function (McCullagh and Nelder 1989). First a main effects-only model was fitted and secondly the saturated model, and evaluated their relative goodness of fit using Schwarz's Bayesian Criterion. This sequential approach was required to avoid over-fitting the model.

To determine whether cases of BBD were aggregated or randomly distributed, the frequency distributions of cases of BBD per transect and reef were tested for goodness of fit to a Poisson distribution using chi-squared tests.

Coral cover was determined at each site from line intercept data recorded along the central line of each belt transect. The relationships between BBD prevalence and both the cover and density of all corals, scleractinian corals and acroporid corals were examined using regressions. A G-test was used to determine if all families found to have BBD infections were equally susceptible. All families without BBD infections were excluded and data from all reefs were pooled to satisfy test requirements that cells contain a minimum frequency of 5.

Cyanobacterial infections exhibiting gross morphological differences to BBD (i.e. not forming a blackish matt or band) were recorded separately from BBD and are discussed only briefly in this paper. Cyanobacteria that formed only a very thin line at the interface between live coral tissue and recently denuded skeleton, were not black in colouration or appear microscopically to be morphologically distinct from the cyanobacteria dominant in BBD mats have been recorded previously from the GBR, but their status is still unresolved (Willis et al. 2004). Therefore the prevalence and host range of these other cyanobacterial infections is not reported here.

Figure 2.1. Reefs surveyed for BBD in three latitudinal sectors of the Great Barrier Reef. Reefs numbered as follows: (1) Day Reef, (2) Yonge Reef, (3) No Name Reef, (4) Macgillivray Reef, (5) Lizard Island, (6) North Direction Island, (7) Martin Reef, (8) Linnet Reef, (9) Maxwell Reef, (10) Dip Reef, (11) Knife Reef, (12) Fork Reef, (13) Kelso Reef, (14) Little Kelso Reef, (15) Davies Reef, (16) Orpheus Island, (17) Magnetic Island, (18) Middle Reef, (19) Heron Island.



2.4. RESULTS

2.4.1. Distribution and Spatial Variability of Black Band Disease

The distribution of BBD on the GBR was widespread in the summer (January-March) of 2004, being recorded from 73.7% of the 19 reefs surveyed across sectors in each of the northern, central and southern sections of the Great Barrier Reef Marine Park. The proportions of reefs with BBD in the northern (88.9%) and central (66.7%) sectors were high and not statistically distinguishable (Yates corrected $\chi^2 = 0.32$, df = 1, p = 0.57). BBD was also present on the one 1 reef surveyed in the southern sector. BBD was recorded on all mid-shelf reefs in both the northern and central sectors and on all outer-shelf reefs in each of the three 3 sectors, with the exception of 1 outer-shelf reef in the northern sector. In contrast, BBD was absent from all inner-shelf reefs in the central sector and one 1 inner-shelf reef (Maxwell Reef) in the northern sector.

Although BBD was recorded from 73.7% of reefs and 70.2% of transects, the distribution of BBD was not random. Cases of BBD were clumped at both the reef ($\chi^2 = 282.3$, df = 3, $p < 0.001$) and transect level ($\chi^2 = 589.5$, df = 3, $p < 0.001$). Despite its clumped distribution, the prevalence of BBD was not dependent on the density or cover of any coral taxa examined. I found no significant relationship between BBD prevalence and either cover ($r^2 = 0.009$, F = 0.146, p = 0.7; Fig 2.2a) or density ($r^2 = 0.08$, F = 0.126, p = 0.72; Fig 2.2b) when all corals (scleractinian, octocoral and hydrocorals) were combined. Since most cases of BBD were recorded from scleractinian corals and specifically from corals within the family Acroporidae, the analyses were repeated, excluding first all non-scleractinian corals and then non-acroporid corals. However, there was still no significant relationship between BBD prevalence and cover ($r^2 = 0.028$, F = 0.465, p = 0.5; Fig 2.2c) or density ($r^2 = 0.016$, F = 0.254, p = 0.62; Fig 2.2d)

of scleractinian corals, nor between BBD prevalence and cover ($r^2 = 0.095$, $F = 1.67$, $p = 0.21$; Fig 2.2e) or density ($r^2 = 0.1$, $F=1.66$, $p = 0.21$; Fig 2.2f) of acroporid corals.

Table 2.1 Total number of black band (BBD) cases and BBD prevalence per reef.

Latitudinal sector	Cross-shelf position	Reef name	Number of BBD cases	BBD prevalence (%)
Cooktown/ Lizard Island	Inner-	Linnet Reef	4	0.061
		Martin Reef	2	0.020
		Maxwell Reef	0	0.000
	Mid-	Lizard Island	1	0.013
		Macgillivray Reef	6	0.101
		North Direction Island	48	0.696
	Outer	Day Reef	18	0.261
		No Name Reef	0	0.000
		Yonge Reef	3	0.047
Townsville	Inner-	Magnetic Island	0	0.000
		Middle Reef	0	0.000
		Orpheus Island	0	0.000
	Mid-	Davies Reef	4	0.132
		Kelso Reef	6	0.104
		Little Kelso Reef	3	0.083
	Outer-	Dip Reef	3	0.063
		Fork Reef	2	0.037
		Knife Reef	3	0.063
Capricorn Bunkers	Outer-	Heron Island	1	0.012

The prevalence of BBD on the GBR was low, affecting on average 0.09% ± 0.04 of corals per reef (n = 19 reefs) (Table 2.1). In the northern and central sectors, only 103 colonies of the 105,421 examined were infected. The highest prevalence was recorded at North Direction Island in the northern sector, where 0.7% (n = 6899) of corals were infected. Only one case of BBD was recorded from the two sites surveyed in the southern sector (i.e. 0.012% of 8326 corals surveyed). On the GBR, BBD predominately affects scleractinian corals, with only one recorded case for soft corals. If all non-scleractinian corals are excluded, BBD prevalence increases slightly to an average of 0.1% of all scleractinian corals, with prevalence ranging between 0% and 0.74% of scleractinian corals per reef.

The prevalence of BBD was consistently higher in the northern sector in comparison to the central sector at all cross-shelf positions (Table 2.2, Fig 2.3). Cross-shelf patterns in BBD prevalence were consistent in both the northern and central sectors, with the percentage of infected corals peaking on mid-shelf reefs and reaching minima on inner-shelf reefs. In fact, no cases of BBD were recorded from inner-shelf reefs in the central sector (Table 2.1, Table 2.2, Fig 2.3). Although there are clear cross-shelf differences in BBD prevalence in the central sector, this pattern is not as robust in the northern sector, due to the higher variation in BBD prevalence at mid- and outer-shelf reefs. This was because, although reefs were selected haphazardly, one reef in each of the mid- and outer-shelf positions in the northern sector is largely driving cross-shelf and long-shelf patterns in BBD prevalence in this sector (87% of BBD cases recorded from the northern sector mid-shelf and 86% of cases from the northern sector outer-shelf were each recorded from single reefs, i.e. North Direction Island and Day Reef respectively). However, the overall similarity in the cross-shelf pattern found in the central sector supports the generality of this pattern.

Figure 2.2. Relationship between BBD prevalence and a) cover of all corals, b) density of all corals, c) cover of scleractinian corals, d) density of scleractinian corals, e) cover of Acroporid corals, f) density of Acroporid corals.

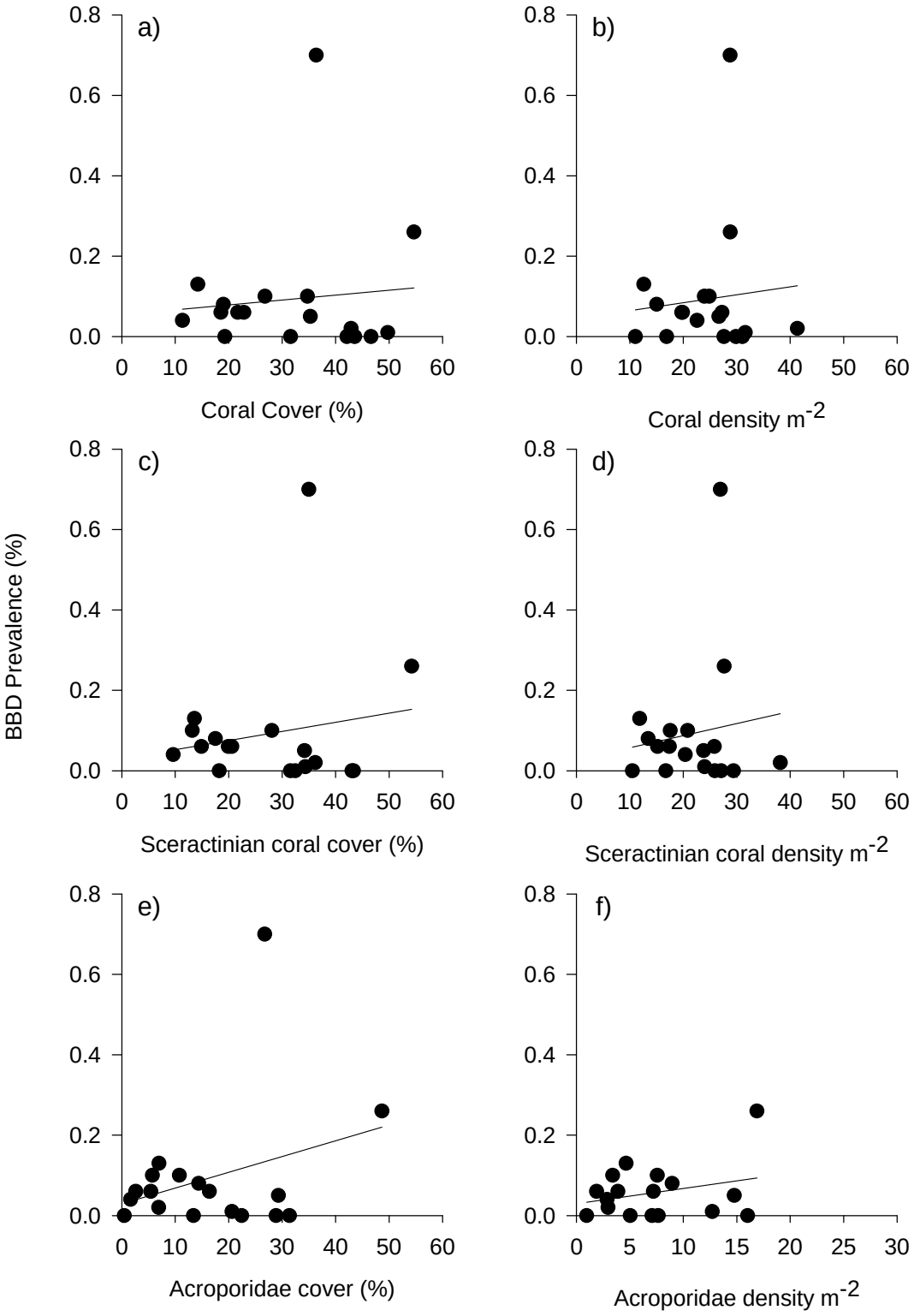
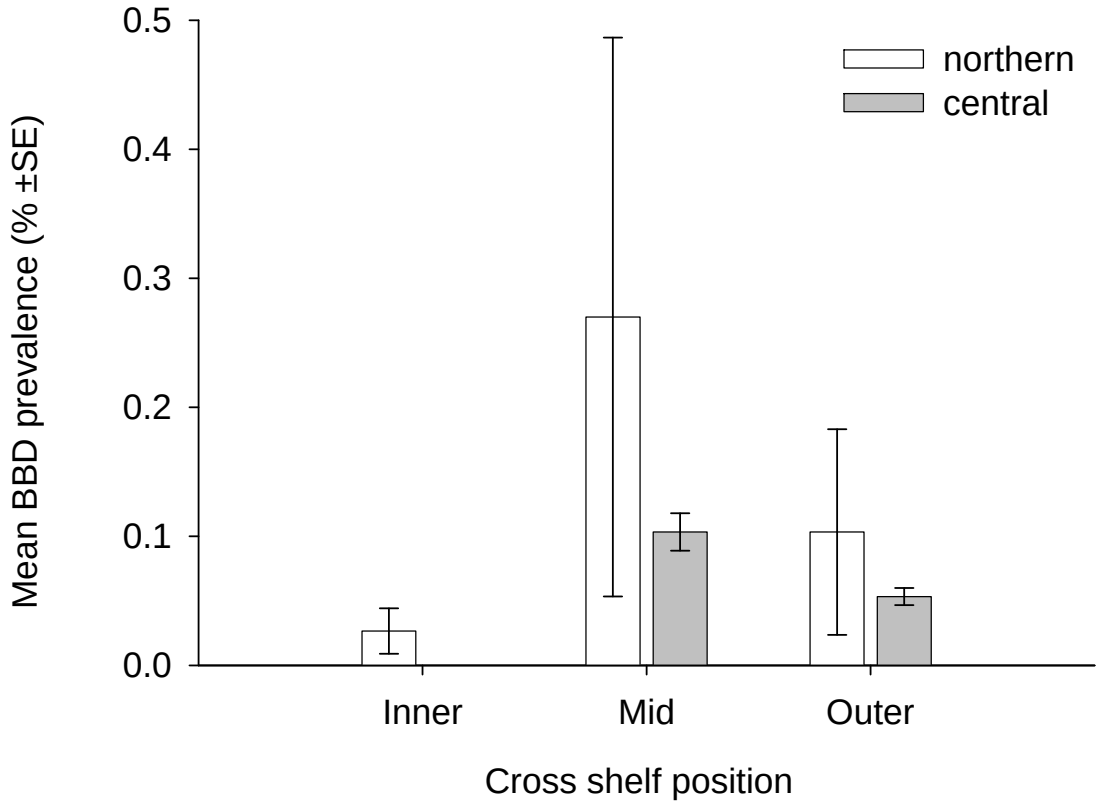


Table 2.2. Linear regression analyses of proportion of corals affected by BBD.

Model 1				Model 2 (Saturated model)		
Factor	df	$\chi 2$	p	df	$\chi 2$	p
Sector	1	14.6809	0.0001	1	0.0013	0.9716
Shelf	2	43.5908	<0.0001	1	10.1871	0.0061
Sector × Shelf				1	0.3535	0.8380
Δ in Schwarz's Bayesian criterion: 79.97				Δ in Schwarz's Bayesian criterion: –0.722a		

a Negative change in Schwarz's Bayesian criterion indicates no improvement of model by interaction term

Figure 2.3. Percentage of all corals (± SE) affected by BBD in each cross shelf position in the northern (Cooktown / Lizard Island) and central (Townsville) sectors of the Great Barrier Reef in the summer of 2004.



2.4.2. Host Range

BBD predominately affects scleractinian corals on the GBR, with only one case of BBD affecting a soft coral recorded from the 19 reefs surveyed. In total, cases of BBD were recorded from 7 scleractinian families (Acroporidae, Pocilloporidae, Poritidae, Faviidae, Mussidae, Siderastreidae and Dendrophylliidae), 12 scleractinian genera and at least 25 species (~7% of GBR scleractinian corals) (Table 2.3). In many cases the species affected could not be identified in the field and so were recorded only to genus and morphological group. Therefore, Table 2.3 represents an underestimate of the number of species affected by BBD on the GBR. These records extend the number of hosts for BBD on the GBR from 32 (Willis et al. 2004) to 40. Based on the belt-transect surveys on sheltered sites at 19 reefs, the seven families affected by BBD were found to differ in their susceptibilities to infection (G-test = 126.808, df = 3, $p < 0.01$). Species within the family Acroporidae were by far the most susceptible, with 90% of all infections found on species within this family. All genera within the family Acroporidae (including the sub-genus *Isopora*) were affected; however branching species of *Acropora* (particularly *A. muricata, A. florida and A. intermedia*) were the most often affected species. Five percent of BBD infections were recorded from species in the family Poritidae, 3% from the family Pocilloporidae and 1% of infections from each of the families Dendrophylliidae and Alcyoniidae.

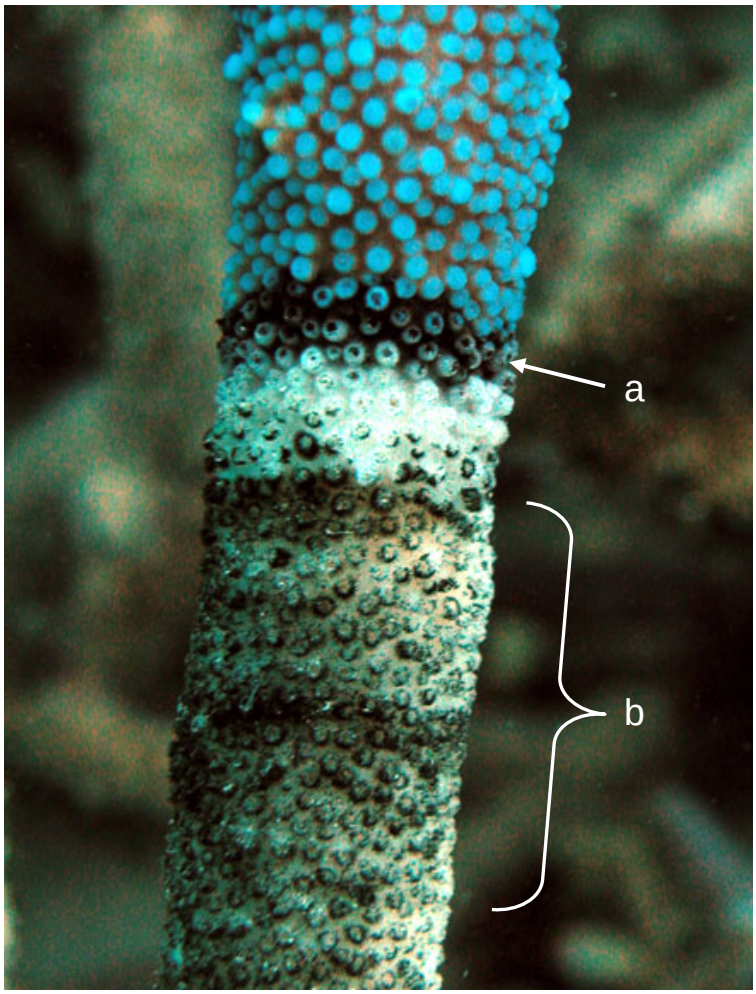
Table 2.3. Total number of cases of BBD and occurrence of BBD in combination with other disease signs, for all taxa. * indicates cases of each recorded taxa affected by BBD outside of transect boundaries.

Family	Genera	Morphological group and species	Total	BBD	BBD and Bleaching	BBD and SEB	BBD and SEB and Bleaching
Acroporidae	*Acropora*	Staghorn *Acropora* spp. (*A. muricata, A. florida, A. intermedia*)	76,3*	65,3*	4	4	3
		Tabulate *Acropora* spp. (*A. cytherea, A. hyacinthus*)	7,2*	7,2*	0	0	0
		Bushy *Acropora* spp.	3	3	0	0	0
		Corymbose *Acropora* spp.	2	2	0	0	0
		Digitate *Acropora* spp.	1,1*	1,1*	0	0	0
		Bottlebrush *Acropora* spp.	1	1	0	0	0
	(Sub-genus *Isopora*)	*A. bruggemanni*	1	1	0	1	0
	Astreopora	*Astreopora* sp.	1	1	0	0	0
	Montipora	*Montipora* spp.	2,3*	2,3*	0	0	0
Pocilloporidae	*Pocillopora* *	*P. damicornis**	2*	2*	0	0	0
		Other *Pocillopora**	3	3	0	0	0
	Seriatopora	*Seriatopora hystrix*	2, 1*	2, 1*	0	0	0
	Stylophora	*Stylophora pistillata*	1,1*	1,1*	0	0	0
Poritidae	*Porites*	Branching *Porites* spp.	2	2	0	0	0
		Massive *Porites* spp.	2	2	0	0	0
		Other *Porites* sp.	1	1	0	0	0
	*Goniopora**	*Goniopora* sp.*	1*	1*	0	0	0
Mussidae*			1	1	0	0	0
Faviidae*	*Favia**	*Favia* sp*	1*	1*	0	0	0
	*Cyphastrea**	*Cyphastrea* sp.*	1*	1*	0	0	0
Sideraestreidae*	*Psammacora**	*Psammacora minuta**	1*	1*	0	0	0
Dendrophyllidae	*Turbinaria*	*Turbinaria* sp.	1	1	0	0	0
Alcyoniidae			1	1	0	0	0

2.4.3. BBD in Combination with Other Disease Signs

Approximately 10% of colonies with signs of BBD also showed signs of either bleaching (3.4%), skeletal eroding band (SEB) (4.3%), or signs of both of these disease states combined (2.6%). Fig 2.4 shows a colony of *Acropora muricata* infected with both BBD and the heterotrich folliculinid *Halofolliculina corallasia*, the putative causative agent of skeletal eroding band (Antonius and Lipscomb 2001). In the specimen pictured, the *H. corallasia* individuals are embedded in the coral skeleton behind the BBD front, suggesting that the cyanobacterial mat is the main cause of coral mortality. In some cases, however, groups of *H. corallasia* have been observed both ahead of the BBD front and amongst the BBD cyanobacterial mat. Eleven of the 12 cases of BBD occurring with other signs of disease were recorded on branching *Acropora* species.

Figure 2.4. The characteristic black cyanobacteria matt of black band disease a) and *Hallofolliculina corallasia* (the causative agent of skeletal eroding band (SEB)) b) on the same colony of *Acropora muricata*.



2.5. DISCUSSION

The detection of BBD on 74% of reefs surveyed (n = 19) and in three latitudinal sectors encompassing 1000 kms of the Queensland coast, indicates that BBD is widely distributed over the length of the Great Barrier Reef. This contrasts with findings of other authors: Miller (1996) found that BBD was present on only 19% of 110 GBR reefs surveyed between 1993 and 1996, while the Australian Institute of Marine Science Long-term Monitoring Program (unpubl. data) found BBD was present on between 2 and 17% of 48 GBR reefs between 1998 and 2004. These differences in findings are almost certainly attributable to differences in survey methods, the timing of surveys and differences in the reef zone surveyed. The up to 4-fold greater percentage of reefs with BBD in this study reflects the greater likelihood of detecting macroscopic signs of the disease by searching a comparatively small belt transect on SCUBA. The Manta Tow and opportunistic surveys of large (> 750 m^2) areas used by Miller (1996) involve observers scanning large areas of reef in a relatively short period of time and generally from a distance well above the substratum, while the AIMS LTMP surveys involve a single observer scanning on SCUBA a 1500 m^2 area rapidly (typically in less than 1.5 h), all of which mitigate against accurate observations of BBD presence. Although it is possible that the distribution of BBD has spread to encompass more reefs in recent years, the greater accuracy of belt transects using SCUBA to closely examine each coral is a more likely explanation of the wider distribution of BBD detected in this surveys. These surveys were conducted on sheltered north-west back reef sites in 3 to 6m, whereas surveys of belt transects by the AIMS LTMP SCUBA are located on the exposed north-east flank in 6 to 9m of depth (Sweatman et al. 2001). Given that BBD is commonly restricted to shallow (<6 m) reef environments (Kuta and Richardson 2002), and was more prevalent at sheltered sites than at exposed sites at the northern sector

(Willis et al. 2004) it is not surprising that these surveys revealed a higher distribution of BBD than surveys by the AIMS LTMP in a deeper more exposed habitat.

An additional factor contributing to the higher percentage of reefs with BBD in this study is the timing of surveys. Temperature is thought to be an important factor influencing the prevalence and spread of BBD, with higher prevalence recorded in summer from reefs in both the Caribbean (Rutzler et al. 1983; Edmunds 1991) and the GBR (Willis et al. 2004). Miller's (1996) surveys and the AIMS LTMP surveys (Sweatman et al. 2001) were spread across both winter and summer months, whereas these surveys were timed for the summer months when average seawater temperatures are highest, which is likely to have contributed to the greater number of reefs with active BBD infections. Differences in the distribution of BBD on the GBR found by our study and Miller (1996) highlight the importance of developing standardized survey methods that can be used on a global scale to both allow direct comparisons of coral disease data collected in different geographical regions, and to increase the likelihood of detection of changes in the distribution and prevalence of coral diseases at a variety of spatial scales. At present circular or arc transects (Santavy et al. 2001) and belt transects are used to measure coral disease prevalence in the Caribbean (Porter et al. 2001; Patterson et al. 2002; Weil 2004), while belt transects are used in the Indo-Pacific (Willis et al. 2004). Given the range of depths the circular or arc method may encompass on some steeply sloping reef communities of the Indo-Pacific, I suggest that the belt transect method described in this and other recent studies of coral disease in the Indo-Pacific (Raymundo et al. 2003; Willis et al. 2004; Raymundo et al. 2005) is the more appropriate method for use in coral disease surveys to enable geographical comparisons of coral disease data. However, a quantitative comparison of coral disease survey methods used globally would be timely.

While widespread on the GBR, BBD was absent from 80% of inshore reefs surveyed, which are the reefs that typically experience the highest levels of nutrients, sediment and pollutants from adjacent mainland catchments (Furnas 2003). The reasons for its absence on these reefs are unclear, particularly given correlations found between the occurrence or activity of BBD and both elevated nutrients (Kuta and Richardson 2002) and poor water quality (Antonius 1988; Al-Moghrabi 2001) in other reef regions. Other studies have supported a link between poor water quality and high BBD prevalence (Taylor 1983; Bruckner et al. 1997), although only one found a quantitative correlation between BBD occurrence and water quality parameters (including higher than average water temperatures, nitrite and orthophosphate concentrations; Kuta and Richardson 2002). Levels of terrestrial inputs experienced by reefs of the GBR not only vary across the shelf, but also between the northern and central sectors of the GBR as a consequence of land-use practices, rainfall and other characteristics of adjacent catchments (Furnas 1990; 2003). Here again, the higher prevalence of BBD in the relatively more pristine northern sector compared to the central sector (Fig 2.3) is contrary to the pattern that would be predicted based on previous studies of BBD. Patterns of highest BBD prevalence on mid-shelf reefs in the northern sector provide further evidence that terrestrial inputs from mainland catchments may not be the primary factors determining the prevalence of BBD on the GBR. However, overall levels of nutrients on reefs of the GBR may be below thresholds that may drive BBD outbreaks on Caribbean reefs. Given the increasing evidence of a link between eutrophication of coral reef waters and increases in coral disease prevalence and activity worldwide (Bruno et al. 2003; Harvell et al. 2004), a study of the relationship between BBD and a wide range of nutrients, would shed further light on potential links between BBD and terrestrial inputs on the GBR.

Differences in coral community structure between inner-, mid- and outer-shelf reefs were also tested as a potential explanation for the lack of BBD on inner-shelf reefs. Given that the greatest number of cases of BBD were recorded from species in the genus *Acropora* (Appendix 1) and in particular branching species (*Acropora muricata, A. intermedia and A. florida*), it is tempting to invoke the low abundance of acroporid corals that has been found on many inner-shelf reefs in past studies of the GBR (Done 1982; Ninio and Meekan 2002) as an explanation for BBD absence on these reefs. However, in contrast to these previous surveys, I found a high (45 to 95% of taxa) abundance of acroporid corals on inshore reefs on which BBD was absent. Moreover, I found no significant relationship between BBD prevalence and either the density or cover of corals in general or either scleractinian or acroporid corals specifically. One factor that may contribute to the absence of BBD from the majority of inshore reefs in this study is the higher turbidity and hence reduced light that is characteristic of inner-shelf reef environments. Antonius (1985a) found that sediment trapped amongst algae on newly exposed areas of coral skeleton prevented BBD development through suppression of photosynthesis by the cyanobacteria in low light environments. Consequently, turbid inshore environments may protect corals from infection by BBD by reducing the activity of the cyanobacteria. However, it is likely that prevalence of BBD is driven by a complex array of factors involving both host and pathogen characters, environmental parameters and coral community attributes.

Globally, there is concern that marine diseases are increasing in distribution and prevalence (Harvell et al. 1999), and five marine taxa have been identified as particularly at risk, including the scleractinian corals (Ward and Lafferty 2004). While there is evidence of increasing prevalence (Weil 2004) and distribution (Porter et al. 2001) of coral disease in the Caribbean, lack of baseline data on the GBR obviates

detection of trends in disease incidence in this reef region. Two studies suggest that the prevalence of coral disease on the GBR may have increased in recent years (Antonius and Lipscomb 2001; Willis et al. 2004). Antonius and Lipscomb (2001) found that the number of cases of skeletal eroding band (SEB) increased from rare (1-3 cases per 30min swim) to moderate (4 - 12 cases per 30min swim) between 1988 and also showed that the distribution and number of cases of white syndrome (WS) increased (20-fold for WS abundance) between 1998 and 2003. In both studies the relationship between the abundance of disease cases and disease prevalence is unclear without concurrent data on the number of healthy corals. In contrast to the detected increase in disease cases, the prevalence of BBD appears to have declined at sheltered sites at the northern sector over the last decade, from 2.8% of all corals in 1994 (Dinsdale 2000) to 1.7% in 2003 (Willis et al. 2004) and 0.095% in 2004 (this study). At two other reefs, BBD prevalence has remained low and stable: at No Name Reef, an outer-shelf reef in the northern sector, BBD prevalence was 0.051% in 2003 (Willis et al. 2004) compared to 0% in 2004 (this study); and at Heron Island in the southern sector, prevalence was 0.01% in 2003 (Willis et al. 2004) compared to 0.012% in 2004 (this study). These values span a range similar to that recorded for BBD prevalence in the wider Caribbean (0.2 to 6.0%, reviewed in Weil 2004). Having established baseline levels of BBD prevalence for reefs spanning 2/3 of the length of the GBR, it will now be possible to detect temporal trends in BBD prevalence and compare its ecological impacts on GBR coral assemblages with those in other reef regions.

This study expands the host range of corals susceptible to BBD on the GBR to 40 species from the 32 previously recorded (Willis et al. 2004). Inclusion of species in the genera *Echinopora*, *Seriatopora*, *Psammocora* and *Cyphastrea* (Willis et al. 2004; this study) increases the number of Indo-Pacific scleractinian genera known to be

susceptible to BBD from 17 (Sutherland et al. 2004) to 21. This study combined with records from Willis et al. (2004) contribute a further 12 species to the list of 45 coral species known to be susceptible to BBD in the Indo-Pacific compiled by Sutherland et al. (2004). This host range of BBD is likely, however, to be an underestimate given that surveys were restricted to upper back reef slopes. Increases in the number of taxa susceptible to BBD are expected to increase as coral disease surveys are completed in previously unsurveyed reef zones and habitats and unsurveyed areas of the Pacific in particular. While BBD affected a wide variety of scleractinian families in these surveys, the family Acroporidae was by far the most susceptible, accounting for 90% of records. Previous authors reported Pocilloporidae as the family most susceptible to BBD on the GBR, followed by the Acroporidae (Dinsdale 2000, Willis et al. 2004). However, my different results reflect the inclusion of a greater number of reefs from all cross-shelf positions in these survey and a concomitant reduction in the relative proportion of corals sampled in the Pocilloporidae because of their lower abundance on inner-shelf reefs. Willis et al. (2004) proposed that investment in rapid growth and reproduction by both the Acroporidae and Pocilloporidae may result in less well developed resistance to disease. Although acroporid corals are relatively resistant to infection by BBD compared to faviid corals in the Caribbean, they are the most susceptible to infection by other diseases such as White Band (Gladfelter 1982) and white pox (Patterson et al. 2002) in this region. The relationship between life history characteristics and susceptibility to coral disease in general or to specific coral diseases remains unclear but is worthy of more detailed analysis given the potential impact of coral disease on coral diversity worldwide.

BBD has been known to develop on corals previously affected by white plague, white plague-like disease or white syndrome (Antonius 1981a; b; 1985a; b; see Willis et

al. 2004 for discussion of the use of the term 'white syndrome' for Indo-Pacific corals), while SEB has been found to develop on corals previously affected by white plague (Antonius and Lipscomb 2001). However, cases of corals affected by two diseases simultaneously have not previously been reported. Of the BBD cases recorded in this study, 10.3% were from colonies showing signs of other disease states, particularly skeletal eroding band (SEB), bleaching or both SEB and bleaching simultaneously, or of compromised health (Appendix 1). It is possible that corals infected with BBD are more vulnerable to invasion by other pathogens at sites of tissue necrosis along the disease front. Conversely, it is also possible that initial BBD infections may be dependant on a previous injury (Antonius 1981a; b; 1985a; b), infection with another disease (Antonius 1977; 1981a; Winkler et al. 2004) or compromised colony health. It is not possible to distinguish whether the co-occurrence of BBD and bleaching suggests that bleaching plays a role in the initiation or progress of BBD infection or vice versa. *Halofolliculina corallasia* ciliates have not previously been described in association with BBD or any other coral disease, despite the common occurrence of these ciliates amongst BBD infections on the GBR (associated with 6% of BBD infections). The co-occurrence of *H. corallasia* amongst the BBD mat makes it difficult to determine the relative contribution of each pathogen to the recent coral mortality and it is also unclear how the presence of one influences the progression or activity of the other pathogen.

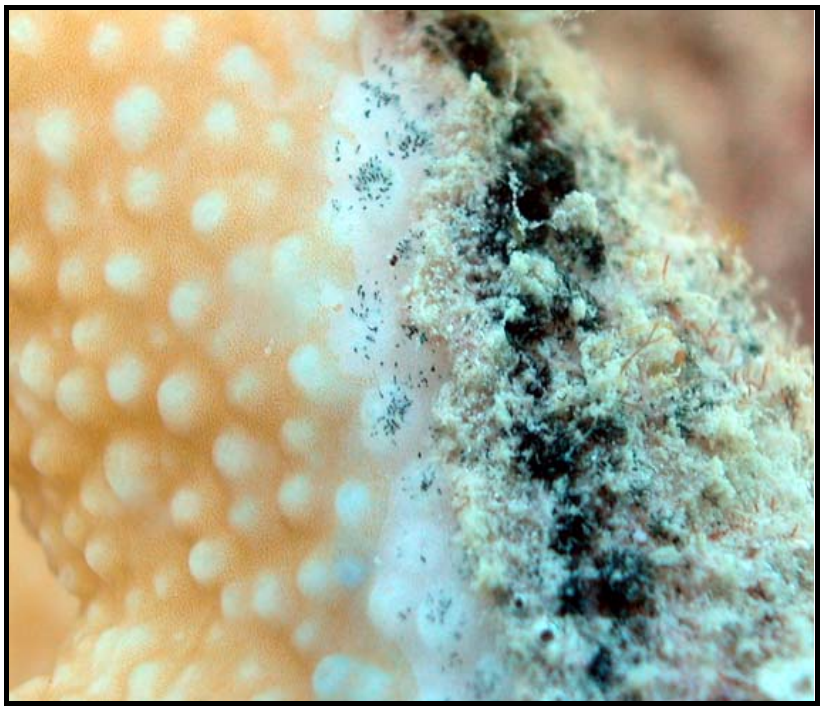
A number of cyanobacterial infections that differed in colour and morphology from the typical black mat of BBD were recorded during this study and also by Willis et al. (2004). In many of these cases, the cyanobacteria formed only a thin line at the interface between live coral tissue and recently denuded skeleton. Given the low abundance of cyanobacteria in these bands, monitoring of tagged infected colonies is required to determine whether they are the primary cause of the recent coral mortality or

if they opportunistically settle at the interface following injury or predation. After black, the most common colouration of cyanobacteria was green, followed by brown or red. In one case, red cyanobacteria formed a distinct red band ~5 cm wide (cf. Richardson 1992). Sussman et al. (2006) discuss cyanobacterial strains identified in a similar red band observed in Palau. Further studies of cyanobacterial infections of corals are required to determine how many strains are involved and whether they can be distinguished using gross morphological characteristics in the field.

In conclusion, BBD is widely distributed on the GBR, being recorded from the northern, central and southern sectors and from outer-, mid- and some inner-shelf reefs. Patterns of highest BBD prevalence in the comparatively pristine northern sector and on mid-shelf reefs in cross-shelf transects, suggest that BBD distribution on the GBR is not linked to gradients in terrestrial influences, at least when overall BBD prevalence is low (0.09 + 0.04%). I speculate that the absence of BBD on 80% of inshore reefs suggests that high turbidity and sedimentation adversely affect cyanobacteria and protect corals from BBD infections. BBD has a wide host range on the GBR, infecting corals in seven scleractinian families and one soft coral family; however, branching species of *Acropora* in the family Acroporidae are the most common hosts. Long-term, large-scale studies of coral disease are required to establish spatial and temporal trends in coral disease prevalence and allow more accurate risk assessment of the potential impact of BBD and other coral diseases on coral communities of the GBR.

Chapter 3

Epidemiology of skeletal eroding band on the Great Barrier Reef and the role of injury in the initiation of this widespread coral disease



Tagged *Acropora* colony with aggregations of *Halofolliculina corallasia* ciliates at the interface between coral tissues and skeleton, characteristic of skeletal eroding band (Lizard Island, northern Great Barrier Reef)

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3.1. ABSTRACT

Skeletal eroding band (SEB), which manifests as dense aggregations of the ciliate *Halofolliculina corallasia*, was the first coral disease described from the Indo-Pacific. Little is known about its etiology or impact. This study describes the distribution, prevalence and host range of SEB on a 500 km extent of the Great Barrier Reef (GBR), together with in situ rates of progression and infection following experimental injury. SEB occurred on 90-100% of reefs surveyed (n = 18) in each of three years, demonstrating that SEB is widely distributed and persistent. SEB had the highest prevalence of any disease, affecting approximately 2% of 283,486 scleractinians and hydrocorals surveyed. Its host range was large, affecting 12 families and at least 82 scleractinian species, as well as the hydrocoral, *Millepora*. Corals in the families Pocilloporidae and Acroporidae were most susceptible, the former being up to five times more susceptible than other families. Progressive tissue loss was recorded on 95% of *Acropora muricata* colonies monitored (n = 18), with rates of SEB progression averaging ~2 mm d^{-1}. Injury experiments demonstrated that *H. corallasia*, the putative pathogen of SEB, readily colonised recently exposed coral skeleton in the absence of a vector, but did not colonise intact coral tissue. Invading ciliates failed to form band-like aggregations associated with progressive tissue loss on any of three coral species tested experimentally, suggesting that, while *H. corallasia* readily colonises recently exposed coral skeleton, it may not be sufficient in itself to cause tissue mortality. Interactions with additional microbial agents, nutritional or environmental factors, that either increase ciliate virulence or lower disease resistance of coral hosts may be required before halofolliculinid infections become associated with tissue loss.

3.2. INTRODUCTION

Recognition of the serious impacts of coral disease on reef assemblages is increasing worldwide, commensurate with the increasing numbers of reports (Ward and Lafferty 2004) and diseases described in the last three decades (Green and Bruckner 2000; Raymundo et al. 2003; Sutherland et al. 2004; Willis et al. 2004; Weil et al. 2006). Until recently, research has focused primarily on coral diseases in the Caribbean, where they have caused significant and widespread mortality of both scleractinian (Williams et al. 1999; Porter et al. 2001; Patterson et al. 2002) and gorgonian corals (Guzmán and Cortés 1984; Garzón-Ferreira and Zea 1992; Nagelkerken et al. 1997; Kim and Harvell 2004). Growing realisation that disease commonly plays a role in the ecology of many Indo-Pacific coral species (Loya et al. 1984; Willis et al. 2004; Winkler et al. 2004; Raymundo et al. 2005; Dalton and Godwin 2006; Page and Willis 2006; Chapter 2) highlights the importance of understanding the etiology and impacts of disease, particularly given recent increases in the number of diseases (Raymundo et al. 2003; Willis et al. 2004)and disease cases in the region (Antonius and Lipscomb 2001; Raymundo et al. 2003; Willis et al. 2004).

Skeletal eroding band (SEB) was the first coral disease described from an Indo-Pacific reef (Antonius 1999), however little is known beyond its macroscopic appearance and aspects of the biology of the putative pathogen. The disease manifests as a black band (~1 - 10cm wide) at the interface between recently exposed skeleton and apparently healthy coral tissue (Fig 3.1a-d; Antonius 1999; Antonius and Lipscomb 2001). The black band may appear speckled, depending on the density of the sessile folliculinid ciliate, *Halofolliculina corallasia*, which comprises the band. The ciliates embed themselves in the coral skeleton producing an eroded appearance. Koch's postulates have not been fulfilled for this disease, however coral mortality is thought to

be caused by spinning and chemical secretions of the asexually produced motile swarming phase of *H. corallasia* (Antonius and Lipscomb 2001). The band of ciliates is so dense at times that SEB may be confused with black band disease (BBD) in the field (Antonius and Lipscomb 2001). Recent apparent increases in the geographical distribution of SEB in the Indo-Pacific may be partly explained by the increasing familiarity of researchers with the distinction between SEB and BBD.

Since its discovery in 1988, SEB has been recorded from reefs throughout the Indo-Pacific, including Motupore Island (Papua New Guinea), Lizard Island (Great Barrier Reef (GBR)), Mauritius, and the Red Sea (Antonius and Lipscomb 2001). Its distribution continues to widen with recent records from the northern and southern GBR (Willis et al. 2004), New Britain (Papua New Guinea; Neale, personal observation), Palau, the Marshall Islands, the Solitary Islands (Page and Willis, personal observation) and the Philippines (Harvell et al. 2007). SEB has not been detected on Indo-Pacific reefs in Indonesia, Guam or Moorea, or on Caribbean reefs in Belize, the Florida Keys or the Dominican Republic, in surveys between 1997 and 2000 (Antonius and Lipscomb 2001) or more recently (Weil 2004). However, another ciliate of the genus *Halofolliculina* was recently found on Caribbean corals (Cróquer et al. 2006a), although it appears to be a different species (Cróquer et al. 2006b). In this study therefore, the term SEB will not include Caribbean ciliate infections.

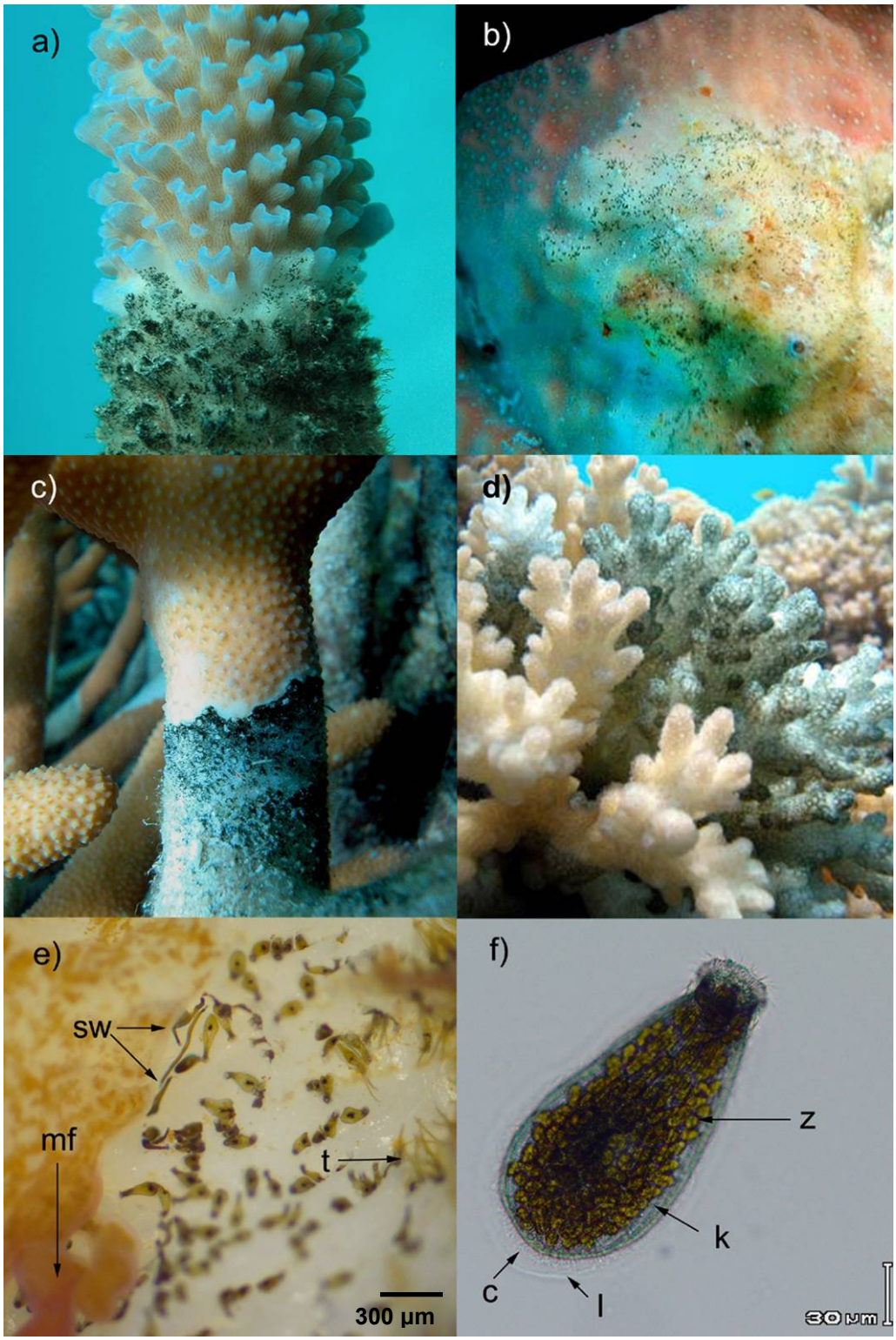
Compared to other coral diseases, the prevalence of SEB is extremely high. It was the most prevalent scleractinian disease on two of three GBR reefs surveyed in 2002 - 2003, affecting 5.4% of all corals (Willis et al. 2004). Moreover, it was found on up to 38% of corals in Red Sea surveys (Winkler et al. 2004). Despite the limited number of reefs in these surveys, SEB has been found on at least 31 scleractinian species on the GBR (Willis et al. 2004) and 22 genera in the Red Sea (Winkler et al. 2004). To date,

only BBD and the white diseases (white plague-like diseases/white syndrome) have greater host ranges (summarised in Sutherland et al. 2004). Rates of partial and whole colony mortality caused by SEB are unknown. Progression rates of the band have been estimated to vary between 1mm $week^{-1}$ and 1mm d^{-1} (Antonius and Lipscomb 2001), but quantitative measurements are lacking.

Observations of "loose settlements" of *H. corallasia* scattered across recently exposed coral skeleton suggest that the ciliate opportunistically settles following tissue loss caused, for example, by predation or physical injury (Winkler et al. 2004). This interpretation is supported by the positive correlation found between the prevalence of SEB and the prevalence of coral damage or injury on reefs in the Red Sea (Winkler et al. 2004). However, currently, it is unclear if loose settlements of ciliates lead to tissue loss and skeletal erosion associated with band-like aggregations of the ciliates. Furthermore, it is unclear if *H. corallasia* is the causative agent of tissue loss or if the ciliate colonises skeleton exposed by another micro-organism.

The goal of this study was to investigate the etiology, impact, and role of injury in the initiation of SEB on corals of the GBR. Specific objectives were to 1) determine the distribution, prevalence and host range of SEB from large-scale surveys of the GBR, 2) determine within colony rates of disease progression, 3) evaluate the role of injury in the initiation of SEB, and 4) determine if an intermediate host is required for transmission of *H. corallasia* to recently injured corals.

Figure 3.1. Macroscopic field signs of skeletal eroding band on: a) *Acropora pulchra*, b) *Turbinaria stellata*, c) *Acropora muricata*, and d) *Pocillopora damicornis*. Microscopic images of: e) motile swarmers (sw) of *Halofolliculina corallasia* ahead of sessile trophonts (t) at the interface between live coral and bare skeleton on *P. damicornis*. [Note that the coral tissue is damaged and a mesenterial filament (mf) is exposed]; and f) motile swarmer undergoing morphogenesis and secreting a lorica (l). Cilia (c) which lie in rows or kineties (k) and zooxanthellae (z) are clearly visible.



3.3. MATERIALS AND METHODS

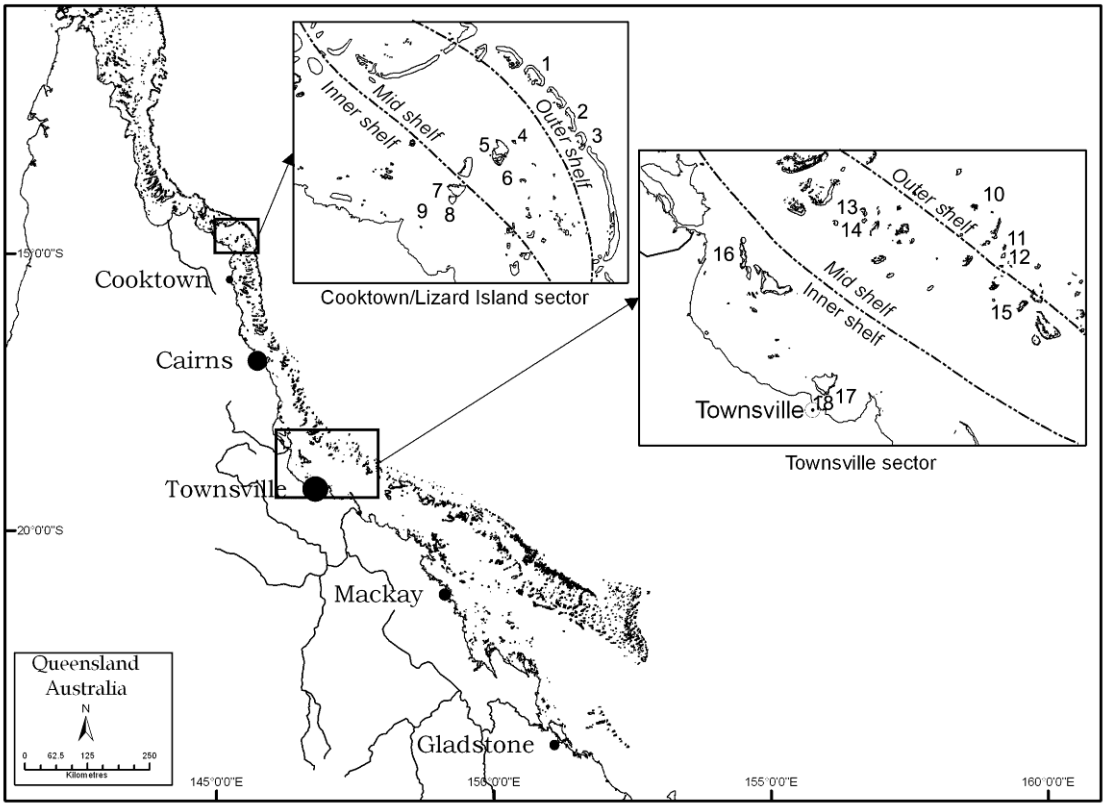
3.3.1. SEB Distribution, Prevalence, Host Range and Rates of Disease Progression

To determine the prevalence and host range of SEB on the GBR, surveys were completed on 18 reefs spanning 500km in each of three years (2004, 2005, 2006). To ensure representation of reef types and habitats, reefs were surveyed within each of three cross-shelf positions (inner-, mid- and outer-shelf) in each of two latitudinal sectors (Cooktown /Lizard Island and Townsville) (Fig 3.2). Reefs were surveyed during austral summers, when prevalence and host range were likely to be highest (Willis et al. 2004). Within each cross-shelf by sector position, two sites on each of three reefs were surveyed. Surveys involved three replicate 20 m x 2 m belt transects placed parallel to depth contours on the upper reef slope at 3-6m depth (as per Willis et al. 2004, Page and Willis 2006; Chapter 2). Reefs were selected haphazardly from those accessible within sectors. Sites were selected haphazardly from within areas characterised as north-western, back reef slopes and transects were placed haphazardly on upper reef slopes. On many reefs, surveys encompassed much of the full extent of the depth range of coral assemblages in this back reef zone. The two surveyed sites encompassed more than approximately 40% of the back reef zone per reef. The restriction of surveys to a single reef zone means that care should be taken in extrapolation of results to other reef zones or when making comparisons with other studies. Within each 20m x 2m belt, all scleractinians, gorgonians, alcyonaceans and hydrocorals were examined, the presence or absence of SEB was recorded, and corals identified to the lowest taxonomic or morphological group, as appropriate. SEB cases were identified *in situ* by diffuse areas of tissue loss separated from live tissues by dark green to black bands 1 to 10 cm in width. Microscopic examination of samples confirmed that bands were comprised of the

sessile stage of the ciliate *Halofolliculina corallasia.* Differences in susceptibility to SEB among taxa were tested using a chi-squared test of independence. Other diseases on corals showing signs of SEB were also recorded.

Rates of partial mortality attributable to SEB were examined on colonies of *Acropora muricata* at Lizard Island (S 14' 21 15.55, E 145^{o} 26' 36.85), a mid-shelf reef located in the Cooktown/Lizard Island sector of the GBR. Eighteen branches, each from a separate colony, were tagged using numbered plastic cattle tags secured with cable ties to exposed skeleton. Tags were attached a short distance behind the disease front to avoid interfering with disease progression. The distance from cable ties to the nearest live tissue was measured in late November 2004 and again four weeks later in late December. The difference between the two measurements was used as a measure of linear disease progression and divided by the number of days between measurements to calculate a daily rate of SEB progression.

Figure 3.2. Reefs surveyed annually between 2004 and 2006 for skeletal eroding band (SEB) in two latitudinal sectors of the Great Barrier Reef. Reefs are numbered as follows: (1) Day Reef, (2) Yonge Reef, (3) No Name Reef, (4) Macgillivray Reef, (5) Lizard Island, (6) North Direction Island, (7) Martin Reef, (8) Linnet Reef, (9) Maxwell Reef, (10) Dip Reef, (11) Knife Reef, (12) Fork Reef, (13) Kelso Reef, (14) Little Kelso Reef, (15) Davies Reef, (16) Orpheus Island, (17) Magnetic Island, (18) Middle Reef



3.3.2. *In Situ* Injury Experiments

A pilot study to test the most appropriate position for artificial injuries on coral branches and the appropriate timespan for following ciliate colonisation was completed on shallow patch reefs (2 -5m) at Lizard Island in November 2004. To determine whether intact coral tissue completely surrounding an injury is an impediment to ciliate colonisation and hence the initiation of SEB, artificial injuries were created in two positions: one completely surrounded by intact coral tissue (mid-branch injury), and one immediately adjacent to exposed skeleton at the base of branches (basal injury). Ciliate densities were assessed 7, 14 and 44 days after injuries were inflicted.

Artificial injuries were created on branches *in situ* using an airgun attached to a SCUBA tank. High-pressure air removed coral tissue, but the underlying skeleton was not visibly damaged, mimicking the removal of coral tissue by corallivorous starfish (*Acanthaster planci)* or gastropods (*Drupella* spp.). The size of the injury was restricted using a square plastic shield with a 2 cm x 2 cm window held in front of the coral branch. Injuries in the two branch positions (basally and mid-branch) were inflicted on 20 physiologically separate, healthy branches of three scleractinian species commonly affected by SEB: *A. muricata, Pocillopora damicornis* and *Porites cylindrica*. Artificially injured branches and an equal number of healthy, uninjured control branches (20 per species) were tagged using plastic cable ties to assist in their relocation. Five injured and five uninjured branches of each species were then collected 7, 14 and 44 days after injury using bone cutters. Each branch was placed in a separate sealed plastic bag and transported immersed in seawater. All injured and non-injured branches were examined microscopically and the number of ciliates per injury (injured branches) or per 2 cm x 2 cm area (uninjured branches) was recorded.

Differences in ciliate densities among coral species and between injury positions over time (7 versus 14 day samples) were examined using a three-factor, fully orthogonal ANOVA, with coral species, injury position and time treated as fixed factors. In addition, a two-factor ANOVA was used to examine differences in ciliate densities on mid-branch injuries among species over the three sampling periods (7, 14 and 44 days after injury). Basal injuries were excluded from the second analysis because they were generally obscured by heavy fouling 14 days after injury. For this reason, only mid-branch injuries were used in subsequent injury experiments.

A full-scale experiment was conducted at Lizard Island over the austral summer of 2004/2005 to determine rates of colonisation of artificial injuries by ciliates, changes in ciliate densities through time, levels of coral mortality associated with their presence, and if the band-like manifestation of SEB developed following ciliate colonisation of injuries. Artificial injuries were inflicted mid-branch, as described above, on 90 healthy branches of each of *A. muricata, P. damicornis* and *P. cylindrica*. 90 healthy, uninjured branches of each species were also tagged (experimental controls). Injured and uninjured branches were examined *in situ* on days 1, 5, 10, 15, 20 and 133 and all injuries remeasured (maximum and perpendicular diameters). At each sampling, 15 randomly selected injured and uninjured branches of each species were collected and examined microscopically, as described above. Numbers of ciliates on the entire injury were counted when densities were low or in six replicate 5mm x 5mm areas subsampled haphazardly within each injury site when densities were high. Ciliate densities were then standardised to the number per cm^2.

The effects of coral species and time on the proportion of injuries with ciliates present were tested using the linear model: Proportion with ciliates = Species + Time + Species x Time. The response variable was the binary classification of injuries with

ciliates either present or absent. Data were analysed using logistic regression with categorical variables, where the logit (the log of the ratio of probability of ciliates present versus ciliates absent) was the link function (McCullagh and Nelder 1989). A two- way fixed factor ANCOVA controlling for injury size was used to test for changes in ciliate densities through time and between species. Single degrees of freedom ANCOVA and Tukey's HSD tests were subsequently used to identify when changes in ciliate densities occurred in each species. Changes in injury size on the 15 branches of each species collected 133 days after injury were examined using a multivariate repeated measures ANOVA, followed by single degrees of freedom ANOVA and Tukey's HSD tests to determine the timing of significant changes in injury size.

3.3.3. Aquarium Injury Experiment

To evaluate the possibility that other reef organisms, particularly fish and corallivores such as *Drupella* spp., act as vectors and transmit ciliates to injuries, an exclusion experiment was carried out in aquaria. Thirty healthy branches of *A. muricata, P. damicornis* and *P. cylindrica* were collected from the field experimental site and transported immersed in seawater to the laboratory. Branches were cleaned of all visible microfauna and flora. Each broken branch edge was covered with non-toxic modelling clay and placed in a 3cm long section of PVC piping, which held branches upright in racks. Ten branches of each species were randomly assigned to one of three aquaria and mid-branch injuries (2 cm x 2 cm) were created on five randomly chosen branches of each species in each aquarium. Thus, each aquarium contained five injured and five uninjured branches of each coral species. Aquaria were supplied with a high flow of unfiltered seawater pumped from the local reef flat and fragments were examined microscopically for the presence of ciliates five days after injury.

3.4. RESULTS

3.4.1. Distribution, Prevalence, Host Range and Rate of Disease Progression

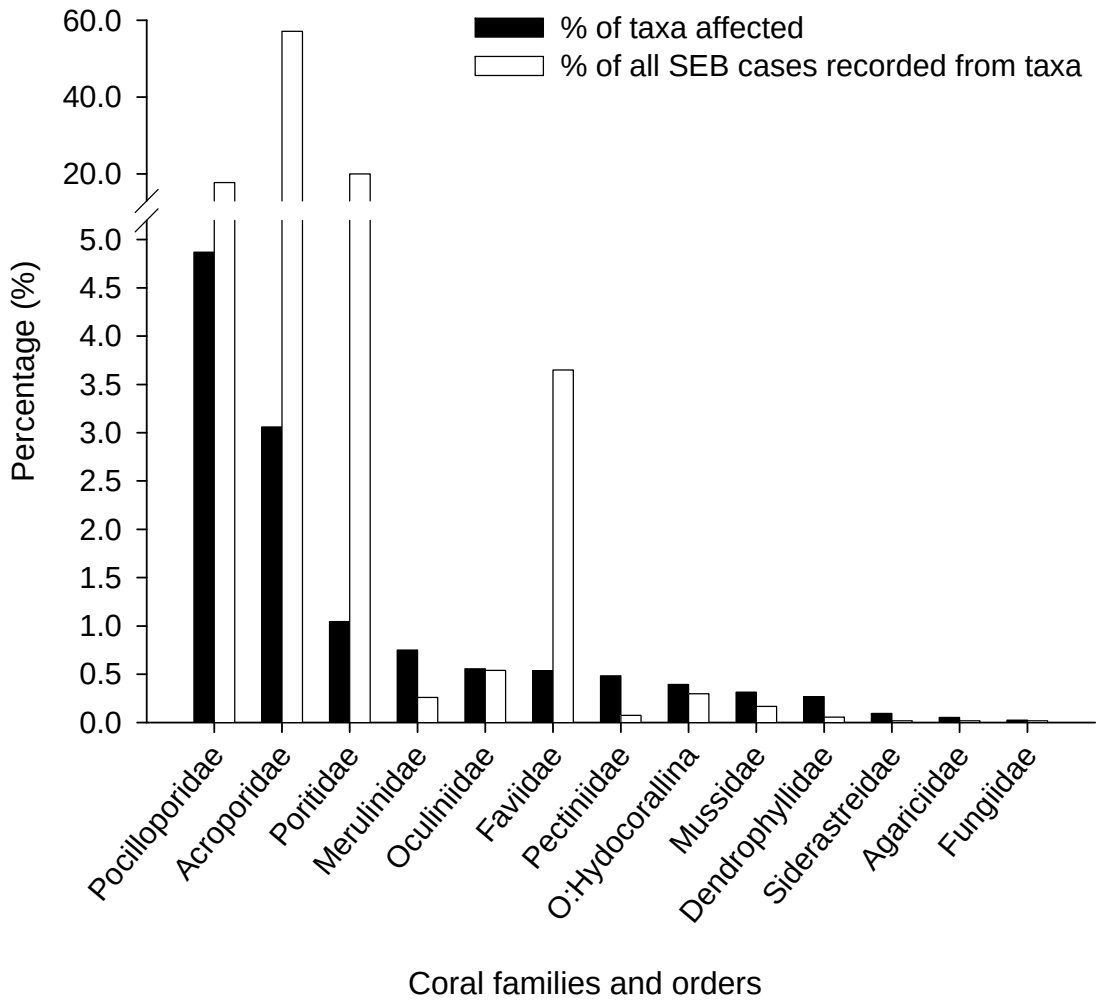
SEB was present on all reefs surveyed (n = 18) in 2005 and on all but one reef (Middle Reef in the Townsville sector) in 2004 and 2006. SEB accounted for the majority (40 - 60%) of disease cases recorded in each year (data for other diseases not shown), making SEB the most prevalent coral disease in regions surveyed. SEB predominantly affected scleractinian corals, but also at least one species of *Millepora* (O: Hydrocorallina) (Appendix 1); it was not observed to affect alcyonaceans, gorgonians or antipatharians. SEB affected between 1.2 % (1,101 of 91,552 colonies in 2006) and 2.3 % (2,177 of 96,148 colonies in 2005) of combined scleractinian and hydrocoral taxa surveyed each year. At the transect level, SEB prevalence ranged from 0% to 11.9% of combined scleractinian and hydrocoral taxa.

SEB affected a wide range of coral hosts, including 12 families, 26 genera and 81 species of scleractinians, plus at least one species of *Millepora* (O: Hydrocorallina) (Appendix 1). Susceptibility to SEB varied significantly among scleractinian and hydrocoral families (χ^2 = 2780.93, df = 12, $p < 0.001$). Pocilloporid corals were ~1.5 times more susceptible than acroporids, ~five times more susceptible than poritids and greater than five times more susceptible than corals in all other families (Appendix 1; Fig 3.3). Although pocilloporids were the most vulnerable to SEB, 57% of cases were recorded from acroporid corals, reflecting the greater abundance of this family in surveys. In comparison, 18% of cases were recorded from pocilloporid corals, 20% from poritid corals, 3.6% from faviids and less than 1% of cases from the remaining susceptible families. Colonies with signs of SEB also showed signs of other diseases,

including tumors and cyanobacterial infections, however the prevalence of colonies with multiple disease signs was very low (Appendix 1).

In situ monitoring over four weeks revealed that SEB disease fronts progressed on all but one tagged branch of *A. muricata* (n = 18 branches). Rates of linear progression ranged from 0.3 to 3.3 mm d^{-1}, with an average (± SE) progression rate of 2 ± 0.3mm d^{-1} (n = 17 branches) in December 2004.

Figure 3.3. Prevalence of skeletal eroding band (SEB) within each family and order (black bars) and the percentage of total SEB cases recorded from each family and order (white bars). Families and Order shown in rank order of SEB prevalence.



3.4.2. *In situ* Injury Experiments

Halofolliculina corallasia colonised injuries created on all three experimental coral species in the pilot field study (Table 3.1), whereas no ciliates were observed on control branches or on healthy parts of injured branches, suggesting that injury enhances the ability of ciliates to colonise corals. The suitability of exposed skeleton for ciliate colonisation differed significantly among coral species, injury position and time after injury, with *A. muricata* and *P. cylindrica* being preferred hosts. 100% of injuries on *A. muricata* and *P. cylindrica* were colonised within 7 days, whereas only 70% of injuries on *P. damicornis* were colonised by this time. Ciliates were recorded on both basal and mid-branch injuries, the latter indicating that ciliates either traverse live coral tissue to reach injured areas or settle directly onto exposed skeleton from the water column. Patterns in ciliate densities for the two injury positions differed among the three species ($ANOVA_{(Injury\ Position\ x\ Species)}$: $p < 0.001$; Table 3.2). On *A. muricata,* basal injuries were colonised by higher densities of ciliates than mid-branch injuries (Tukey's HSD: $p < 0.001$), while on *P. damicornis* and *P. cylindrica,* ciliate densities did not differ significantly between injuries at the two branch positions. Patterns in ciliate densities differed among the three coral species through time, both when densities were compared between the first two sampling times (7 and 14 days after injury) for the two injury positions ($ANOVA_{(Time\ x\ Species)}$: $p < 0.001$; Table 3.2) and when densities on mid-branch injuries alone were compared among all three sampling times (7, 14 and 44 days after injury) ($ANOVA_{(Time\ x\ Species)}$: $F = 6.213$, $df = 2$, $p < 0.001$). Given the problem of defining the area of basal injuries by day 44 because of heavy fouling, only mid-branch injuries were used in subsequent experiments.

Table 3.1. Colonisation of mid and basal branch injuries created on three coral species (*Acropora muricata, Porites cylindrica* and *Pocillopora damicornis*) by the ciliate *Halofolliculina corallasia.* Mean ciliate densities per wound and % of injuries with ciliates are compared through time (7 to 44 days after injury) for each coral. Number of injuries with ciliates present shown in parentheses.

Species	Parameter (ciliate density per cm^2, or % of injuries with ciliates)	Days after injury				
		7		14		44
		Mid-branch	Basal	Mid-branch	Basal	Mid-branch
A. muricata	Mean # ciliates	110.02 ± 95.8	425.07 ± 120.8	386.4 ± 55	661.33 ± 238.1	20.55 ± 13
	% of injuries with ciliates	100 (5)	100 (5)	100 (5)	100 (5)	3
P. cylindrica	Mean # ciliates	19.5 ± 6.6	14.1 ± 34.1	192.9 ± 47.5	340 ± 81.4	30.45 ± 1.6
	% of injuries with ciliates	100 (5)	100 (5)	100 (5)	100 (5)	5
P. damicornis	Mean # ciliates	6 ± 1.1	0.95 ± 5.8	2.25 ± 0.1	5.5 ± 5.5	0
	% of injuries with ciliates	100 (5)	40 (2)	80 (4)	20 (1)	0

Table 3.2. Three factor ANOVA comparing densities of the ciliate *Halofolliculina corallasia* on mid and basal branch injuries created on three coral species (*Acropora muricata, Porites cylindrica* and *Pocillopora damicornis*), 7 and 14 days after injury. Data are log + 0.0001 transformed. * denotes significance at $\alpha = 0.05$.

Source of variation	df	F	p
Time	1	4.356	0.042*
Injury Position	1	1.277	0.264
Species	2	80.012	<0.001*
Time x Injury Position	1	0.048	0.828
Time x Species	2	10.015	<0.001*
Injury Position x Species	2	10.031	<0.001*
Time x Injury Position x Species	2	1.370	0.264

The full-scale, field injury experiment confirmed that recently exposed skeleton is a necessary precursor for colonisation of otherwise healthy corals by *H. corallasia.* More than 90% of artificially-created lesions (n = 45) contained ciliates within 5 days (Fig 3.4), whereas no ciliates were observed on control branches or on healthy areas of injured branches (not shown). Rates of injury colonisation again varied among the three coral species and through time (Fig 3.4; Table 3.3). For *P. cylindrica,* 100% of injuries were colonised within 24 hours and ciliates were recorded on all wounds except in the final 4.5 month sample, at which time no ciliates were detected. For *A. muricata,* colonisation was slower, taking 10 days for 100% of injuries to be colonised, although again ciliates were present on all injuries prior to the final, 4.5 - month sample. In contrast, although 86% of *P. damicornis* injuries were colonised by day 5, this proportion declined rapidly to ~ 13% within three weeks of injury, suggesting that exposed skeleton of *P. damicornis* represents a less suitable substrate for ciliate colonisation and population growth.

Figure 3.4. Comparison of the percentage of artificially-created injuries having the ciliate, *Halofolliculina corallasia,* present among three coral species (*Acropora muricata, Porites cylindrica* and *Pocillopora damicornis*), 1 to 133 days after injury.

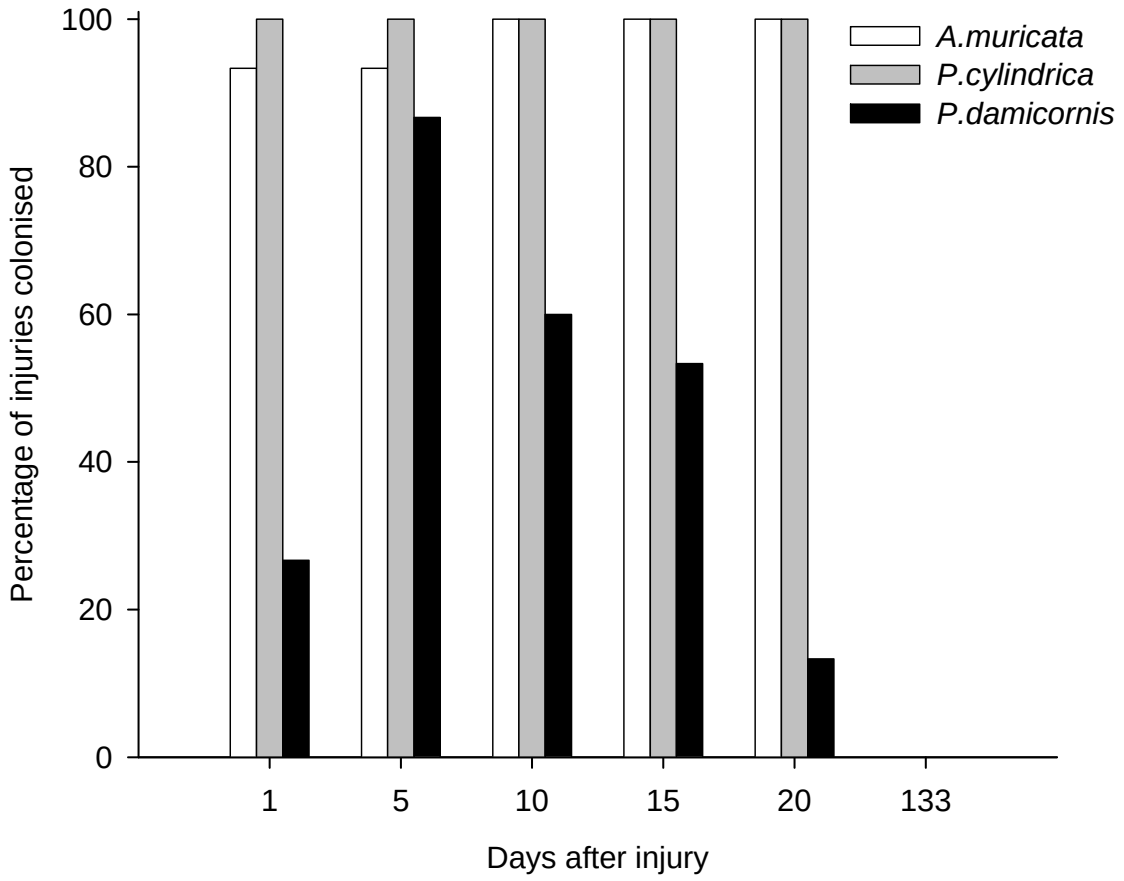


Table 3.3. Logistic regression comparing the proportion of mid branch injuries colonized by the ciliate *Halofolliculina corallasia*, 1 to 133 days after injury. *: denotes significance at $\alpha = 0.05$.

	Model 1			Model 2 (saturated model)		
Factor	df	$\chi 2$	p	df	$\chi 2$	p
Time	1	47.9655	< 0.0001	2	12.858	<0.01*
Species	2	47.8443	< 0.0001	1	0.3940	0.53
Time x Species				2	7.4325	0.02*
Δ in Schwarz's Baysian criterion between model 1 and 2 = 109.32 Positive change in Schwarz's Baysian criterion indicates improvement of model by interaction term						

Patterns in the magnitude and timing of changes in mean ciliate densities varied among the three coral species ($ANCOVA_{(Time\ x\ Species)}$: $F = 22.694$, $df = 10$, $p < 0.001$). Similarly, overall mean ciliate densities differed among species ($ANCOVA_{(Species)}$: $F = 177.5$, $df = 2$, $p < 0.0001$), further indicating that skeletons of the three species differ in their suitability for ciliate colonisation and replication (Fig 3.5). Average ciliate densities were greatest on *P. cylindrica* injuries, reaching on average 834 ± 59 ciliates cm^{-2} 20 days after injury, and were lowest on *P. damicornis* lesions, reaching on average only 41.9 ± 15.7 ciliates cm^{-2} by day 15. Although a more suitable host than *P. damicornis, A. muricata* was significantly less suitable than *P. cylindrica,* as indicated by the at least ten-fold fewer ciliates colonising *A. muricata* injuries (63 ± 10 ciliates cm^{-2}). *P. cylindrica* clearly provided the most suitable substratum for ciliates, with mean ciliate densities at least doubling every 5 days until peaking at 20 days after injury (Fig 3.5). However, ciliate densities declined to zero by 4.5 months after injury on all three coral species. Ciliate declines on *P. damicornis* were commensurate with the healing of wounds in this species (Fig 3.6i). The absence of ciliates on *A. muricata* and *P. cylindrica* injuries by the end of the study could be partly attributed to reductions of exposed skeleton through injury repair, however heavy fouling of the remaining exposed skeleton, predominantly by turfing and coralline algae (Fig 3.6g, h), was primarily responsible for reduced densities of ciliates. Thus, although *H. corallasia* is a good early coloniser, it is a poor competitor relative to the general fouling community.

The presence of ciliates on coral skeleton exposed via injury did not lead to additional tissue loss on any of the injured branches. In fact, overall, the size of injuries declined through time ($ANOVA_{(Time)}$: Pillai's trace = 0.918, $F = 69.41$, $df = 6$, $p < 0.001$; Fig 3.7) indicating repair rather than degeneration of wounds in the presence of *H. corallasia* ciliates. The extent to which injuries healed varied among the three species, as

indicated by variation in the timing and magnitude of declines in injury size among species ($ANOVA_{(Time \times Species)}$: Pillai's trace = 1.305, F = 11.9, df = 12, $p < 0.001$; Fig 3.7). All injuries on *P. damicornis* healed completely by 4.5 months, although tissue in the repaired area was visibly paler than surrounding tissues. In contrast, none of the lesions completely healed in the other two species. For *A. muricata,* there was no significant decrease in mean injury size over the 4.5 months. Regeneration of injuries in *P. cylindrica* was relatively slow compared with regeneration in *P. damicornis,* with injury size declining by 40% between day 20 (4 ± 0.04 cm^2) and 4.5 months after injury (2.3 ± 0.26 cm^2).

3.4.3. Aquarium Injury Experiment

Exclusion of macroscopic organisms in the aquarium injury experiment confirmed that macroscopic vectors are not required for colonisation of newly exposed coral skeleton by *H. corallasia*. 85% of injured branches were colonised by ciliates in the absence of other organisms. Ciliates were again absent from areas of healthy tissue on both the injured and control branches.

Figure 3.5. Mean densities of the ciliate, *Halofolliculina corallasia*, on injuries artificially created on three scleractinian species (*Acropora muricata*, *Porites cylindrica* and *Pocillopora damicornis*), 1 to 133 days after injury.

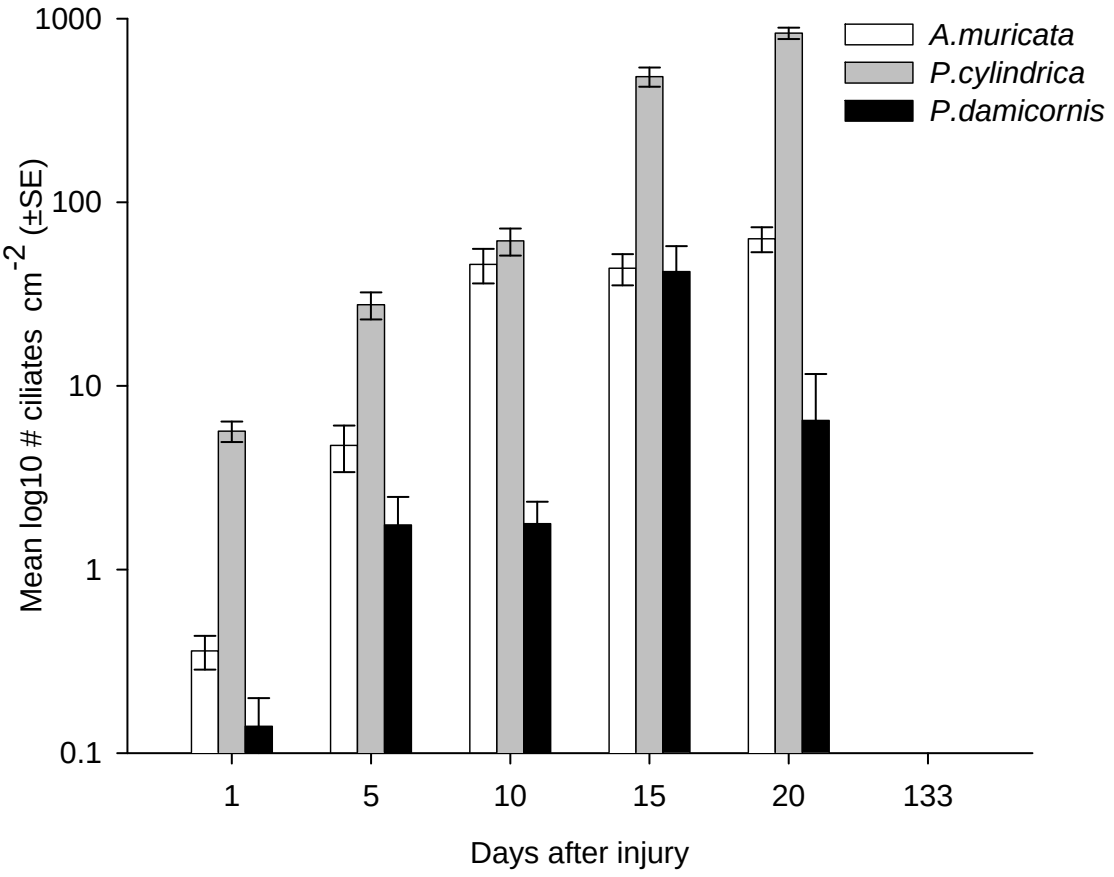


Figure 3.6. Representative images showing temporal sequences in the colonisation of injuries artificially created on branches of (a,d,g) *Porites cylindrica,* (b,e,h) *Acropora muricata*, and (c,f,i) *Pocillopora damicornis*: a-c) bare white skeleton at time 0 following injury; d-e) light fouling with some healing on all species at 10- 20 days after injury [note high ciliate densities on *P. cylindrica* in d)]; g, h) healing along the edges of injuries, decreased injury size and heavy fouling of skeleton in *P. cylindrica* and *A. muricata* 133 days after injury; and i) complete healing of injury in *P. damicornis* 133 days after injury.

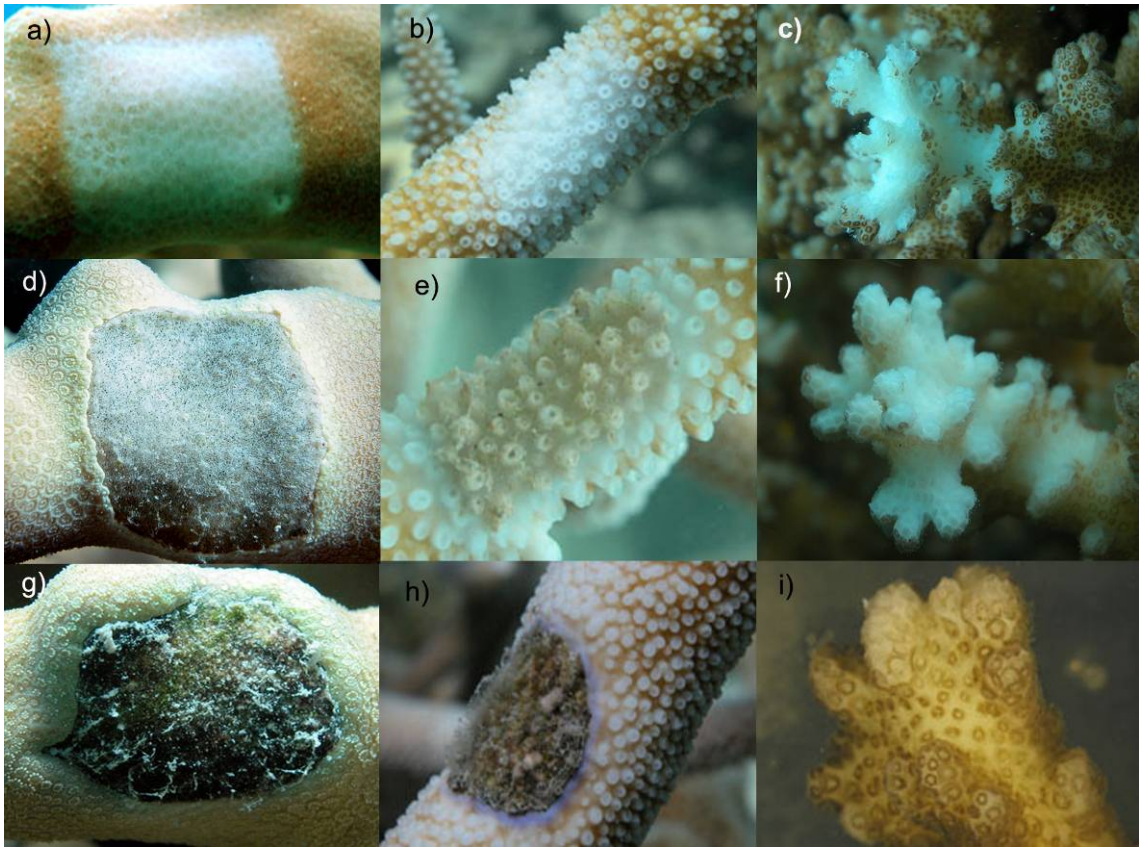
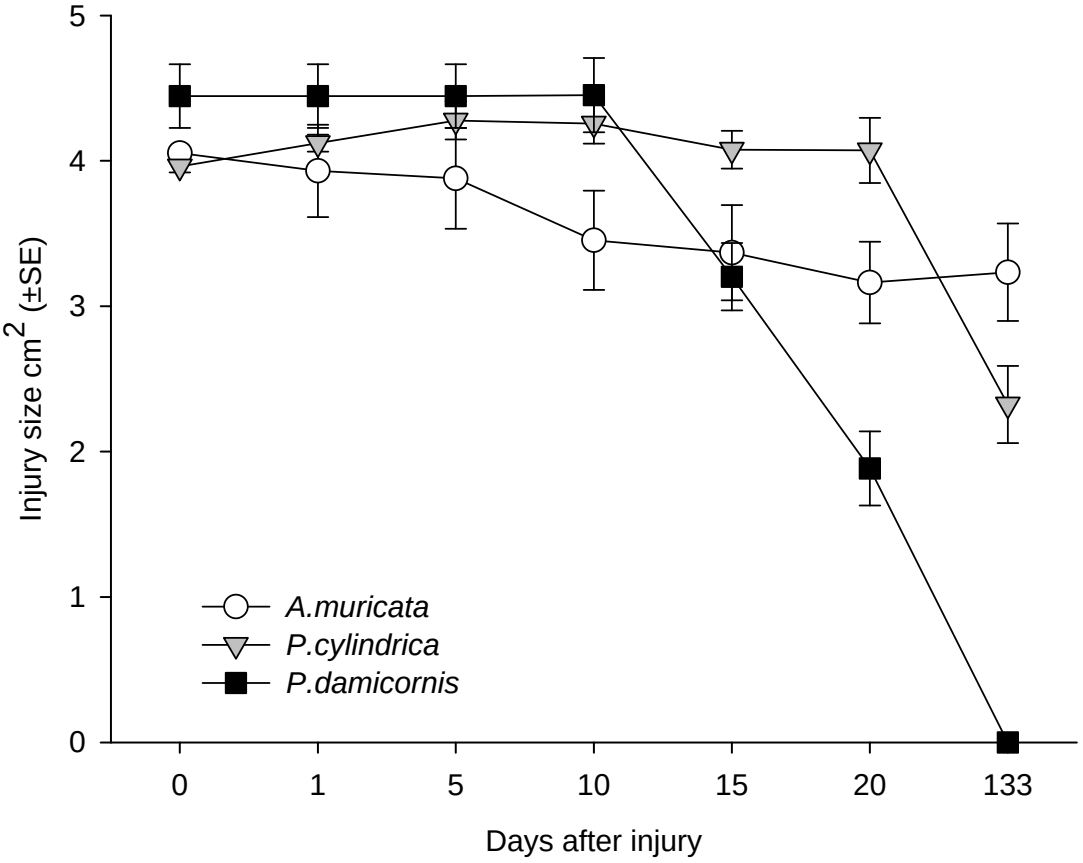


Figure 3.7. Mean size of injuries, artificially created on three scleractinian species (*Acropora muricata, Porites cylindrica* and *Pocillopora damicornis*), 1 to 133 days after injury.



3.5. DISCUSSION

3.5.1. SEB Distribution, Prevalence, Host Range and Rate of Disease Progression

Tissue loss associated with the progression of band-like aggregations of *H.corallasia* along branches of the staghorn coral, *A. muricata,* is recorded here and confirms that SEB infections are associated with progressive tissue loss of corals on the Great Barrier Reef (GBR). Mean and maximum rates of disease progression, of 2 ± 0.3 mm d^{-1} and 3.3 mm d^{-1} respectively, are low compared with rates of tissue loss caused by other coral diseases on the GBR. For example, mean rates of SEB progression are less than half those reported for BBD at Lizard Island (4.1 - 9.9 mm d^{-1}, Boyett et al. 2007) and are likely to be slower than those reported for white syndrome in the southern GBR (up to 57.8 mm^2 d^{-1}, Roff et al. 2006), although faster than those recorded much further south (>1,000 km south of the GBR) in the Solitary Islands (maximum of 0.52 mm d^{-1} , Dalton and Smith 2006). Although rates of disease progression are generally low for SEB, they are, nevertheless, within the range of progression rates recorded for several diseases that have caused significant mortality on Caribbean reefs (cf. 3 to 10 mm d^{-1} for BBD; 3 - 30 mm d^{-1} for white plague II; 3.1 mm d^{-1} for white plague I (summarised in Weil 2004; and 6 mm $month^{-1}$ for yellow band disease in the Caribbean, Cervino et al. 2001). Although rates of tissue loss caused by SEB may be at the lower end of the range for coral diseases, its widespread distribution suggests that it could nevertheless have a significant impact on coral assemblages.

The ubiquity of SEB on all reefs surveyed along more than 1,200km of the GBR (combined data from this study and Willis et al. 2004) and on all reefs surveyed in the Red Sea (Winkler et al. 2004) suggests that SEB is endemic within these regions. The absence of SEB from one reef (Middle Reef, central GBR) in two survey years (2004

and 2006) is more likely to reflect its low abundance on this reef, rather than its complete absence in some years. The potential to miss a disease when its prevalence is low highlights the need for temporally and spatially replicated surveys to gain accurate distributional ranges of coral diseases. The widespread distribution of SEB, not only along the GBR, but also throughout the Indo-Pacific (Motupore: Antonius 1999 and New Britain, PNG: Neale, personal communication; Mauritius, Red Sea: Antonius and Lipscomb 2001; Philippines: Harvell et al. 2007; Palau, Marshall Is., Solitary Is.: personal observation), in combination with the recent discovery of a similar disease in the Caribbean (Cróquer et al. 2006b) suggest that halofolliculinid infections may be endemic in coral populations worldwide. As researchers become more familiar with field signs of SEB, its distribution range is likely to increase, particularly in the Indo-Pacific where coral disease has been understudied. SEB may be the only coral disease detectable in the fossil record due to the characteristic erosion of coral skeleton during lorica section by motile swarmers of *H. corallasia* (Riegl and Antonius 2003). Hence, the fossil record may provide clues on the emergence and spread of SEB on geological timescales.

The status of SEB as one of the most common coral diseases on the GBR has remained unchanged over the past 5 years (Willis et al. 2004; this study). In 2002-03, SEB accounted for 33% of disease cases recorded (n = 1,800) and was the most prevalent disease on two of the three reefs surveyed (Willis et al. 2004). Annual surveys of 18 reefs between 2004 and 2006 indicate that SEB is still the most abundant coral disease on the GBR, accounting for 40-66% of all disease cases. Although the overall prevalence of SEB is low, affecting only 2% of GBR corals surveyed, studies in the Red Sea demonstrate that it is capable of infecting up to 51% of corals on a reef, and up to 70% of pocilloporid corals in the genus *Seriatopora* (Winkler et al. 2004). Surveys in this study were restricted to upper reef slopes in back reef zones, therefore surveys of

additional reef zones and depth ranges are required to determine the maximum prevalence of SEB in these other reef areas. In the present study, pocilloporid corals were also the most susceptible, with more than 4.5% of pocilloporids showing signs of SEB. Although acroporid corals were only three-quarters as likely to be infected on the GBR (~3.0% of acroporids showed signs of SEB), their greater abundance within back reef zones meant that they accounted for the majority (57%) of SEB cases. The greater susceptibility of corals in the family Pocilloporidae (1.5 times more susceptible than acroporids, five times more than poritids, and greater than five times more susceptible than corals in other families) meant that when combined with the Acroporidae, these two families accounted for ~75% of SEB cases. Interestingly, acroporid corals are the most common hosts for a number of other diseases, including black band (Page and Willis 2006; Chapter 2) and brown band on the GBR (Willis et al. 2004) and white band in the Caribbean (Precht et al. 2002), highlighting the vulnerability of this family to disease.

Disease records from this study significantly expand the known host range for SEB. The 82 species with signs of SEB recorded is over 2.5 times greater than host ranges reported in previous smaller studies (Antonius and Lipscomb 2001; Willis et al. 2004). This increase in host range is undoubtedly a function of repeatedly surveying a wider range of reef types and coral communities than in previous studies, highlighting the important contributions that large-scale spatial and temporal studies make to epidemiological studies of coral disease. Continuation of large-scale surveys is required, however to confirm that increased susceptibility of corals to SEB has not contributed to the documented increase in host range. SEB currently has a greater documented host range than any other coral disease worldwide (cf. Sutherland et al. 2004). As predicted above for its distributional range, its host range is likely to continue to increase as

researchers worldwide become more familiar with its appearance in the field and as more reefs are surveyed.

3.5.2. The Role of Injury in SEB Development

Results of this study demonstrate that injury enhances the ability of the ciliate, *H. corallasia,* to colonise corals. High proportions (up to 100%) of artificially created injuries were colonised within 10 days of injury in two of the three experimental coral species, highlighting both the rapid colonisation of injuries by ciliates in the field and the ubiquitous presence of ciliates in the early fouling community of bare coral skeleton. The rapid colonisation of injuries by *H. corallasia* (in as little as 6 hours, personal observation) is consistent with the 1-8 hour morphogenesis times (time to metamorphose from motile swarmers to sessile trophonts) known for other folliculinid ciliates (Andrews 1914; Andrews 1923). Based on observations that *H. corallasia* trophonts contain zooxanthellae engulfed using ciliary currents (Fig 3.1f), it is possible that motile swarmers are attracted to sites of recent injury by the release of zooxanthellae from damaged coral tissues.

Despite initial increases in ciliate densities on artificially-inflicted wounds, ciliates remained in loose, scattered distributions and did not form band-like aggregations associated with tissue lysis on any of the experimental corals. On the contrary, tissues adjacent to injuries regenerated, decreasing the size of lesions through time, suggesting that *H. corallasia* may not be a primary agent of tissue necrosis. If this is the case, then tissue loss recorded on 95% of *in situ A. muricata* colonies with band-like ciliate aggregations on a nearby reef suggests that additional microbial or environmental factors, absent from experimental wounds, may be required before *H. corallasia* aggregations become associated with progressing disease bands. Typically,

wildlife diseases involve a web of causation including multiple micro-organisms, nutrition and environmental stressors and very few causative agents are both necessary and sufficient to produce disease over a wide range of conditions (Wobeser 2006). Thus, coral health may need to be compromised in some way before progressing band-like aggregations develop. For example, reduced energetic reserves in bleached or otherwise compromised corals (Grottoli et al. 2004) may limit investment in boundary maintenance or impair wound healing (Meesters and Bak 1993; Fine et al. 2002), increasing the susceptibility of coral hosts to ciliate infections. Shifts in microbial community composition in the mucus of compromised corals (e.g., in bleached corals, Ritchie 2006) may also allow microbes to invade normally resistant coral tissues. Although vectors were unnecessary for colonisation of injuries by *H. corallasia*, corallivores, in particular, may play a role in SEB development through means other than injury creation, for example through the introduction of new microbes during feeding. Experiments on corals with compromised health and involving injuries created by corallivores may help to elucidate factors leading to the development of band-like, potentially pathogenic ciliate aggregations. Microbial studies are also required to evaluate the possibility that a primary causative agent initiates tissue necrosis before *H. corallasia* invades secondarily. Additional studies of tissue mortality associated with *H. corallasia* bands on a diversity of coral taxa are also required to confirm that this ciliate is responsible for tissue loss in all coral taxa observed with signs of this disease.

Clear differences in susceptibility to SEB among the three experimental coral species are likely to relate to both ciliate substrate preferences and host resistance mechanisms. Ciliate substrate preferences may reflect differences in the rugosity or density of exposed coral skeleton or differences in colony morphology. The porosity and fine-scale complexity of *P. cylindrica*'s skeletal micro-architecture may provide

appropriate interstices within which the ciliates can embed, contributing to the high densities of ciliates found on this species. The more open corallite structure of *P. damicornis* and particularly its high skeletal density (Marshall 2000) may discourage or inhibit ciliate colonisation, contributing to the low densities found on injuries of this species. In addition, greater numbers of *H. corallasia* swarmers may be ingested by coral polyps in the closely intertwining branches of *P. damicornis,* which provide only small gaps through which ciliates can approach injured areas. Conversely, the more open branching patterns of *A. muricata* and *P. cylindrica* may allow *H. corallasia* swarmers to more readily evade polyps and thereby gain easier access to wounds.

Interestingly, differences in susceptibility to SEB among the three coral species did not reflect their capacity for injury repair. In particular, injury regeneration in the presence of ciliates was slowest in *P. cylindrica,* which is a member of the least susceptible family (of the three experimentally injured families) as determined empirically in surveys. Moreover, although ciliate densities were lower on the faster regenerating *P. damicornis,* in general, changes in ciliate densities were not matched by changes in rates of injury regeneration. For example, ciliate densities on *P. damicornis* injuries increased 40 fold between days 10 and 15, over which time injury size decreased by ~ 70 %. Also, ciliate densities on *P. cylindrica* injuries doubled between days 15 and 20, whereas injuries did not change in size. Differences in rates of injury repair among species reflect differing prioritization of energy allocation to maintenance and repair within the life-history strategies of these three species (Hall 1997), but clearly, this is not the main factor driving susceptibility to SEB.

Rather than the capacity for injury repair, vulnerability to injury may be more important in determining differing susceptibilities to SEB among the three coral species. Pocilloporid and acroporid corals, which are the most susceptible coral families on the

GBR (Willis et al. 2004), are also amongst the preferred prey of both the crown-of-thorns starfish (*A. planci)* (De'ath and Moran 1998; Pratchett 2007) and *Drupella* snails (Cummings 1999) in the Indo-Pacific. Moreover, fragmentation is a common part of the life history of branching acroporid corals (Wallace 1985). The potential role of injury in disease initiation has been highlighted previously (Antonius 1981b; Bak and Criens 1981; Antonius 1985a) and is likely to underlie correlations between corallivores and disease prevalence in the Red Sea (Antonius and Riegl 1997; Antonius and Riegl 1998). The high susceptibility of pocilloporid corals to SEB in the Red Sea supports the injury vulnerability hypothesis, however acroporid corals were less susceptible than some genera (i.e., *Hydnophora* and *Galaxea*) (Winkler et al. 2004) that are not typically amongst the preferred prey of crown-of-thorns starfish or *Drupella* snails. More detailed analyses of regional differences in taxa susceptibilities to both SEB and injury are needed to further explore the role of injury vulnerability in the establishment of this disease.

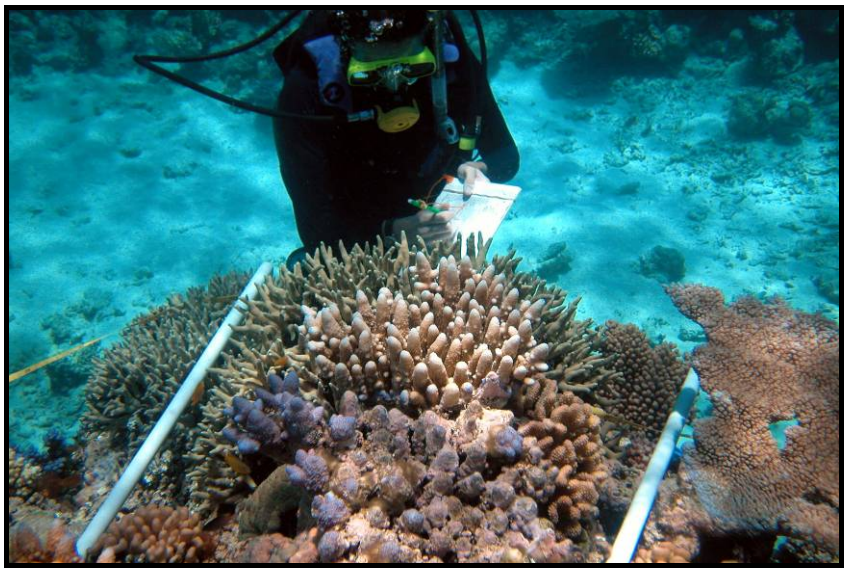
The number and impacts of coral diseases have been increasing worldwide in recent years (Harvell et al. 1999; McClanahan et al. 2004b; Willis et al. 2004; Miller and Williams 2006) and are likely to continue to increase given that there are few options for mitigating their impacts (Bruckner 2002). The identification of injury as a factor that facilitates the colonisation of corals by the ciliate, *H. corallasia,* suggests that protection of reefs from activities which injure corals represents an important management tool. Correlations between increased tourist activity (Winkler et al. 2004) and increased wave action at shallow sites (Antonius and Lipscomb 2001) with increased prevalence of SEB provide corroborative evidence of the importance of reef protection as a management strategy. In particular, management of activities which result in coral breakage (e.g.,

destructive fishing practices, snorkelling, diving, reef walking, and boat groundings) is an important strategy for minimising the transmission and spread of SEB on local scales.

In summary, SEB is endemic within the GBR, affecting ~2% of corals in recent surveys, and currently has the widest host range documented for any coral disease. Reported here are measured rates of SEB progression (average 2 ± 0.3 [± SE] mm d^{-1}), which, although low in comparison to rates of progression of other GBR diseases, are nevertheless equivalent to or faster than progression rates of diseases that have had significant impacts on Caribbean reefs. Injury facilitates the colonisation of otherwise healthy corals by *H. corallasia,* the putative pathogen of SEB. Vectors were not required for ciliate colonisation of injuries in aquaria, suggesting that their motile phases disperse in the water column. However, the lack of development of progressing, band-like aggregations of ciliates on experimental injuries in the field suggests that either *H. corallasia* is not the causative agent of tissue loss, or, additional factors that increase its virulence or reduce host resistance are required before ciliate infections become pathogenic. Microbial studies are needed to elucidate the potential role of an alternative pathogen in tissue lysis associated with band-like aggregations of ciliates.

Chapter 4

Differential susceptibilities of corals to disease on the Great Barrier Reef



A SCUBA diver surveys shallow water corals for disease signs (No Name reef, northern Great Barrier Reef)

4.1. ABSTRACT

Disease has played an important role in structuring Caribbean coral communities, yet limited knowledge of how disease susceptibilities vary among Indo-Pacific corals currently limits understanding of the role of disease in structuring Indo-Pacific coral communities. Disease susceptibilities of scleractinians and other common anthozoans were documented in annual (2004- 2006) surveys encompassing 18 reefs that span the major cross- and long-shelf gradients in environmental factors, anthropogenic pollution and coral community structure on the Great Barrier Reef (GBR). Susceptibilities to other factors affecting the resilience of coral populations (bleaching, changes in pigmentation, injury caused by predation, and algal overgrowth) were also recorded. Scleractinian corals were twice as susceptible to disease and other factors that compromise health as other anthozoans (Scleractinia: disease prevalence = 3.1 ± 0.3%; prevalence of other signs of compromised health = 4.5 ± 1%). Within the Scleractinia, the Pocilloporidae and Acroporidae were the most susceptible families to disease and this pattern was consistent in all three cross shelf positions (inner-, mid- and outer-shelf), in both latitudinal sectors (northern Cooktown/Lizard Island and the central Townsville sectors), and in all three annual surveys. Similar consistencies in patterns were found for other signs of compromised health. Thus, susceptibility hierarchies were not influenced by spatial and temporal variation in environmental factors nor did they merely reflect high abundance of a family at a given site. Disease prevalence was positively correlated with the number of diseases to which a family was susceptible, with seven diseases recorded to affect the most susceptible family, the Acroporidae. Concordance in family rankings for susceptibility to disease and susceptibility to other factors that compromise coral health suggests that colony morphologies and resource allocation strategies of fast growing, branching corals and their resultant high abundance in coral assemblages enhance their vulnerability to a range of biological and physical stressors. Results of this study highlight the vulnerability of acroporid and pocilloporid populations to disease epizootics which, when combined with their vulnerability to cyclones, crown-of-thorn starfish predation and bleaching events, increases the likelihood of catastrophic declines in these groups and the potential for significant re-structuring of GBR coral assemblages.

4.2. INTRODUCTION

Disturbances such as cyclones/hurricanes, large-scale bleaching events and outbreaks of crown-of-thorns starfish (COTS, *Acanthaster planci*) play important roles in structuring coral reef communities (Gleason 1993; Connell et al. 1997). Mortality caused by such disturbances reduces the abundance of highly susceptible taxa, although increased substratum availability may enhance recruitment of less susceptible taxa, leading to changes in the composition and structure of reef communities (Hughes 1994; Connell et al. 1997). Recently, disease has joined the list of disturbances that structure coral reef communities, as illustrated by a series of widespread and catastrophic epizootics affecting echinoderms and anthozoans that have dramatically changed the structure of reef communities throughout the Caribbean (Lessios 1988; Hughes 1994; Harvell et al. 1999; Aronson and Precht 2001). During the 1980's, a disease epizootic eliminated over 93% of the sea urchin, *Diadema antillarum,* at locations throughout the Caribbean for which quantitative data were collected (Lessios 1988). Disease epizootics have also reduced population sizes of gorgonian and scleractinian corals throughout much of the Caribbean (Gladfelter 1982; Porter et al. 2001; Patterson et al. 2002; Kim and Harvell 2004), reducing their reproductive output and the recruitment of new individuals, and hence, significantly slowing population recovery (Edmunds 2000; Kim and Harvell 2004; Richardson and Voss 2005). Recovery of coral populations has also been inhibited by the recruitment of macro-algae to substrates previously occupied by corals (Hughes 1994). Thus, the impact of disease on urchin densities (Lessios 1988; Jackson et al. 2001) has contributed to phase-shifts from coral to algal dominated reefs in many areas of the Caribbean (Hughes 1994; McClanahan and Muthiga 1998). The impacts of disease on a variety of reef taxa have clearly played a significant role in recent changes in the structure of Caribbean reef communities (Williams et al. 1999).

In recent decades, dramatic reductions in both the size of coral populations (Miller et al. 2002; Kim and Harvell 2004) and the composition of coral assemblages in the Caribbean as a consequence of disease epizootics (Aronson and Precht 2001), highlight the importance of understanding how corals differ in susceptibility to disease. *Acropora cervicornis* and *Acropora palmata* have dominated shallow reefs throughout the Caribbean for up to 95,000 years (Pandolfi and Jackson 2006), but the susceptibility of these acroporids to white band disease in particular (Gladfelter 1982), but also more recently to white pox (Patterson et al. 2002), has resulted in disproportionate reductions in their population sizes throughout the Caribbean (Gladfelter 1982; Miller et al. 2002). Caribbean coral assemblages are now dominated by slower growing species that are less susceptible to disease (Aronson and Precht 2001). Despite evidence of the critical role disease plays in structuring Caribbean coral assemblages, little is known about the role disease currently plays in structuring coral assemblages on Indo-Pacific reefs or how disease epizootics, predicted to increase in frequency with climate warming (Harvell et al. 2002; Bruno et al. 2007), might change the composition of Indo-Pacific coral assemblages.

Currently, knowledge of how Indo-Pacific corals vary in their susceptibilities to disease comes from a relatively small number of studies (Loya et al. 1984; Dinsdale 2000; Yamashiro et al. 2000; Raymundo et al. 2003; Jones et al. 2004; Willis et al. 2004). These studies indicate that the families most susceptible to disease vary between Indo-Pacific regions, with pocilloporid and acroporid corals being most susceptible to a variety of diseases at locations including the Great Barrier Reef (GBR) (Jones et al. 2004; Willis et al. 2004; Page and Willis 2006; Chapter 2), the Arabian Gulf (Riegl 2002), the Red Sea (Winkler et al. 2004), the north-west Hawaiian Islands (Aeby 2005) and some Solitary Island sites (Dalton and Smith 2006). In contrast, poritid corals are

most susceptible to disease on reefs of the Philippines (Raymundo et al. 2005) and the main Hawaiian island group (Aeby 2007). The geographic scale of the majority of these studies is very limited; surveys are typically completed on three or fewer reefs and do not encompass the range of reef types or major gradients of anthropogenic pollution, wave action, light and coral assemblage composition found in these regions. For example, disease surveys in the Philippines included only turbid inshore reefs dominated by poritid corals (Raymundo et al. 2003), whereas studies of disease on the GBR encompassed either turbid inshore reefs dominated by species of *Platygyra* and *Montipora* (Loya et al. 1984; Jones et al. 2004), or clear water offshore reefs dominated by pocilloporid and acroporid corals (Dinsdale 2000; Willis et al. 2004). Variation among families in life-history strategies, and in particular, differences in tolerances to extremes of water quality, light, wave exposure and temperature, may result in spatial variability in the families most susceptible to disease within Indo-Pacific regions. Given that many diseases are density dependent (Anderson and May 1979; Bruno et al. 2007), families that dominate assemblages and are susceptible to disease are likely to become the target for disease outbreaks. Thus, differences in the Indo-Pacific families most susceptible to disease that have been identified to date may simply reflect variation in the reef types, environmental settings or coral assemblages surveyed. Large-scale disease surveys are needed in Indo-Pacific regions to compare the susceptibilities of families to disease across gradients in environmental factors, anthropogenic pollution and coral assemblage composition to gain insights into how the susceptibilities of Indo-Pacific corals to disease are influenced by these factors.

The proportion of species currently known to be affected by disease is lower on Indo-Pacific reefs than on Caribbean reefs (Sutherland et al. 2004; Page and Willis 2006; Chapter 2), but is likely to reflect greater efforts to document disease host ranges

in the latter region (Page and Willis 2006; Chapter 2). For example, prior to this study, BBD was recorded to affect 7% of ~350 scleractinian species on Indo-Pacific reefs, but approximately half of all scleractinian species on Caribbean reefs (reviewed in (Sutherland et al. 2004). However, there have been few large-scale surveys of BBD on Indo-Pacific reefs prior to the present study (see Chapter 2), whereas large-scale surveys of BBD have been completed throughout the Caribbean (Antonius 1985a; Edmunds 1991; Bruckner et al. 1997; Green and Bruckner 2000; Weil et al. 2002). Host ranges of diseases affecting Indo-Pacific corals are likely to continue to increase as researchers in this region become more familiar with disease signs in corals, but also as more reefs are surveyed (Page and Willis 2008; Chapter 3). Large-scale spatial surveys incorporating the range of reef environments and species present on Indo-Pacific reefs are essential to establish baseline host ranges for Indo-Pacific coral diseases. Moreover, establishing Indo-Pacific baseline host ranges is fundamental to identifying changes in pathogen virulence or host susceptibility, with climate warming (Epstein 2001).

Coral reefs around the world are increasingly threatened by the compounding impacts of natural and anthropogenic stressors (Hughes and Connell 1999; Wilkinson 2004) and climate warming is predicted to increase the impacts of major disturbances like cyclones/hurricanes, bleaching events and outbreaks of the crown-of-thorns starfish (COTS, *Acanthaster planci*) (Lucus 1973; Agee 1991; Hoegh-Guldberg 1999). Coral disease epizootics are also expected to increase with ocean warming (Harvell et al. 1999), given increases in coral pathogen virulence and host susceptibility at elevated temperatures (Kushmaro et al. 1998; Ben-Haim et al. 2003; Ward et al. 2007). Understanding how corals differ in their susceptibility to these disturbances is critical for understanding how coral assemblages may change as the cumulative impacts of multiple disturbances increase. For example, disease has played a major role in the loss

of acroporid corals from Caribbean reefs (Gladfelter 1982), however acroporid corals are also vulnerable to damage from hurricanes and numerous predators, and such losses further reduce the capacity of acroporid populations to recover from disease-mediated losses (Precht et al. 2002). The vulnerability of Caribbean acroporids to predation and damage from hurricanes may also explain their vulnerability to disease, given links between disease and physical injury (Bak and Criens 1981; Antonius 1985a; Page and Willis 2008; Chapter 3), predation (Antonius and Riegl 1997; 1998) and bleaching (Miller et al. 2006; Muller et al. 2008). Studies of Indo-Pacific corals are urgently needed to identify species vulnerable to multiple disturbance types and therefore most at risk of catastrophic declines.

The aims of this study were to: 1) compare the susceptibilities of scleractinian families and other common anthozoans to disease and factors that compromise their health, 2) determine if susceptibility hierarchies for GBR corals are stable over spatial and temporal scales, 3) identify characteristics of families that contribute to their vulnerability to infection by disease, and 4) expand knowledge of disease host ranges on Indo-Pacific reefs. Results of this study will provide insights into characteristics of coral and other anthozoan assemblages on Indo-Pacific reefs that contribute to their disease susceptibility and will also identify corals that are vulnerable to compounding losses from multiple disturbances. Such information is essential for developing targeted management strategies for the conservation of Indo-Pacific coral reefs.

4.3. METHODS

4.3.1. Study Sites and Survey Methods

Disease susceptibilities of Great Barrier Reef (GBR) scleractinians and other common anthozoans were documented in large-scale annual surveys completed on 18 reefs between 2004 and 2006. To examine the influence of gradients in environmental factors, particularly turbidity, wave action, temperature and water quality on disease susceptibility, and to maximise documentation of host ranges for common diseases, surveys were spread across two latitudinal sectors (Cooktown/Lizard Island and Townsville sectors; see Fig 3.1) and three cross-shelf positions (inner-, mid- and outer-shelf). In combination, this sampling design spans the major gradients across which GBR coral assemblages and reef types vary (Ninio and Meekan 2002). Three reefs, selected haphazardly from those accessible, were surveyed within each cross-shelf by sector position. On each reef, two sites were selected haphazardly from within areas characterised as north-western, back reef slopes. Surveys involved three replicate 20m x 2m belt transects placed parallel to depth contours on the upper reef slope at 3-6m depth (as per Willis et al. 2004). Within each belt, all scleractinians, gorgonians, alcyonaceans and hydrocorals were examined, the presence or absence of disease was recorded, and corals identified to the lowest taxonomic or morphological group, as appropriate. Reefs were surveyed during austral summers, when disease prevalence is likely to be highest (Willis et al. 2004) and surveys were completed in three consecutive summers (2004, 2005 and 2006) to determine if susceptibility to disease varies among years.

Black band disease (BBD) was characterised by black matts or bands predominately comprised of cyanobacteria adjacent diffuse areas of tissue loss and bordering live coral tissues. Cyanobacteria that were not black in colouration, formed

only a very thin line at the interface between live coral tissue and recently denuded skeleton or appear microscopically to be morphologically distinct from the cyanobacteria dominant in BBD mats were classified as other cyanobacterial infections (OtCy). Brown band disease (BrB) was characterised by brown bands comprised of elongate ciliates of the sub-class Scuticociliatia adjacent diffuse areas of tissue loss and live coral tissues. Thin white bands of bare coral skeleton may or may not be visible between live coral tissues and brown ciliate bands. Skeletal eroding band (SEB) was characterised *in situ* by dark green to black speckled bands of the ciliate *Halofolliculina corallasia* bordering live coral tissues and on the other side bare coral skeleton having a speckled and eroded appearance. In the absence of competitive interactions and predators, areas of tissue loss having no visible signs of microorganism adjacent live coral tissues were classified as white syndrome (WS). Atramentous necrosis (AN) was characterised by areas of bleaching and bare coral skeleton adjacent black deposits covered by white filaments. Areas of abnormal skeletal growth were classified as growth anomalies (GAs).

In addition to disease, the presence or absence of other signs of compromised health were recorded to enable comparisons between susceptibility to disease and susceptibility to other factors affecting the resilience of coral populations for each taxa. Signs grouped in this second category were bleaching caused by thermal or other physical stressors, changes in pigmentation, injury caused by predation, and algal overgrowth. Colonies exhibiting these signs are not typically regarded as healthy, but would also not be considered diseased, therefore for the remainder of this chapter, this group of signs (excluding disease) will be summed into a single category called compromised health signs. Although in the broadest definition, disease is compromised health, here I am distinguishing infectious and non-diseases known or thought to be

caused by pathogens and parasites from impaired health states caused by predation, competition or physical factors to more clearly explore taxonomic patterns in disease susceptibility. In this study bleaching is grouped with other signs of compromised health, although considered a disease by some (Rosenberg 2004).

4.3.2. Statistical Analysis

The health of over 310, 039 coral colonies was assessed as part of this study. Susceptibility to disease and to other factors that compromise coral health was determined by comparing the frequency of occurrence of disease and other factors that compromise coral health among coral taxa. Pearson's chi-squared tests of independence were used to test for differences in susceptibility to disease and to other factors that compromise coral health among all scleractinian families and anthozoan taxa recorded in surveys. Differential susceptibility among taxa was examined for diseases individually and for all disease types pooled. To minimise the number of cells with an expected count of five or less, families with low disease counts were excluded from analyses as appropriate. Differential susceptibility to atramentous necrosis (AN) was not tested because of the limited occurrence of this disease in surveys.

To test for consistency in patterns of susceptibility among coral families in relation to cross-shelf position, GBR sector, and survey year, I first calculated the prevalence of colonies with disease and compromised health signs for each scleractinian family, region and survey year (i.e. prevalence per coral family was calculated for each sector by shelf by year combination). Families were then ranked in order of descending prevalence of disease or compromised health, separately for each region by year combination. MANOVA was then used to test for variation in the ranks of families among sectors, shelf positions and survey years. Univariate ANOVA's were also used

to test for spatial and temporal variation in the ranks of the two families identified as the most susceptible to disease and to other factors that compromise coral health (i.e. the Pocilloporidae and Acroporidae) when data were pooled across all sites. Although data were non-normally distributed and variances were heterogeneous, univariate analyses were considered appropriate to detect large-scale differences when reef and site data were pooled across regions. The three-way interaction (Year x Shelf x Sector) was excluded from these MANOVAs and ANOVAs due to the lack of replication at this level following pooling of data.

Spearman's coefficient of rank correlation was used to test if a family's abundance affected its susceptibility to disease or to other factors that compromise coral health. Also the relationships between a family's susceptibility to multiple diseases and to the prevalence of all disease types pooled were examined using Spearman's coefficient of rank correlation. For these tests, families were ranked in terms of their abundance, the number of diseases to which they were susceptible, the prevalence of all diseases pooled, or the prevalence of other signs of compromised health, and ranks were used in these tests.

4.4. RESULTS

4.4.1. Susceptibilities of Anthozoan Taxa to Disease and Other Factors That Compromise Health

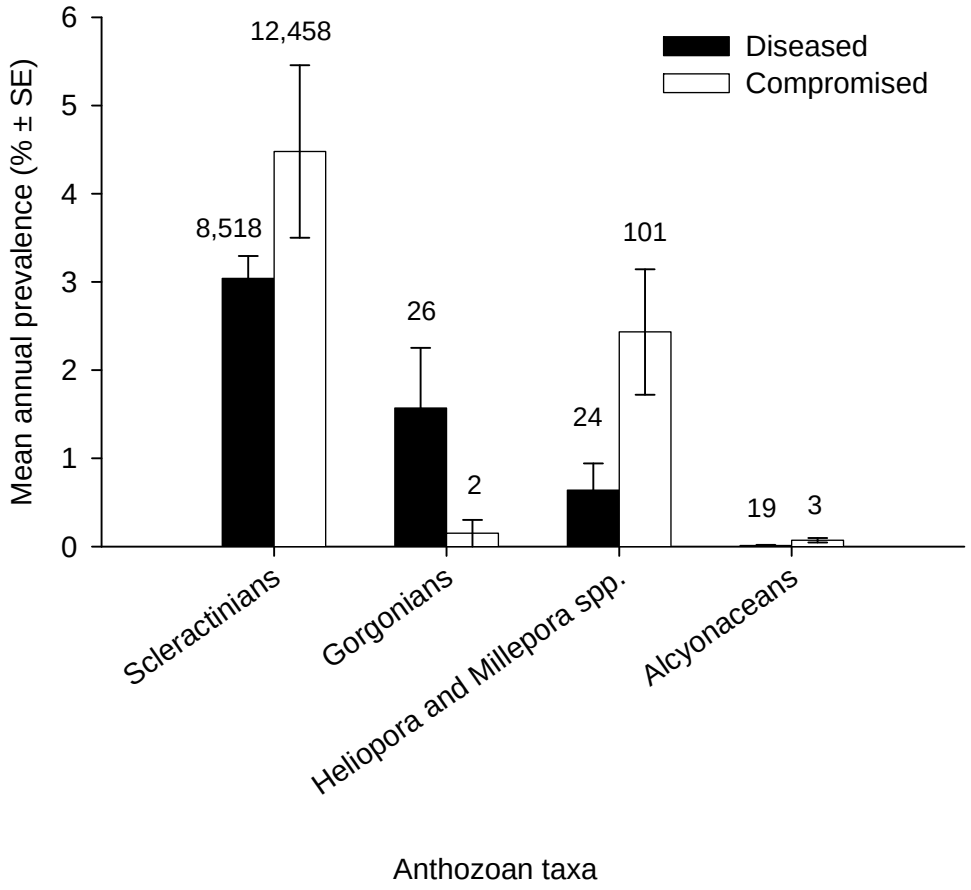
Scleractinian corals were found to be almost twice as susceptible to disease as other anthozoan taxa ($\chi^2 = 916.095$, df = 3, $p < 0.001$; Fig 4.1), with over 98% of all disease cases being recorded from scleractinian corals. Generally over the 3 years, mean prevalence of disease in scleractinian corals was low (3.1 ± 0.3 % of colonies) and mean

prevalence of other signs of compromised health was only marginally higher (4.5 ± 1% of scleractinian colonies; Fig 4.1). Gorgonians were second amongst the anthozoans in their susceptibility to disease (mean disease prevalence = 1.57 ± 0.7% of gorgonians). Scleractinian corals were almost five-fold more susceptible to disease than the hydrocoral *Millepora* and the blue coral *Heliopora* combined (0.64 ± 0.3% of colonies showed signs of disease) and 300-fold more susceptible to disease than alcyonaceans (0.01 ± 0.006% of colonies showed signs of disease).

Scleractinian corals were also two-fold more likely than other anthozoan taxa to exhibit compromised health signs ($\chi^2 = 1294.272$, df = 3, $p < 0.001$; Fig 4.1). Whereas 4.5 ± 1% of scleractinian colonies were recorded with compromised health signs, only 2.4 ± 0.7% of *Millepora* and *Heliopora* colonies showed signs of compromised health caused by factors other than disease (Fig 4.1). Gorgonians were 30-fold less likely (0.15 ± 0.15% of colonies) and soft corals 60-fold less likely (0.07 ± 0.03% of colonies) to exhibit compromised health signs than scleractinian corals (Fig 4.1).

Scleractinian colonies were over 10-fold more abundant in surveys (n = 279,397 colonies) than other anthozoan taxa and were also twice as susceptible to both disease and other factors causing compromised health, however susceptibilities of other anthozoan taxa did not reflect their relative abundance in the large-scale surveys. In particular, gorgonians were the second most susceptible anthozoan taxa to disease but were infrequently recorded in surveys (n = ~1,500 colonies). In contrast, soft corals were rarely recorded as diseased (n = 19) or having compromised health signs (n = 3), but were the second most abundant anthozoan taxa recorded during surveys (n = 26,575) (Fig 4.1).

Figure 4.1. Average prevalence of disease and other signs of compromised health in scleractinians (n = 279,398 colonies examined), alcyonaceans (n = 26,577 colonies), gorgonians (n = 1,566 colonies) and the combined *Heliopora* and *Millepora* category (n = 4,068 colonies) in annual surveys of anthozoan assemblages on the GBR between 2004 and 2006. Numbers above bars indicate the total number of diseased and compromised cases recorded per taxa, summed for the three years of surveys.



4.4.2. Susceptibilities of Scleractinian Families

There was a clear hierarchy in disease susceptibility among scleractinian families when colonies were pooled across all regions and survey years ($\chi^2 = 29400.44$, df = 8, $p < 0.001$; Fig 4.2). The family Pocilloporidae was the most susceptible (6.7 ± 0.7 % of colonies diseased) and almost 1.5 times more likely to be diseased than the Acroporidae (4.5 ± 0.4 % of colonies diseased), the second most susceptible family (Fig 4.2). These two families were 2-3-fold more susceptible to disease than all other families, which typically had less than 2% of colonies showing signs of disease (Fig 4.2). The families Pocilloporidae and Acroporidae also exhibited a greater number of compromised health signs than all other scleractinian families ($\chi^2 = 8420.38$, df = 8, $p < 0.001$; Fig 4.2). Pocilloporid corals were twice as likely to show signs of compromised health (13.35 ± 4.07 % of colonies surveyed per year) as acroporid corals (7.07 ± 1.49 % of colonies surveyed per year; Fig 4.2). The family Acroporidae was 3.5 times more likely to display compromised health signs than all other scleractinian families, which typically had 2% or fewer colonies showing signs of compromised health (Fig 4.2).

The pattern that the families Pocilloporidae and Acroporidae were the most susceptible to disease when all data were pooled, remained consistent when cross-shelf and long-shelf patterns in disease prevalence were examined separately (MANOVA Shelf, Sector and Shelf x Sector interaction: $p > 0.05$; Table 4.1; Table 4.2). Susceptibility hierarchies were also stable between years of surveys (MANOVA Year: $p > 0.05$; Table 4.1; Table 4.2), with the families Acroporidae and Pocilloporidae being the most susceptible to disease in all three annual surveys completed between 2004 and 2006. Thus, disease susceptibility hierarchies at the level of the family were stable through time across both long- and cross-shelf gradients of environmental parameters and irrespective of coral community composition (Table 4.1; Table 4.2). For example,

pocilloporid and acroporid corals were most susceptible to disease both on outer-shelf reefs where they dominate coral communities and on inner-shelf reefs where they form a more minor component of coral assemblages (Ninio and Meekan 2002; Page and Willis unpublished data). Thus, although poritid corals were numerically dominant on inner-shelf reefs in the central Townville and northern Cooktown/Lizard Island sectors (on average, 9791 ± 1035 colonies surveyed on inner-shelf transects (720 m^2) per year), pocilloporids (359 ± 87 colonies per 720 m^2 per year) and acroporids (3811.5 ± 575 colonies per 720 m^2 per year) were more susceptible to disease. Hierarchies in the likelihood of scleractinian families showing signs of compromised health were also stable over cross- and long-shelf gradients in environmental parameters and coral community composition in the three years of this study (Table 4.1). Again, the Pocilloporidae and Acroporidae were consistently most likely to show signs of predation, bleaching, changes in pigmentation, and algal overgrowth in all three cross-shelf positions and in both the northern Cooktown/Lizard Island and central Townsville sectors of the GBR in surveys over the three years (Table 4.2).

The greater susceptibility of acroporid and pocilloporid corals in analyses based on pooled disease data reflects the vulnerability of these families to a wide variety of pathogens. I found a positive relationship between overall pooled disease prevalence per family and the number of diseases to which the family was susceptible ($r_s = 0.848$, df = 12, $p < 0.001$; Fig 4.3). Pocilloporid corals were susceptible to six and acroporid corals to all seven of the diseases affecting GBR corals (Fig 4.3). Atramentous necrosis was the only disease not recorded to affect the Pocilloporidae in this study (Fig 4.4g). However, susceptibility to a wide range of pathogens did not always result in high prevalence when all diseases were pooled. For example, poritid and mussid corals were susceptible to all seven, and faviid corals were susceptible to six of the seven diseases

affecting GBR corals, and yet the combined prevalence for all diseases in these families was over two-fold lower than in acroporid corals and three-fold lower than in pocilloporid corals (Fig 4.3). The high prevalence of disease in the Pocilloporidae also reflects the 1.5 and 3-fold higher susceptibility of this family (in comparison to all other scleractinian families) to the two most prevalent coral diseases on the GBR: skeletal eroding band (SEB) and other cyanobacteria infections (i.e. cyanobacteria infections that differ in appearance from black band disease, herein referred to as other cyanobacteria infections, OtCy) (SEB: $\chi^2 = 2580.17$, df = 7, $p < 0.001$; OtCy: $\chi^2 = 806.97$, df = 7, $p < 0.001$; Fig 4.4a and Fig 4.4b). Pocilloporid corals were also the second most susceptible family to brown band disease, black band disease and white syndrome, although these diseases each affected less than 0.2% of pocilloporid colonies. The ranking of the family Acroporidae as the second most susceptible scleractinian family reflects their high susceptibility to four of the seven diseases affecting GBR corals, i.e. to black band disease (BBD), atramentous necrosis (AN), white syndrome (WS) and brown band disease (BrB) (BBD: $\chi^2 = 373.661$, df = 3, $p < 0.001$; BrB: $\chi^2 = 508.134$, df = 3, $p < 0.001$; WS: $\chi^2 = 200$, df = 3, $p < 0.001$; Fig 4.4). Growth anomalies represented the only disease type for which acroporid or pocilloporid corals were not the most susceptible family; poritid corals were over six-fold more susceptible to growth anomalies than any other scleractinian family (growth anomalies: $\chi^2 = 534$, df = 2, $p < 0.001$; Fig 4.4c).

Figure 4.2. Average prevalence of colonies showing signs of disease and compromised health per scleractinian family, in annual surveys of GBR coral assemblages between 2004 and 2006. The number above each bar indicates the total number of colonies examined in the family.

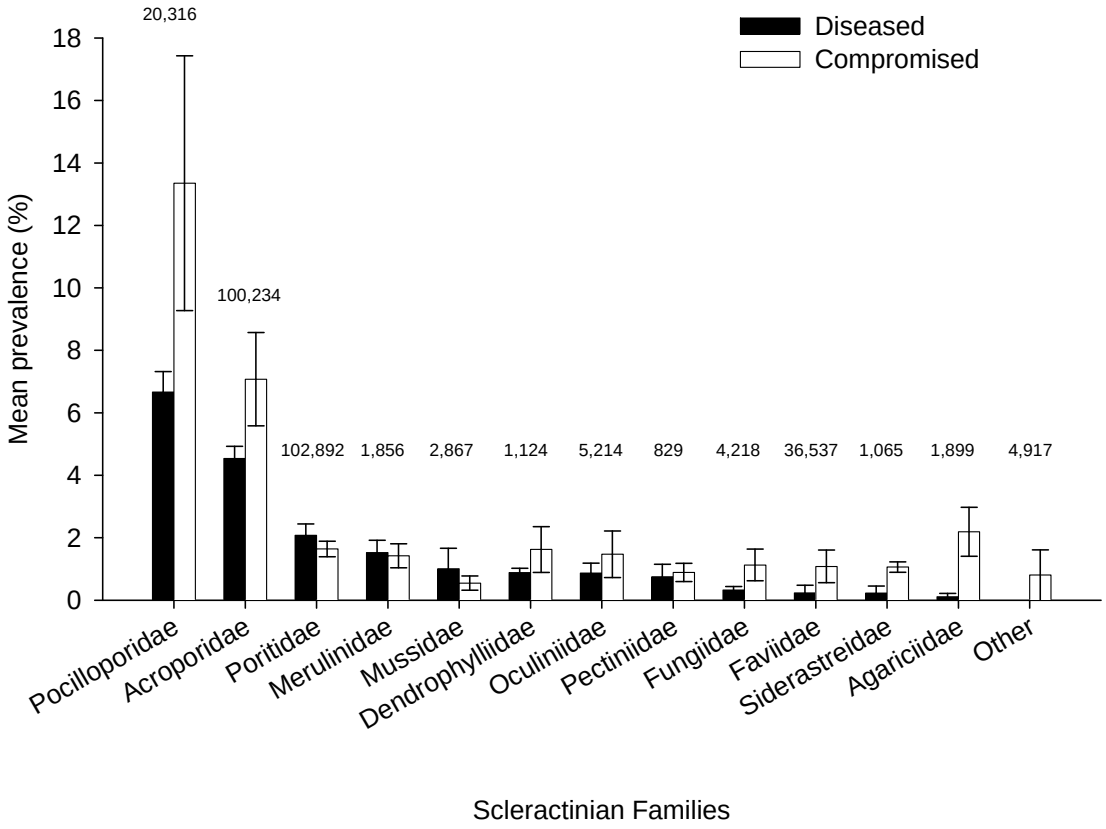


Table 4.1. Three factor MANOVA comparing the rank prevalence of disease and compromised health per scleractinian family, between the northern and central sectors and inner-, mid- and outer-shelf positions of the GBR in three years (2004, 2005 and 2006). $\alpha = 0.05$.

Dependent	Source of variation	Pillai's trace	F	df	p
Disease	Year	1.4293	1.252	8	0.44
	Sector	0.9937	39.721	4	0.11
	Shelf	1.6230	2.153	8	0.23
	Year x Sector	1.1540	0.682	8	0.70
	Year x Shelf	2.0510	1.052	16	0.45
	Sector x Shelf	1.7511	3.519	8	0.11
Compromised	Year	1.1602	0.7	8	0.69
	Sector	0.9970	84.7	4	0.08
	Shelf	1.5597	1.8	8	0.30
	Year x Sector	1.4230	1.2	8	0.44
	Year x Shelf	2.0816	1.1	16	0.43
	Sector x Shelf	1.1090	0.6	8	0.73

Table 4.2. Univariate ANOVAs testing for differences in the rank prevalence of disease and other signs of compromised health in the coral families Pocilloporidae and Acroporidae among GBR sectors (northern versus central sectors), cross-shelf positions (inner-, mid- and outer-shelf positions) and survey year (2004, 2005 and 2006). α = 0.05.

Diseased/ Compromised	Family	Source of variation	SS	df	MS	F	p
Diseased	Acroporidae	Year	10.778	2	5.389	1.6724	0.29
		Sector	6.722	1	6.722	2.0862	0.22
		Shelf	1.444	2	0.722	0.2241	0.80
		Year x Sector	10.778	2	5.389	1.6724	0.29
		Year x Shelf	4.222	4	1.056	0.3276	0.84
		Sector x Shelf	14.111	2	7.056	2.1897	0.22
		Error	12.889	4	3.222		
	Pocilloporidae	Year	2.778	2	1.389	0.847	0.49
		Sector	1.389	1	1.389	0.847	0.40
		Shelf	2.111	2	1.056	0.644	0.57
		Year x Sector	0.111	2	0.056	0.034	0.96
		Year x Shelf	3.222	4	0.806	0.492	0.74
		Sector x Shelf	7.444	2	3.722	2.271	0.21
		Error	6.556	4	1.639		
Compromised	Acroporidae	Year	0.583	2	0.292	0.1180	0.89
		Sector	1.681	1	1.681	0.6798	0.45
		Shelf	4.083	2	2.042	0.8258	0.50
		Year x Sector	3.694	2	1.847	0.7472	0.53
		Year x Shelf	11.333	4	2.833	1.1461	0.44
		Sector x Shelf	1.861	2	0.931	0.3764	0.70
		Error	9.889	4	2.472		
	Pocilloporidae	Year	9.000	2	4.500	1.2656	0.37
		Sector	1.389	1	1.389	0.3906	0.56
		Shelf	4.000	2	2.000	0.5625	0.60
		Year x Sector	2.778	2	1.389	0.3906	0.69
		Year x Shelf	8.000	4	2.000	0.5625	0.70
		Sector x Shelf	7.111	2	3.556	1.0000	0.44
		Error	14.222	4	3.556		

Figure 4.3. The linear relationship between the prevalence of disease and the number of diseases to which each scleractinian family is susceptible, in annual surveys of coral assemblages on the GBR between 2004 and 2006. Data are pooled across all cross-shelf positions, latitudinal sectors and years of surveys.

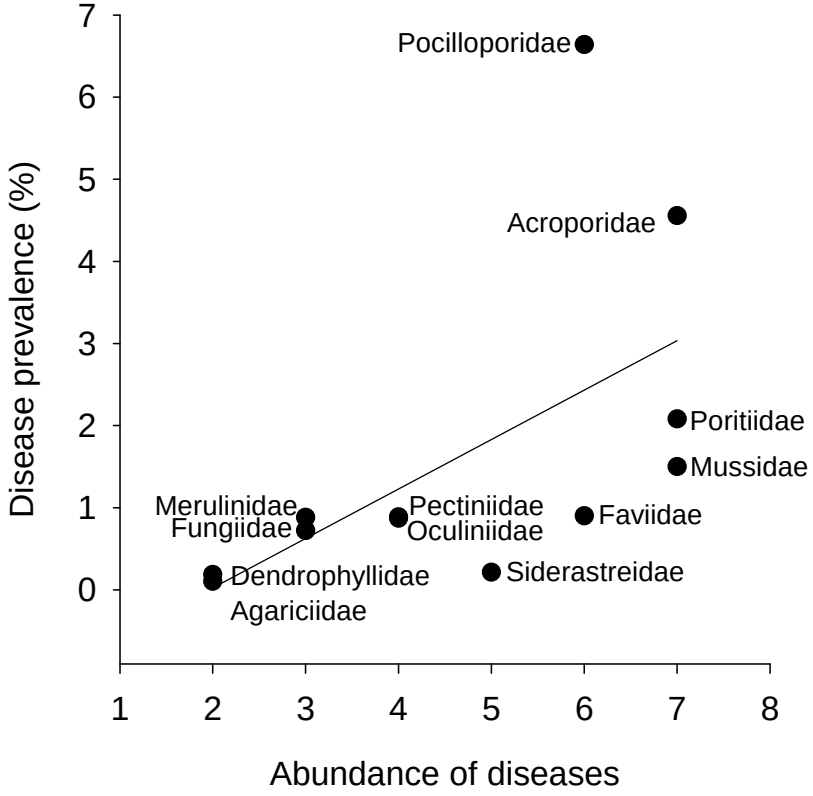
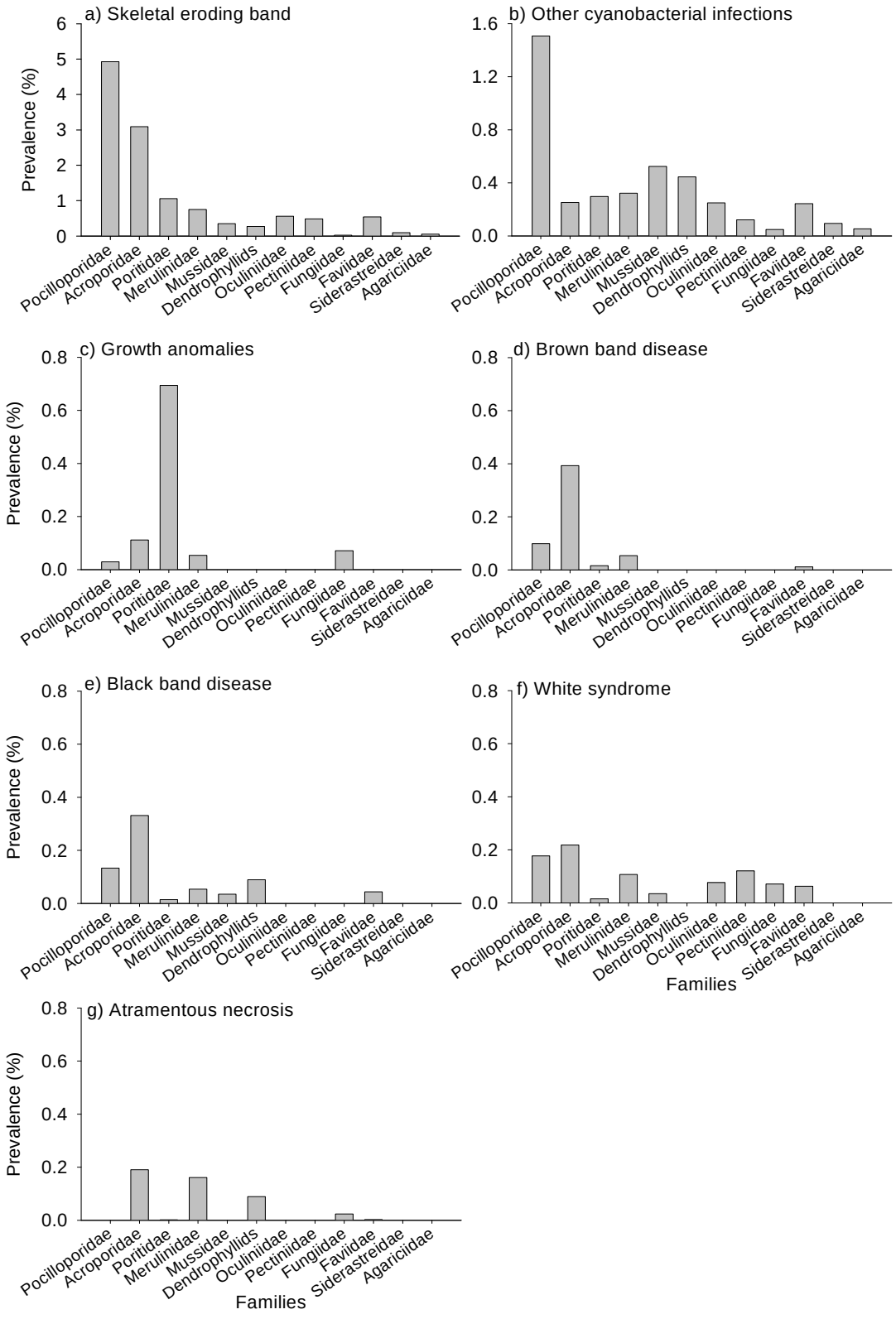


Figure 4.4. Prevalence of a) skeletal eroding band, b) cyanobacteria infections differing in appearance from black band disease (referred herein as other cyanobacterial infections, c) growth anomalies, d) brown band disease, e) black band disease, f) white syndrome, and g) atramentous necrosis, per scleractinian family on the GBR, pooled across all cross-shelf positions, latitudinal sectors and years of surveys. Note: the y-axis scales on 4a and 4b differ from scales on other panels.



Rankings of the twelve scleractinian families surveyed according to disease prevalence and according to the prevalence of other signs of compromised health were both positively related to the abundance of colonies recorded per family (Disease: r_s = 0.714, df = 13, $p < 0.01$; Compromised: $r_s = 0.621$, df = 13, $p = 0.041$; Fig 4.5). Thus the dominance of GBR coral assemblages by acroporid, pocilloporid and poritid corals is also likely to have contributed to these three families being amongst the most susceptible to disease and other factors that compromise coral health. However, the greater susceptibility of acroporid and pocilloporid corals on reefs dominated by poritid corals (inner-shelf Townsville sector), indicates that the abundance of colonies on reefs is not the only important driver of variation in susceptibility to disease or other factors that compromise coral health among scleractinian families.

Within scleractinian families, the susceptibility of genera and morphological groups of corals to disease also varied considerably, although these differences were not formally tested (Appendix 1). For example, staghorn acroporids were more than two and eight-fold more susceptible to SEB (4.38% of colonies) than digitate acroporids (1.68% of colonies) and the genus *Montipora* (0.41% of colonies) respectively.

4.4.3. Disease Host Ranges and Distributions

Diseases affected a wide range of scleractinian and other anthozoan hosts on the GBR, however the number of families and species that were susceptible to each of the seven GBR coral diseases varied considerably. From the broad-scale GBR surveys, the host range of SEB was two-fold greater (88 species) than the disease having the next largest host range (other cyanobacteria infections: 45 species; Appendix 1). SEB affected 12 scleractinian families and the hydrozoan coral *Millepora*. Other cyanobacteria infections (i.e. those differing in appearance to BBD (OtCy)) were

similarly recorded to affect 12 scleractinian families and the hydrozoan *Millepora,* but unlike SEB, were also recorded affecting alcyonacean and gorgonian corals. Seven and nine scleractinian families were susceptible to BBD and WS respectively, the families Acroporidae, Poritidae, Faviidae and Merulinidae being susceptible to both diseases. In addition, BBD affected the family Dendrophylliidae and WS the families Mussidae, Fungiidae, Pectiniidae and Oculinidae. Although WS was recorded to affect a greater number of families than BBD, both diseases affected a similar number of scleractinian species in this study (BBD: 31 species; WS: 29 species). Five scleractinian families were susceptible to brown band disease (BrB) and growth anomalies (GAs), however, the number of species susceptible to these two diseases varied three-fold (BrB: 41, GA: 13 species). BrB and GAs both affected the families Pocilloporidae, Acroporidae, Poritidae and Merulinidae; in addition BrB was recorded to affect the family Faviidae and growth anomalies affected the family Fungiidae. Atramentous necrosis had the smallest host range of the seven diseases recorded from the GBR, affecting only eight scleractinian species from six scleractinian families (Appendix 1). Numbers of hosts recorded here are likely to underestimate GBR host ranges because surveys were limited to a single reef habitat, the upper slope of back reefs., Also, because diseased colonies could not be identified to species in the field in many cases, identification to the genus level or to morphological group within the genus *Acropora* would also have contributed to underestimating host ranges.

Rankings of disease according to the extent of their host ranges paralleled patterns in the prevalence and distributions of diseases on the GBR. SEB and other cyanobacterial infections, which had the greatest host ranges of the seven GBR diseases, were also the most prevalent (Fig 4.4) and widely distributed diseases (Fig 4.6). Both SEB and OtCy infections were recorded from over 80% of reefs surveyed

each year (Fig 4.6). In contrast, atramentous necrosis had the smallest host range of any GBR disease, was lowest in prevalence (Fig 4.4) and was present on the less than 25% of surveyed reefs (Fig 4.6).

The number of species recorded showing signs of compromised health was lower than the number of species showing signs of disease in annual GBR surveys between 2004 and 2006 (Compromised: a minimum of 83 species, Diseased: a minimum of 97 species; Appendix 1). Of the compromised health categories recorded, bleaching affected the largest number of species; bleaching was recorded for at least 67 scleractinian species, approximately half of which were *Acropora* species, as well as for soft corals, gorgonians, and the genera *Millepora* and *Heliopora*. Algal overgrowth was recorded for at least 49 scleractinian species, as well as for soft corals and the genera *Millepora* and *Heliopora*. Changes in pigmentation (other than bleaching) represented the least abundant of the compromised health categories, affecting a minimum of 16 species, including scleractinians and species in the gorgonian genus *Isis.* Signs of predation were recorded from only 39 scleractinian species, the majority of which were *Acropora* species.

Figure 4.5. The linear relationship between the abundance of colonies and, a) the prevalence of colonies showing signs of disease, and b) the prevalence of colonies showing other signs of compromised health, per scleractinian family. All data are ranked and colony counts pooled across all three years of annual surveys (2004 to 2006), cross-shelf positions and latitudinal sectors of the GBR. Smallest ranks represent highest prevalence values.

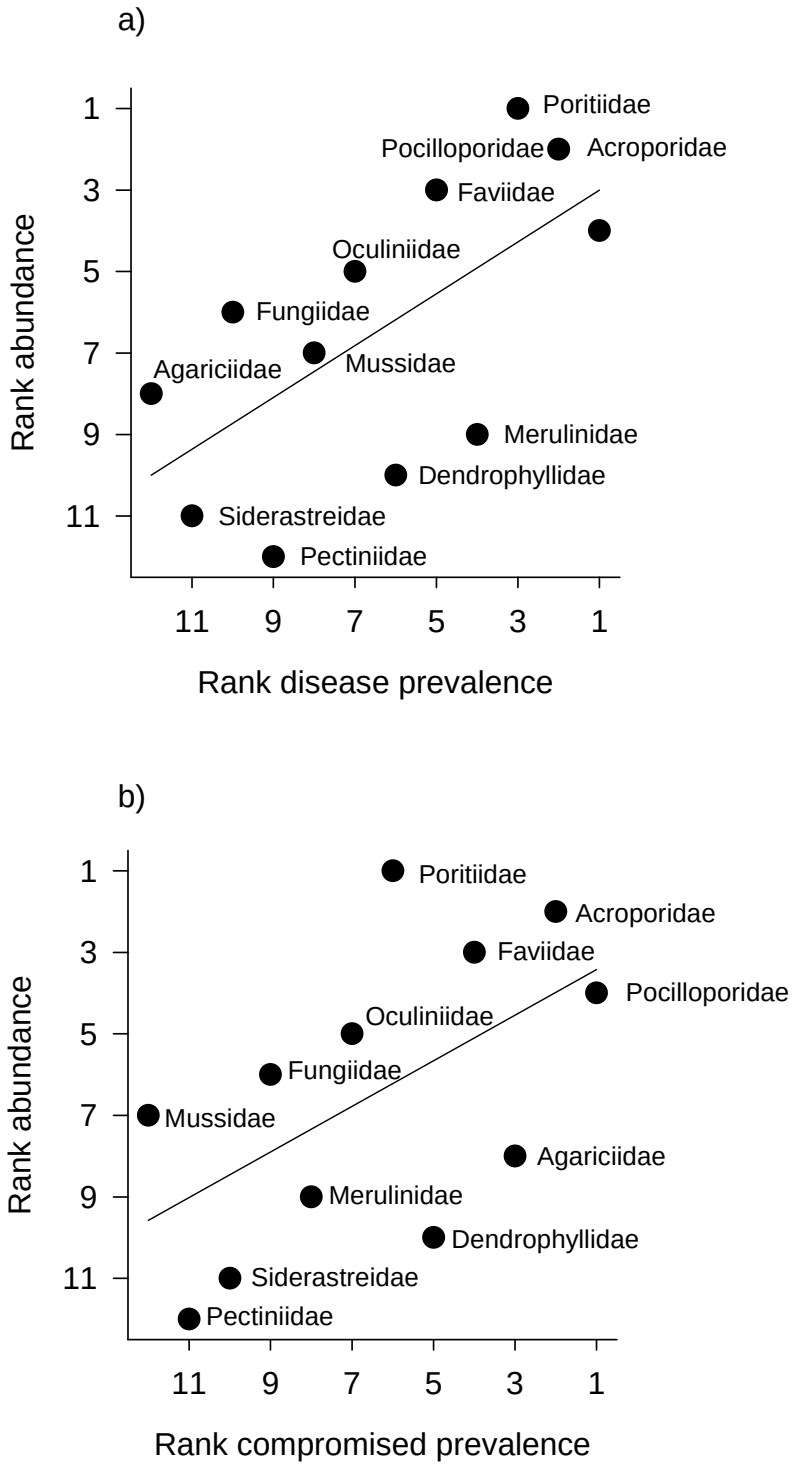
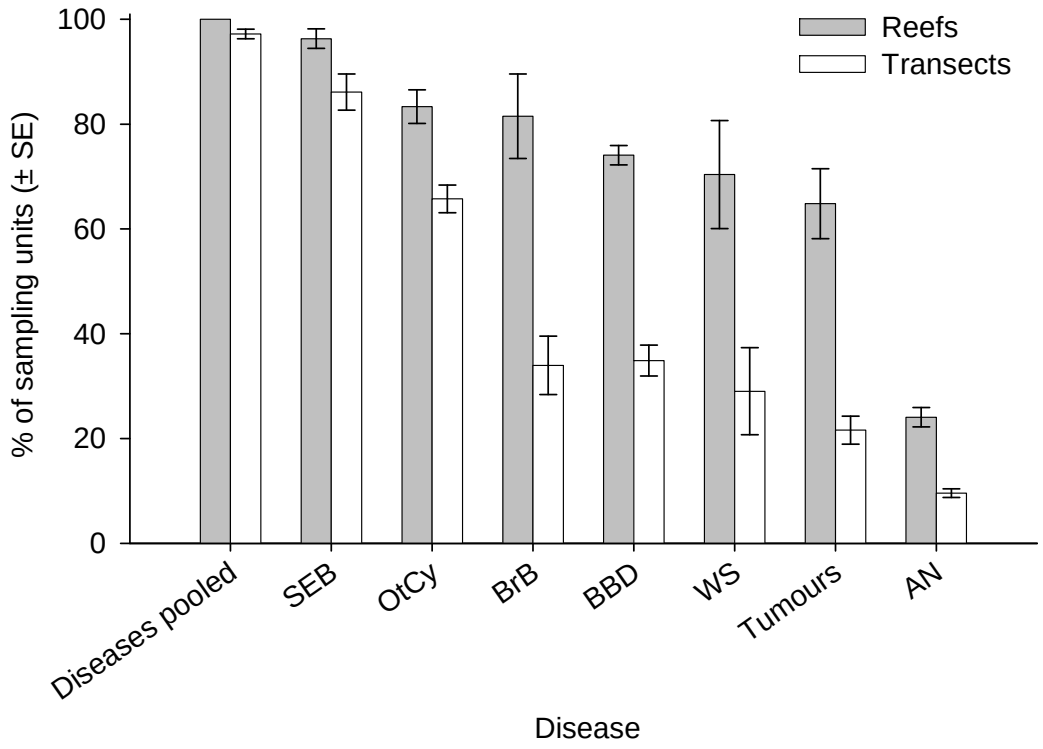


Figure 4.6. Mean percentage (±SE) of GBR reefs (n=18) and transects (n=108) from which diseases, pooled and individually, were recorded in each of three annual surveys between 2004 and 2006.



4.5. DISCUSSION

4.5.1. Susceptibilities of Anthozoan Taxa to Disease and Other Factors That Compromise Health

The greater susceptibility of scleractinian corals to disease than other anthozoan taxa on both the GBR (this study) and in other Indo-Pacific regions (Aeby 2007; Harvell et al. 2007) may partly reflect the dominance of scleractinians on Indo-Pacific reefs (Wilkinson 2004; Sweatman et al. 2005) and the key role that density dependence plays in the spread of infectious diseases (Anderson and May 1979). High prevalence of disease in Caribbean populations of gorgonians (up to 31% on Florida Key reefs; Kim and Harvell 2004), where they occur at greater densities and comprise a greater proportion of anthozoan assemblages than on the GBR (Jordán-Dahlgren 2002; Sweatman et al. 2005), supports this interpretation. However, the few observations of disease on Indo-Pacific gorgonians, soft corals, the hydrocoral, *Millepora,* and the blue coral, *Heliopora* (Morrison-Gardiner 2001; Willis et al. 2004; Phongpaichit et al. 2006), even when these taxa are numerically dominant (pers.obs.), indicates that factors other than low densities are also likely to contribute to the relative immunity of non-scleractinian anthozoans in the Indo-Pacific.

The greater vulnerability of Indo-Pacific scleractinians to disease in comparison to other anthozoans could also reflect the lower levels of antimicrobial activity and secondary compounds that have been found in their tissues compared to octocorals (Jensen et al. 1996; Koh 1997; Kelman et al. 1998; Kelman et al. 2006). Antibiotic activity of bacteria resident in coral mucus has been shown to play an important role in regulating pathogenic microbes (Ritchie 2006), and soft corals, in particular, produce high levels of secondary metabolites that have anti-microbial activity and inhibit

predation and overgrowth by algae (Sammarco and Coll 1988; Michalek-Wagner and Willis 2001). The greater frequency of signs of predation and algal overgrowth in scleractinians than in other anthozoan taxa on the GBR (Appendix 1) provides corroborative evidence of the important role of secondary metabolites in disease resistance of anthozoans. However, poor knowledge of the macroscopic signs of disease in soft corals and gorgonians on Indo-Pacific reefs and the rapidity with which these taxa repair and conceal injuries also highlight the need for further studies of disease signs in non-scleractinian anthozoans on Indo-Pacific reefs. The recent emergence of a novel disease in gorgonians on reefs in Thailand following a tsunami (Phongpaichit et al. 2006), highlights the potential role that inputs of novel pathogens could play in changing anthozoans hierarchies in disease susceptibilities on Indo-Pacific reefs.

4.5.2. Susceptibilities of Scleractinian Families

Previous studies from the GBR (Willis et al. 2004) and a diversity of other Indo-Pacific regions (Aeby 2003; McClanahan et al. 2004b; Raymundo et al. 2005) have shown that scleractinian families differ in their susceptibilities to disease, however this is the first study to show that susceptibility hierarchies are consistent on reefs spanning large gradients in environmental factors, anthropogenic pollution and coral assemblage composition. Consistency in susceptibility hierarchies throughout the GBR indicates that extremes in water quality, light, wave exposure and temperature, and differential tolerances among families to such extremes, are not the primary factors underlying susceptibility hierarchies in this region. Surveys were limited to upper slopes of back reef habitats and it is possible that taxonomic patterns in susceptibilities to disease differ in other reef habitats. Surveys smaller in scale but including exposed front reef slopes on reefs in both the northern and southern GBR revealed similar taxonomic patterns in

disease susceptibility among scleractinian families (Willis et al. 2004), however, indicating that susceptibility patterns may vary little among reef habitats within regions. Factors such as taxonomic patterns in life-history tradeoffs involving the investment of energetic resources into disease resistance, colony abundance and morphology, as well as susceptibilities to other factors that compromise coral health, are likely to be important in determining hierarchies in disease susceptibilities on the GBR.

Life history tradeoffs, colony morphology and disease susceptibility hierarchies: Taxonomic patterns in the combined prevalence of all diseases show that families dominated by branching life-forms (i.e. the Pocilloporidae and Acroporidae) are the most susceptible to disease. The prioritisation of energetic resources for colony growth by fast growing branching species allows these families to rapidly colonise and monopolise bare substratum (Loya 1976; Jackson and Hughes 1985; Wallace 1985), but this life history strategy has possibly evolved to the detriment of immunity and pathogen resistance. It is likely that these species have less energy available for maintenance of colony boundaries and for mechanisms of disease resistance in comparison to hemispherical corals (c.f. Palmer et al. 2008). Families dominated by non-branching morphologies may have more highly developed mechanisms of innate immunity to complement greater investment in the maintenance of colony margins, given slower rates of growth (Chornesky 1983). Lower investment in the maintenance of colony boundaries by species with branching morphologies would also explain the three-fold higher prevalence of disease and two-fold higher prevalence of macro-algal overgrowth in branching poritids compared to poritids with massive, sub-massive or encrusting life-forms (Appendix 1). In combination, these results highlight the vulnerability of families dominated by fast-growing, branching life-forms to invasion by

pathogens. Comparisons of transfer rates of photoassimilates towards disease fronts among species with different morphologies are needed to better assess the role that variation in resource investment strategies plays in determining disease susceptibility hierarchies.

Corals with three-dimensional branching morphologies also expose a comparatively larger surface area of tissue to the water column than massive or encrusting species (Jackson 1979), increasing their likelihood of encountering water borne pathogens and potentially contributing to the higher prevalence of disease in families dominated by branching morphologies. Current knowledge of the etiology and modes of transmission of Indo-Pacific coral diseases is limited (Willis et al. 2004), but there is some evidence to suggest that five of the seven diseases affecting GBR corals, including the two most prevalent diseases (skeletal eroding band and other cyanobacteria infections), could be water borne. The typical clustering of BBD cases (Edmunds 1991; Bruckner et al. 1997; Page and Willis 2006; Chapter 2) and the appearance of new BBD cases downstream of diseased colonies (Bruckner et al. 1997) suggests that currents may spread BBD locally. Similarly, cyanobacterial infections differing in appearance to BBD (other cyanobacterial infections, OtCy) are also likely to be water borne. Colonisation of wounds completely surrounded by intact coral tissues by the ciliate *Halofolliculina corallasia*, the putative pathogen of SEB, in the absence of vectors also suggests that this disease may spread via the water column (Page and Willis 2008; Chapter 3). Isolation of *Vibrio* bacteria causing white syndrome (WS) from the water column above infected Palauan colonies suggests that this and other *Vibrio* bacteria causing white syndromes and atramentous necrosis (included in the white syndrome category in Sussman et al. 2008) on the GBR could also be water borne (Sussman et al. 2008). Thus the finding that families dominated by three-dimensional

life forms, i.e. the pocilloporids, acroporids and branching poritids, were also ranked the highest in the disease susceptibility hierarchy, further supports the hypothesis that their large surface areas expose them to a wide variety of potentially water borne pathogens and contribute to their vulnerability to disease on the GBR.

Concordant hierarchies in compromised health and disease susceptibility: It has long been known that wildlife diseases involve complex interactions between causative agents, the environment and the host, thus disease is more likely to occur in taxa that are sensitive to environmental stressors or frequently exposed to novel micro-organisms or poor nutritional status (Wobeser 2006). The development of disease in corals whose nutritional status is compromised by bleaching, or injured during predation or interactions with macro-algae, is likely to underlie correlations between taxonomic patterns in disease susceptibility and other factors that compromise coral health. Acroporid and pocilloporid corals have the highest susceptibilities to disturbances such as cyclones/hurricanes, bleaching and predation of any coral family, not only throughout much of the Indo-Pacific (Brown and Suharsono 1990; Forde 1992; Cummings and McCorry 1998; De'ath and Moran 1998; Cummings 1999; Marshall and Baird 2000; McClanahan et al. 2004a; Harvell et al. 2007), but also in the Caribbean (Knowlton et al. 1981; Woodley 1993; Precht et al. 2002). Similarly, in addition to the acroporids and pocilloporids being the coral families most susceptible to disease on the GBR (Willis et al. 2004; this study), these two families are also the most susceptible to disease in a diversity of Indo-Pacific regions, including the Arabian Gulf (Riegl 2002), the Red Sea (Winkler et al. 2004), the north-west Hawaiian Islands (Aeby 2005), on Kenyan reefs along the east African coast (McClanahan et al. 2004b) and at some Solitary Island sites (Dalton and Smith 2006). Caribbean acroporids are also highly

vulnerable to a number of highly virulent white diseases (Gladfelter 1982; Patterson et al. 2002; Williams and Miller 2005). Concordance in family rankings for susceptibility to diseases and to other factors that compromise coral health in such a range of reef regions is consistent with family differences in life history tradeoffs and colony morphology underpinning these susceptibilities, as described above. In addition, it is likely that susceptibility of acroporids and pocilloporids to a variety of disturbances leads to synergistic interactions that further facilitate disease development and contribute to family rankings in the disease susceptibility hierarchy found in this study.

Synergistic interactions between disturbance types could contribute to disease susceptibility in a variety of ways. The vulnerability of acroporid and pocilloporid corals to breakage during cyclones and storms and their use of fragmentation as a means of asexual reproduction and space monopolisation (Wallace 1985; Marshall 2000) means that species in these families are commonly injured, which, in turn, makes them vulnerable to pathogen invasion. Previous studies have highlighted the important role injury is likely to play in disease development in corals. For example, disease outbreaks were documented in Caribbean colonies immediately following fragmentation (Bak and Criens 1981) and in colonies injured or stressed following transplantation between Philippine reefs (Raymundo et al. 2003). On the GBR, the rapid colonisation of experimental injuries by the ciliate, *Halofolliculina corallasia* (Page and Willis 2008; Chapter 3), highlights the likely importance of injury in the development of SEB, the most prevalent disease on the GBR. Correlations between the prevalence of injury and SEB on Red Sea reefs (Winkler et al. 2004) further support the hypothesis that injury plays an important role in facilitating the development of SEB. Given the likelihood that physical injury facilitates disease development in corals, management strategies that

limit activities resulting in coral breakage e.g. destructive fishing practices, snorkelling, diving, reef walking, and boat groundings, are recommended to limit disease spread.

The probability of disease developing in injured corals increases if pathogens are transferred directly to coral tissues via corallivores. Previous studies have identified the important role that corallivores, including butterfly fishes (Aeby 1998; Aeby and Santavy 2006), the snail *Coralliophila abbreviata* (Williams and Miller 2005), the fireworm *Hermodice carunculata* (Sussman et al. 2003) and the nudibranch *Phestilla* sp. (Dalton and Godwin 2006) play in the transmission of coral pathogens. Recent observations of the development of BrB on corals preyed on by crown-of-thorns starfish (COTS) in Indonesia (Nugues and Bak 2009) and on the GBR (pers.obs.) suggest that preferences for acroporids and pocilloporids as prey species by common corallivores like COTS and *Drupella* snails (De'ath and Moran 1998; Cummings 1999; this study) may partly underlie the vulnerability of these families to disease. Interactions with macro-algae might also increase disease transmission if algae act as reservoirs of coral pathogens (Nugues et al. 2004) or increase concentrations of dissolved organic carbon (DOC) in the vicinity of corals (Kline et al. 2006), thereby increasing the abundance of pathogenic bacteria and leading to coral mortality (Smith et al. 2006a). Thus, macro-algal overgrowth of branching corals that invest little in the maintenance of colony boundaries is likely to contribute to the susceptibility of pocilloporid and acroporid corals to disease. However, further experimental studies are needed to determine if macro-algae and corallivores act as biological agents of stress and / or vectors of pathogens in the Indo-Pacific. Predicted increases in the frequency of COTS outbreaks with deteriorating water quality (Brodie et al. 2005) highlight the importance of understanding the links between disease and predation. Furthermore, concurrent increases in macro-algae and disease on Caribbean reefs over the last decade (Hughes

1994; Gardner et al. 2003) highlight the importance of establishing whether macro-algae facilitate disease in Indo-Pacific corals, particularly given the potential for macro-algal increases driven by water quality deterioration and overfishing on Indo-Pacific reefs (Hughes et al. 2007).

The two- to six- fold higher prevalence of bleaching found in the families Pocilloporidae and Acroporidae, respectively, compared to other scleractinian families in this study concurs with family rankings in bleaching susceptibility found in previous GBR studies (Fisk and Done 1985; Marshall and Baird 2000). Increasing evidence of links between bleaching and disease in corals (Miller et al. 2006; Muller et al. 2008; McClanahan et al. 2009) is consistent with bleaching increasing the vulnerability of corals to pathogen invasion, potentially in two main ways. First, reductions in densities of zooxanthellae, which provide a large proportion of the daily carbon requirements of most coral species (Davies 1984; Muscatine et al. 1984), reduce energetic resources available to colonies for biological processes (Fitt et al. 2000; Grottoli et al. 2004) including disease resistance. Secondly, bleaching decreases the antibiotic properties of bacteria resident in the mucus of corals, allowing increases in *Vibrio* spp. (Ritchie 2006), which are known pathogens of both Indo-Pacific and Mediterranean corals (Kushmaro et al. 1996; Ben-Haim and Rosenberg 2002; Sussman et al. 2008). The greater prevalence of bleaching in the Pocilloporidae and Acroporidae in the current study suggests that changes in mucus bacterial communities and reductions in the nutritional status of bleached colonies could contribute to the correlation between the families most susceptible to bleaching and disease that was found here. The increased vulnerability of bleached corals to subsequent pathogen infection (Muller et al. 2008) suggests that disease outbreaks are likely to further exacerbate coral declines caused by bleaching of corals (Miller et al. 2006).

Density dependence and disease susceptibility hierarchies: The positive relationship between the prevalence of disease and the abundance of colonies in each family found in the large-scale GBR surveys suggests that the high abundance of these taxa contributes to their susceptibility to disease. This hypothesis is consistent with widespread evidence that many wildlife diseases are density dependent (Anderson and May 1979). The dominance of shallow Caribbean reefs by acroporid colonies prior to widespread disease-mediated declines in their cover in the early 1980's (Gladfelter 1982; Williams et al. 1999) provides corroborative evidence for links between host abundance and disease prevalence. The number one ranking of poritids in disease susceptibility hierarchies on reefs in the Philippines and the main Hawaiian island group, where this family dominates coral assemblages (Aeby 2003; Raymundo et al. 2005), further supports this interpretation. However, acroporid and pocilloporid colonies were more susceptible to disease in regions of the GBR where poritids were numerically dominant, i.e. on inner-shelf reefs in the central and northern sectors of the GBR, indicating that numerical abundance may be only one of a number of factors determining hierarchies in disease susceptibility in this region.

In addition to variation in the families dominating coral assemblages, differences in taxonomic patterns of disease susceptibility in the Philippines and the main Hawaiian Island group (Raymundo et al. 2005; Aeby 2007) may also reflect the presence of different pathogens in these reef regions. For example, disease cases are dominated by *Porites* ulcerative white spot disease in the Philippines (Raymundo et al. 2005) and by the diagenetic trematode, *Podacotyloides,* in the main Hawaiian Island group (Aeby 2007). The absence of confirmed cases of these diseases from the GBR and from many other Indo-Pacific regions may also partly explain variation in the families most vulnerable to disease among these Indo-Pacific regions. Current knowledge of the

etiology of Indo-Pacific diseases is poor, thus it is difficult to compare the distributions of Indo-Pacific coral pathogens, particularly in light of the variable macroscopic signs caused by similar pathogens in different Indo-Pacific regions (see Sussman et al. 2008). Clearly greater efforts are needed to understand the pathogens involved in Indo-Pacific coral diseases to allow a greater understanding of the factors driving regional variability in disease susceptibilities hierarchies.

Pathogens can moderate the abundance of dominant taxa within communities, thereby enabling the persistence of competitively inferior species (Kiesecker and Blaustein 1999; Mitchell and Power 2006) and increasing species diversity (Gilbert 2002). Consequently, the selective mortality of disease-susceptible acroporid and pocilloporid species might facilitate the persistence of otherwise less competitive species, thereby promoting the coexistence of a greater number of species on Indo-Pacific reefs. However, the susceptibility of acroporid and pocilloporid corals to multiple disturbances, including disease, bleaching, corallivore outbreaks and cyclones, in combination with predicted increases in the frequency or intensity of many of these disturbances with climate warming and deteriorating water quality (Agee 1991; Harvell et al. 2002; Brodie et al. 2005), suggest that Indo-Pacific acroporid and pocilloporid populations are at increasing risk of catastrophic declines and extinction. Consequently, increases in the frequency and intensity of disease outbreaks and other disturbances on Indo-Pacific reefs could result in decreases in the diversity of corals on Indo-Pacific reefs, consistent with the Intermediate Disturbance Hypothesis (Connell 1978). Recent dramatic and widespread declines in acroporid populations throughout the Caribbean, driven by their vulnerability to a diversity of disturbances (Hughes 1994; Precht et al. 2002), highlights this possibility. Management strategies specifically aimed at minimising the cumulative impacts of stressors affecting pocilloporid and acroporid

corals on Indo-Pacific reefs are therefore urgently needed to minimise losses from these vulnerable families.

4.5.3. Disease Host Ranges

Records of disease from this and other recent studies (McClanahan et al. 2004b; Willis et al. 2004; Raymundo et al. 2005; Weil and Jordán-Dahlgren 2005; Dalton and Smith 2006; Kaczmarsky 2006) have greatly expanded knowledge of host ranges for Indo-Pacific coral diseases in the past five years. For example, the known host ranges of BBD and GAs have increased by ~50% (68 host species now known for BBD and 35 host species for GAs compared to 45 and 24 host species reported, respectively, in Sutherland et al. 2004), while the host range of SEB has increased almost 400% (93 host species now known compared to 24 host species reported in Sutherland et al. 2004). Expanding host ranges are undoubtedly a function of increasingly widespread and temporally repeated surveys on a range of reef types and coral communities. The expansion of the host range of BBD on the GBR from seven families and 25 scleractinian species in one year surveys (2004; Page and Willis 2006; Chapter 2) to 11 families and at least 40 scleractinian species with the addition of two more years of surveys (2005 and 2006), highlights the need for repetitive surveys of reefs spread across large spatial scales to adequately document disease host ranges. This result also indicates that further increases in disease host ranges are probable as the geographical range of disease studies in the Indo-Pacific expands.

Despite increased studies documenting the host ranges of Indo-Pacific diseases, the proportion of species currently known to be affected by disease remains lower on Indo-Pacific reefs than on Caribbean reefs (~30% of Indo-Pacific versus ~80% of Caribbean scleractinian species). The greater proportion of species known to be disease

hosts on Caribbean reefs may reflect more numerous or severe anthropogenic impacts within this semi-enclosed environment and consequent greater rates of novel pathogen emergence and virulence and / or greater reductions in the resistance of Caribbean corals to pathogens. Alternatively, this pattern may reflect greater inputs of novel pathogens into the wider Caribbean (Patterson et al. 2002; Weir-Bush et al. 2004) in comparison to Indo-Pacific reef regions (Phongpaichit et al. 2006). Greater connectivity between Caribbean reefs may also increase rates of pathogen spread among reefs than in the larger Indo-Pacific region. Baseline data on host ranges established in this study will enable identification of future changes in disease host ranges, potentially driven by increases in pathogen virulence or host susceptibility with climate warming (Epstein 2001; Harvell et al. 2002; Harvell et al. 2009).

In summary, results of this study indicate that the seven known categories of diseases recorded to affect GBR corals, while relatively low in prevalence, affect a wide range of anthozoan hosts, which differ markedly in their susceptibilities to disease. The higher prevalence of disease in the pocilloporid and acroporid families may reflect one or more of a number of factors, including: prioritisation of energetic resources for growth and reproduction rather than for defence of colony boundaries and disease resistance; greater exposure to water borne pathogens because of their branching morphologies; and / or their high abundance and hence vulnerability to density dependent pathogen transmission in GBR coral assemblages. Congruence in susceptibilities to diseases and other factors that compromise coral health (bleaching, changes in pigmentation, injury from predators, and interactions with macro-algae) highlight potential links between other factors that compromise health and disease susceptibility hierarchies. In light of widespread and dramatic losses of acroporid

colonies from Caribbean coral assemblages (Williams et al. 1999; Pandolfi and Jackson 2006), which are undoubtedly related to their vulnerability to multiple disturbances (Precht et al. 2002), results of this study indicate that GBR populations of pocilloporid and acroporid corals are the most vulnerable to catastrophic declines through disease epizootics. The vulnerability of acroporid and pocilloporid populations to the compounding impacts of multiple disturbances could restructure coral assemblages both on the GBR and throughout the Indo-Pacific. Management strategies that limit more localised and controllable sources of anthropogenic-mediated mortality are urgently needed to limit loss of these taxa from Indo-Pacific reefs.

Chapter 5

Chronic and acute impacts of disease on coral populations in the northern Great Barrier Reef

Growth anomalies on *Acropora muricata* colonies
(North Direction Island, northern Great Barrier Reef)

5.1. ABSTRACT

Chronic and acute impacts of disease infections have played a significant role in reducing the fitness of Caribbean coral populations, but the impacts of disease infections on the fitness of Indo-Pacific coral populations are poorly understood. In this study, I document lethal and sub-lethal impacts of three common Indo-Pacific coral diseases, i.e. black band disease (BBD), skeletal eroding band (SEB) and growth anomalies, on the fitness of coral populations in the northern Great Barrier Reef. Acute disease impacts were greatest for BBD, with progressive tissue loss recorded from 100% of BBD lesions compared to less than 75% of SEB lesions. On average, BBD lesions progressed across colonies at a rate five-fold faster (6 ± 0.4 mm day^{-1}) than SEB lesions (1.26 ± 0.2 mm day^{-1}) and rates of partial and whole colony mortality were six- and two-fold higher respectively for BBD, than for SEB. In contrast, tissue loss was not typically associated with growth anomalies. Colonies of *Acropora muricata* with BBD infections typically died before colony growth and reproductive output could be measured, hence sub-lethal impacts could not be assessed. In contrast, SEB infections generally reduced rates of growth and reproductive output of *A. muricata* colonies, but impacts were variable throughout the study. Growth rates were half those of healthy control colonies during late spring-summer (November 2004 to January 2005)(SEB: 0.32 ± 0.07 mm day-1; Healthy: 0.73 ± 0.09 mm day^{-1}), but no difference was detected during cooler months (spring). Randomly selected *A. muricata* colonies infected with SEB had oocytes in 30% fewer polyps than healthy control colonies (SEB: 68 ± 11.5 % of polyps $colony^{-1}$; Healthy: 94.4 ± 3.9 of polyps $colony^{-1}$) and polyps that were fecund produced on average 13% fewer oocytes (SEB: 5.1 ± 0.1 oocytes per polyp; Healthy: 5.8 ± 0.2 oocytes per polyp). In contrast, no differences in measures of reproductive output were detected between tagged *A. muricata* colonies with and without SEB infections. Growth anomalies did not reduce rates of acroporid colony growth, however oocytes were absent from tumorous tissues of both *A. muricata* and *A. intermedia*, and from all but one *A. intermedia* colony, indicating that growth anomalies suppress oogenesis in acroporid colonies. Results of this study highlight the significant role that common Indo-Pacific diseases may have in reducing the fitness of GBR coral populations, a factor currently not accounted for in studies of coral population dynamics in the Indo-Pacific region.

5.2. INTRODUCTION

Disease is a fundamental ecological process that plays a significant role in determining the distribution and abundance of both animal and plant populations (Collinge and Ray 2006). However, understanding the impacts of disease on populations of modular, colonial organisms like reef corals is complicated by the fact that size and age are decoupled (Hughes 1984). Disease infections may kill whole colonies or parts of colonies (partial colony mortality; Jackson and Hughes 1985; Hall 1997), with both processes reducing the number of modules and hence the fitness of coral populations. Because of their modular organisation, coral colonies are able to translocate resources to areas of high demand, including areas undergoing rapid growth (Taylor 1977) or bordering injuries and disease lesions (Meesters et al. 1997; Oren et al. 1997). Therefore, in addition to reducing colony fitness, loss of polyps will reduce energetic reserves available for translocation, thereby compromising disease resistance. Mechanisms of disease resistance in scleractinian corals are poorly understood (reviewed in Mydlarz et al. 2006), but stem cells may be involved in the regeneration of injuries (Rinkevich 1996), as well as the production of germ cells. It follows that partial mortality caused by disease may induce tradeoffs in the allocation of resources to reproduction versus disease resistance and lesion repair. Consequently, in addition to causing both partial and whole colony mortality, disease will have sub-lethal impacts on coral reproduction and growth, which will further significantly reduce the fitness of coral populations.

Disease has played a significant role in the population dynamics of both scleractinian (Gladfelter 1982; Williams et al. 1999; Porter et al. 2001) and gorgonian corals throughout the Caribbean (Guzmán and Cortés 1984; Nagelkerken et al. 1997; Kim and Harvell 2004). High rates of partial and whole colony mortality from disease

infections have reduced both the number and size of colonies comprising susceptible populations, resulting in substantial reductions in their reproductive output and, in extreme cases, failure in the recruitment of new individuals into surviving populations (Richardson and Voss 2005). Reductions in recruitment have also driven changes in the size-frequency distributions of coral populations, with significant implications for their resilience (Kim and Harvell 2004; Richardson and Voss 2005). For example, an epizootic of white plague type II reduced the number of *Dichocoenia stokesi* colonies in the Florida Keys by 75% between 1995 and 2002. Subsequent recruitment failure and the greater susceptibility of small corals to whole colony mortality resulted in a 78 % and 35% decline in the number colonies up to 5 and 10 centimetres in diameter respectively, leading to a shift to larger colonies. Furthermore, larger colonies were thought not to be reproducing as a consequence of the disease (Richardson and Voss 2005). The near elimination of the largest and most fecund colonies of the gorgonian *Gorgonia ventalina* from some Florida Keys sites by the fungus *Aspergillosis sydowii*, reduced the total tissue area of sea fans by over 50%. Dominance of remaining populations by small colonies that contribute little to the larval pool, combined with low recruitment to sites having the highest prevalence of aspergillosis, have reduced the capacity of surviving populations to recover from disturbances via sexual reproduction (Kim and Harvell 2004). Thus disease has significantly reduced the fitness of Caribbean coral populations and is recognised as a serious threat to the continued survival of susceptible coral taxa in the region (Diaz-Soltera 1999; Precht et al. 2002), highlighting the need for studies to examine the impacts of disease on the fitness of corals in other reef regions.

Until the early 2000's, disease was assumed to be of little consequence for coral populations in the Indo-Pacific, but mounting evidence that the prevalence, host range and progression rates of diseases affecting Indo-Pacific corals can, in some cases, be equivalent to those affecting Caribbean corals (Willis et al. 2004; Raymundo et al. 2005; Page and Willis 2006; Page and Willis 2008; Chapters 2 and 3), increasingly suggests that disease plays a greater role in their population dynamics than previously recognised. Moreover, the recent emergence of novel diseases on Indo-Pacific reefs (Antonius and Lipscomb 2001; Raymundo et al. 2003; McClanahan et al. 2004b; Willis et al. 2004), the impacts of which are almost entirely unknown, highlight the urgency with which studies of the consequences of disease for the fitness of Indo-Pacific coral populations are needed.

To date, most studies of disease impacts on Indo-Pacific corals have been completed over relatively short time frames (3 days to 5 months) and have focused on determining rates at which disease fronts kill coral tissues as they progress across colonies (Korrubel and Riegl 1998; Roff et al. 2006; Boyett et al. 2007; Page and Willis 2008; Chapter 3). Few studies of Indo-Pacific diseases have been completed over time frames sufficient to incorporate the impacts of whole colony mortality, or alternatively, the cessation of disease infections and colony recovery, into population dynamic studies. In contrast, recovery from infection has been documented for numerous diseases affecting Caribbean corals, including black band disease (BBD), yellow band disease, white plague, dark spot syndrome and aspergillosis (Bruckner and Bruckner 1997b; Gil-Agudelo et al. 2004; Kim and Harvell 2004; Borger 2005). In these studies, partial mortality of colonies may account for a greater proportion of tissue loss from disease infections than whole colony mortality (Kim and Harvell 2004; Borger 2005), highlighting the importance of including partial mortality in studies of disease dynamics

in coral populations. In the few studies of Indo-Pacific diseases that have included rates of partial and whole colony mortality, both rates have been found to be highly variable. A 19-month study of atramentous necrosis documented losses of ~30 to 60% of surface area for colonies of *Montipora aequituberculata* on fringing reefs surrounding Magnetic Island in the central GBR (Jones et al. 2004). In a 17-month study in the Philippines, approximately half of all colonies affected by *Porites* ulcerative white spot disease (PUWS) suffered large (70-90%) reductions in colony surface area (Raymundo et al. 2003). In addition to rates of partial colony mortality, rates of whole colony mortality in the Indo-Pacific have varied between 8%, for colonies with PUWS in the Philippines (Raymundo et al. 2003), and ~29%, in a one year study of white syndrome in the Solitary Islands (Raymundo et al. 2003; Dalton and Smith 2006). There is a clear need for further studies of disease dynamics over time frames sufficient to document rates of both whole and partial colony mortality to determine their relative contributions to declines in the fitness of Indo-Pacific coral populations.

The sub-lethal impacts of diseases are not well understood for corals in any reef region but appear to vary with both disease type and region. The Caribbean sea fan, *Gorgonia ventalina,* produced few or no gametes when infected with the fungus *Aspergillus sydowii* (Petes et al. 2003). Reproduction was also suppressed in scleractinian corals with growth anomalies, although reported impacts on oocyte quantity and quality vary among coral taxa and geographical locations. Growth anomalies on *Montipora informis* in Japan infrequently contained oocytes, but when they did, oocytes were small and immature (Yamashiro et al. 2000). In contrast, the size of oocytes did not vary between growth anomalies and healthy tissues of *Porites* in Hawaii, but the number of oocytes per gonad and the number of gonads per polyp were reduced within growth anomalies (Hunter 1997). The presence of growth anomalies

also suppressed growth rates of *Acropora muricata* in Guam by ~ 20% (Cheney 1975) and growth rates of *A. palmata* in the Caribbean by 50% (if growth anomalies were within 25cm of branch tips) (Bak 1983). Similarly, infections of the digenetic trematode, *Plagioporus* sp., reduced growth rates of *Porites compressa* colonies by 50% on Hawaii'an reefs (Aeby 1991). The sub-lethal impacts of Indo-Pacific coral diseases other than those described for growth anomalies and the digenetic trematode *Plagioporus* sp. are unknown. Given evidence of reductions in growth rates and reproductive output of diseased corals, assessments of the impacts of diseases on the fitness of coral populations must include studies of their sub-lethal impacts.

In this study, I sought to determine the lethal and sub-lethal impacts of three common Indo-Pacific diseases, skeletal eroding band (SEB), black band disease (BBD) and growth anomalies (GAs), on the fitness of GBR coral populations. BBD was selected because of accumulating evidence that it significant impacts the fitness of Caribbean coral populations, for example it has caused 30-48% rates of whole colony mortality in the Florida Keys and Jamaica (Edmunds 1991; Bruckner and Bruckner 1997b), and the likelihood that BBD might similarly impact the fitness of Indo-Pacific coral populations. BBD prevalence on the GBR is within the range of values recorded from Caribbean reefs (0.2 to 6.0% of corals, reviewed in Weil 2004, Page and Willis 2006; Chapter 2), however long-term studies are required to determine whether BBD cases represent chronic, slow-moving infections or acute, rapidly progressing infections on the GBR. SEB is also extremely prevalent in some Indo-Pacific regions and is the most prevalent disease on the GBR (Page and Willis 2008; Chapter 3), where it affects a wide range of coral taxa (reviewed in Page and Willis 2008; Chapter 3). Growth anomalies were the third most prevalent disease affecting GBR corals in annual surveys between 2004 - 2006 (Chapter 4). Although studies from the Caribbean and the Indo-

Pacific have shown that growth anomalies reduce both colony growth (Cheney 1975; Bak 1983) and reproductive output (Hunter 1997; Yamashiro et al. 2000), growth and reproduction have not been investigated in a single study to determine if both are compromised concurrently or if decreasing energetic reserves lead to trade-offs prioritising one function.

The aims of this study were to: 1) document rates of partial and whole colony mortality attributable to SEB, BBD and growth anomalies in coral populations in the northern GBR, and 2) document sub-lethal impacts of these diseases on growth and reproduction in these same populations. This is the first study to assess and compare both lethal and sub-lethal impacts of disease on an Indo-Pacific coral population and to provide insights into the potential impacts of these three diseases on the fitness of coral populations on reefs throughout the Indo-Pacific.

5.3. METHODS

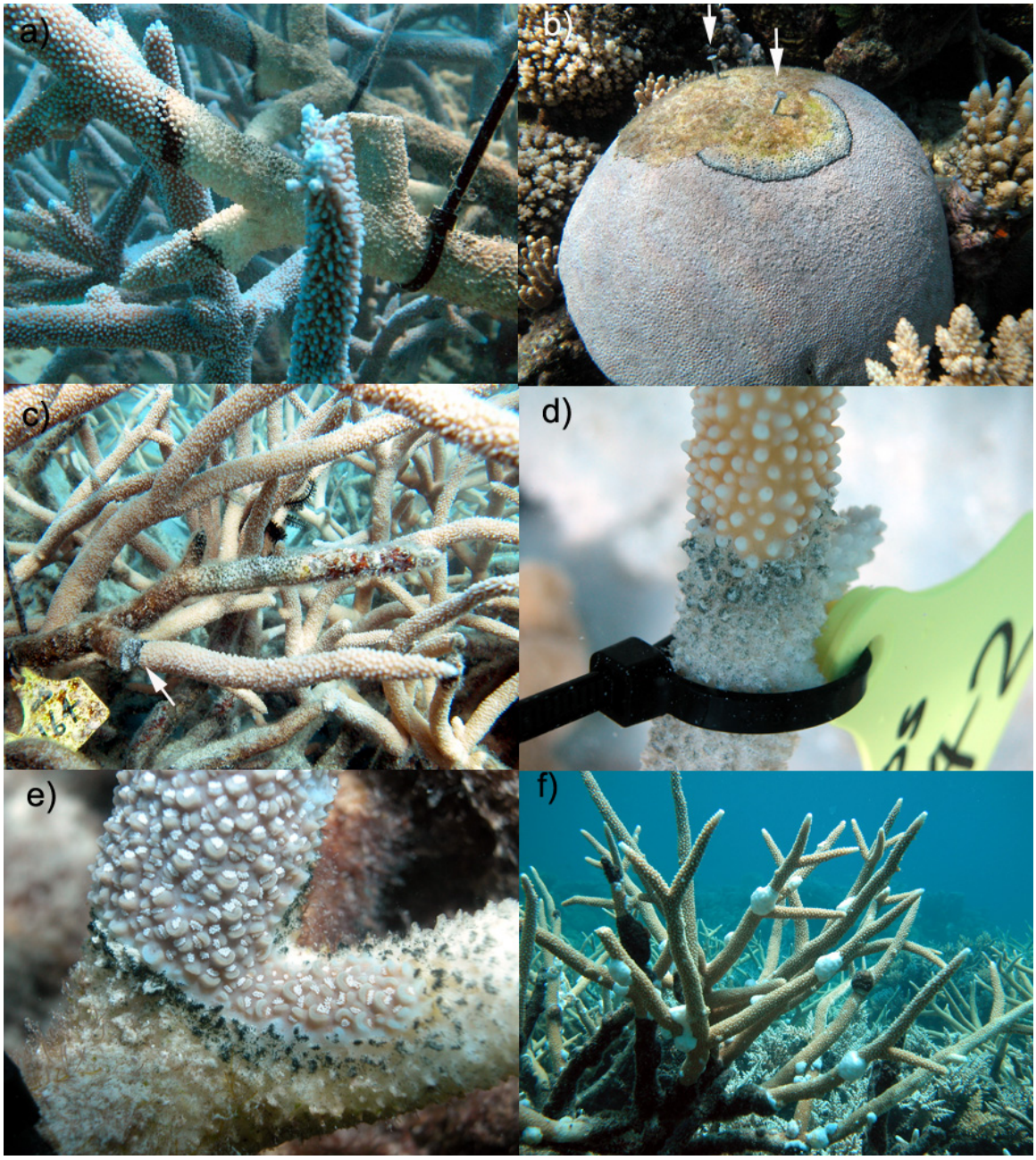
5.3.1. Study Sites and Species

Chronic and acute impacts of disease on coral populations were assessed using tagged and randomly selected colonies located on two reefs in the northern Great Barrier Reef (GBR): Lizard Island (S 14° 41.259, E 145° 26.614) and nearby North Direction Island (S 14° 44.676, E 145°30.389). A total of 201 colonies with signs of black band disease (BBD), 178 with skeletal eroding band (SEB) and 40 with growth anomalies were tagged and their fate followed between July 2003 and November 2005. The majority of colonies were branching species in the genus *Acropora,* reflecting the susceptibility of this genus to disease on the GBR (see Chapter 2). Impacts of BBD were monitored on the branching species *Acropora muricata* (n = 168 colonies), *A. florida* (n = 14), *A. yongei* (n = 8), *A. intermedia* (n = 3), and *A. pulchra* (n = 3), and

also on the plating *A. hyacinthus* (n = 1) and hemispherical *Goniopora* sp. (n = 11). The impacts of SEB were also predominately monitored on colonies of *A. muricata* (n = 135 colonies), but also on *A. pulchra* (n = 23) and *Pocillopora damicornis* (n = 20). Impacts of growth anomalies were monitored on colonies of the branching species *A. intermedia* (n = 21) and *A. muricata* (n = 19). Examples of tagged colonies with signs of BBD, SEB and growth anomalies are shown in Fig 5.1.

All tagged and randomly selected colonies were located on upper-reef slopes (2 - 6m depth) of sheltered back reefs, except *Goniopora* colonies at North Direction Island, where colonies were located on a windward reef flat. In addition to replicate diseased colonies of each species (n = 1 to 139 diseased corals per species per disease type), healthy control colonies (n = 20 to 32) were tagged for each species. The final number of diseased colonies tagged varied according to the abundance of each disease at the study sites.

Figure 5.1. Examples of tagged colonies of, a) *Acropora muricata* and b) a *Goniopora* sp. with black band disease (BBD), c), d) *Acropora muricata*, and e) *Acropora pulchra* with skeletal eroding band (SEB), and f) *Acropora muricata* with growth anomalies, included in this study. White arrows in b) indicate the location of nails from which BBD progression was measured and in c) the location of the ciliate infestation actively killing coral tissues, above which can be seen a branch already killed by this infection.



5.3.2. Measuring Acute Impacts of Disease: Partial and Whole Colony Mortality

Branching colonies were tagged using numbered plastic cattle tags secured with cable ties to exposed skeleton at colony bases. Cable ties were placed a short distance behind disease fronts, which always commenced at the bases of branching species in this study, to avoid interfering with disease progression and used as a baseline from which progression of the disease front was measured. BBD infections on massive *Goniopora* spp. generally started in small areas at the top of colonies, therefore tags were secured with cable ties to the substratum or corals immediately adjacent to diseased colonies to ensure tags did not interfere with disease progression. To calculate surface area and measure the progression of disease fronts on massive *Goniopora* colonies, nails were inserted into bare coral skeleton a short distance behind disease fronts (n = 3 to 10 nails per colony depending on the length of the disease front) and distances between the nails and the nearest live tissue were measured. Colonies were first tagged in July 2003 and revisited every one to four months. When a large proportion of tagged colonies died, additional colonies were tagged to replace them. *Acropora intermedia* and *Acropora muricata* colonies with GAs and healthy control colonies were tagged in May and September 2005 respectively. Colonies were last revisited in November 2005.

The percentage of diseased colonies suffering whole colony mortality was calculated over the time interval between tagging and the final revisit for colonies that could be relocated. To calculate the surface area of branching species for assessments of partial mortality, the height of each branch was measured as the distance along the contours of the branch from the tip of the apical corallite to the interface between live tissues and exposed skeleton at colony bases. The basal diameter of each branch, defined as the basal point of live tissue, was measured at the time of tagging. To

calculate areas of tissue loss corrected for areas of new growth for branching species, the height of each branch and the distance from the cable tie to the nearest interface between live tissues and the disease front were measured at each revisit. The surface area of each branch was modelled as a cone and calculated using the formula: SA = S x branch radius x π (where S = sqrt (branch radius^2 + branch height^2)) and the total surface area of each colony at each sampling point calculated by summing the area of all branches. The area of partial mortality for each branching colony was calculated as the sum of the changes in branch surface areas between time = 0 and the time of survey, minus any increase in colony surface area due to the growth of each branch. To calculate the projected planar surface area of hemispherical colonies of *Goniopora* spp., photos were taken parallel to the uppermost surface of colonies at each revisit. A 10cm ruler photographed on the top of each colony was used to calibrate photos using the software Optimas (version 6.5). Note that all BBD lesions started on the uppermost surface of these colonies. To calculate areas of tissue loss on the uppermost colony surface corrected for areas of new growth from two-dimensional images of massive *Goniopora* species, the projected planar surface areas of live tissues and growth beyond initial colony boundaries were traced from photos taken at each revisit. Partial colony mortality was calculated as the change in planar surface area between time = 0 and time = 3 months, minus any increase in planar surface area due to growth.

Daily rates of tissue loss (= rates of disease front progression) were measured from a subset of colonies on which disease signs were present at both the time of tagging and revisiting (see Table 5.1 for sample sizes). Disease progression was measured for branching colonies by calculating the difference in the distance from cable ties to the nearest live tissue between successive visits. For massive *Goniopora* spp., disease progression was calculated as the mean (n= 3 to 5 measurements per colony)

change in the distance between nails and the nearest live coral tissue. Measurements were converted to a daily rate to enable comparisons among different sampling intervals.

5.3.3. Measuring Chronic, Sub-lethal Impacts of Disease: Growth and Reproduction

The sub-lethal impacts of disease on coral growth and reproduction were assessed for colonies of *A. muricata* and *A. intermedia* (see Figures for numbers of colonies measured per disease type). Linear growth rates of tagged diseased and healthy *A. muricata* colonies were measured for SEB and BBD in summer (24th December 2004 to 10th January 2005) and spring (9th September to 4th of November 2005). Linear growth rates of colonies with and without growth anomalies were measured for *A. intermedia* in winter and spring combined (7th May to 1st November 2005), but only in spring for *A. muricata* (10th September to 2nd November 2005) because the site could not be accessed in May 2005 due to severe weather. Linear growth was measured by staining the skeleton of the growing tips of branches with alizarin sulphonate (Oliver 1984; Harriott 1999). Plastic bags were placed over all branches of each colony (n = 2 to 10 branches per colony) and secured with flagging tape. Alizarin was then released from a vial containing concentrated alizarin dissolved in freshwater from within each plastic bag, producing a final concentration of approximately 10mg/L. Bags were left in-situ for 3 to 4 hours, after which time they were removed leaving branch tips stained red/pink. In the week prior to the predicted date of spawning, all branches from each colony were carefully broken below the line demarcating white areas of new growth from red, alizarin-stained areas using bone cutters and placed individually in labelled

plastic bags. Fragments were then placed in a mild bleach solution to remove coral tissues, rinsed in freshwater and allowed to dry.

To calculate the linear extension of each branch, the distance from a permanent mark inscribed at the base of each fragment to the tip of the apical corallite was measured using callipers. Starting at the tip, the fragment was then cut in 1mm increments perpendicular to the branch axis using a diamond wafering blade attached to a Buehler Isomet low speed saw, until the red alizarin stained skeleton was visible in the cut skeleton. The distance from the basal permanent mark to the cut edge was measured and the linear extension of each branch calculated as the difference between these two measurements. The lengths of white incipient corallites or branches originating below the point of staining were also measured using callipers. Total colony growth was calculated as the total linear extension of all branches plus incipient corallites or branches of a colony. To calculate a daily rate of colony growth or linear extension per colony, total colony growth was divided by the number of days between staining and collection.

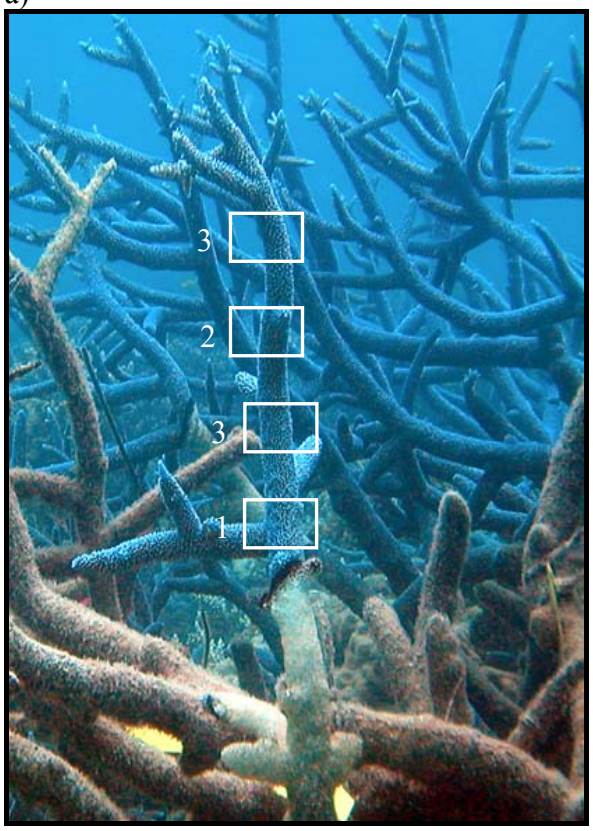
The reproductive output of tagged colonies of *A. muricata* that had been infected for 2-6 months with SEB (n = 24) or remained healthy for this same period (n = 42) was measured from sections of colonies collected in the week prior to the predicted date of spawning in November 2005. Using the same colonies as the growth study, samples approximately 2cm in length were collected in-situ using bone cutters or a hammer and chisel from all branches for diseased and healthy colonies. Samples were collected from the base (sample type 1; Fig 5.2a) and midway along each branch (sample type 2; Fig 5.2a) to avoid sterile areas at branch tips. If oocytes were not visible in a sample in situ, branches were further sub-sampled (sample type 3; Fig 5.2a) to assess whether eggs were absent from the entire branch or just from the one portion of the branch. To assess

the extent to which growth anomalies might suppress oocyte production along branches of *A. muricata* and *A. intermedia*, at least three samples were collected from branches with growth anomalies, namely samples from the growth anomaly (sample type 1; Fig 5.2b), the tissues immediately adjacent to the growth anomaly (sample type 2; Fig 5.2b) (the largest if a colony had more than one growth anomaly) and the tissues midway between the growth anomaly and the base of the branch (sample type 3; Fig 5.2b). Coral samples were placed in numbered resealable plastic bags in-situ. Because many tagged, diseased colonies died before spawning, reproductive samples were also collected from randomly selected colonies of *A. muricata* that had signs of BBD (n = 20) or SEB (n = 19), or appeared healthy (n = 20) just prior to spawning.

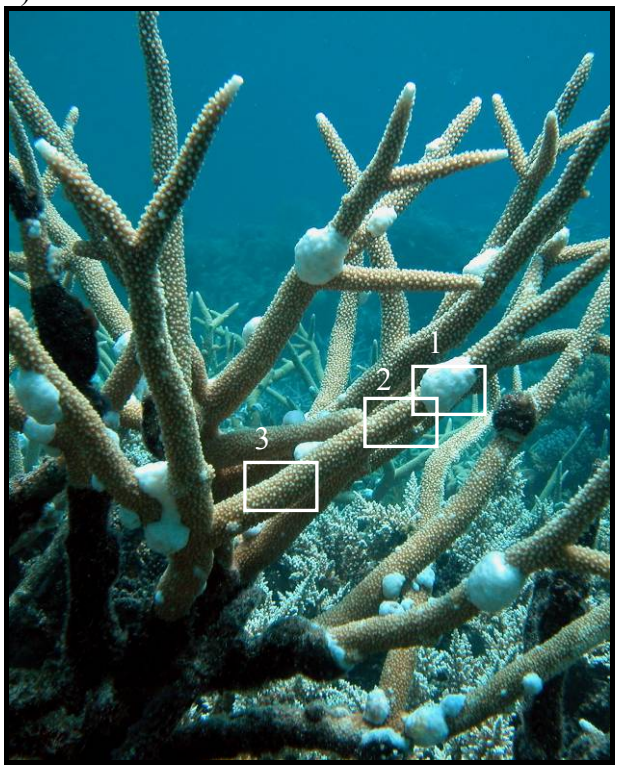
Reproductive samples were preserved in 10% formalin seawater solution, decalcified in 5% formic acid and stored in 70% ethanol. Decalcified sections were examined under a dissecting microscope and the following measures of reproductive output assessed: 1) % of population fecund, measured as the percentage of colonies with oocytes present, 2) % of each colony that was fecund, measured as the percentage of polyps containing oocytes calculated from ten randomly selected polyps per colony, and 3) polyp fecundity, measured as the number of oocytes in each of ten randomly selected polyps in which oocytes were present.

Figure 5.2. a) The location of 1) basal, 2) mid-branch, and 3) further sub-samples collected from colonies with black band disease or skeletal eroding band, and b) the location of samples of 1) growth anomalies (GAs), 2) tissues immediately adjacent GAs, and 3) further sub-samples taken from colonies with GAs, for the analysis of the reproductive output of diseased *Acropora* colonies.

a)



b)



5.3.4. Statistical Analysis

Differences in daily rates of progression of disease fronts were compared among the three diseases, as well as among coral species and sampling periods using ANOVA and post-hoc Tukey's tests or t- tests, following confirmation of homogeneity of variances and normality of distributions. Variations in the number of colonies suffering whole colony mortality versus those surviving disease infections were compared between colonies with BBD and SEB, as well as between coral species separately for each disease, using chi-square tests of independence.

Rates of linear growth were compared between healthy and diseased corals using analysis of covariance, with colony surface area as the covariate to control for the effect of colony size on growth. The health status of colonies (i.e. diseased versus healthy) was treated as a fixed factor and colony as a random factor nested within health status. Only colonies that were healthy or infected by SEB or BBD at both the time of tagging and sample collection were included in this analysis. Student's t-tests were used to test for differences in the number of growing points per colony (i.e. the total number of axial corallites per colony), colony height and surface area between diseased and healthy colonies. Where differences were significant, the relationship between the variable and growth was examined using linear regression.

To test for impacts of BBD, SEB and growth anomalies on reproduction at the population level, chi-squared tests of independence were used to compare variation in the numbers of colonies with oocytes present versus those lacking oocytes between diseased and healthy colonies of *A. muricata*. At the colony level, t-tests were used to test for differences in the percentage of polyps containing oocytes between diseased versus healthy colonies. At the polyp level, analysis of variance was used to test for differences in the number of oocytes per polyp between healthy and diseased colonies.

Health status (i.e. whether a colony was diseased or healthy) was treated as a fixed factor and colonies as a random factor nested within health status. Colonies included in the analyses were only those that were healthy or infected by either SEB or BBD at both the time of tagging and sample collection.

5.4. RESULTS

5.4.1. Acute Impacts of Disease on Coral Populations

Rates of disease progression: Lesions spread progressively across colonies in 100% of cases of black band disease (BBD) on 3 species of *Acropora (A. muricata, A. florida* and *A. yongei)* and 1 species of *Goniopora* monitored over the two years of the study (n = 201 cases in total; Table 5.1). In contrast, skeletal eroding band (SEB) lesions progressed on less than 75% of colonies monitored in two species of *Acropora (A. muricata, A. pulchra)* and the pocilloporid, *Pocillopora damicornis* (n = 178 cases in total; Table 5.1). Only small areas of tissue loss (< 1cm^2) were documented on colonies of *A. intermedia* that displayed growth anomalies, and only on a small number of the tagged colonies.

No seasonal patterns in rates of disease progression were detected for either BBD or SEB on *A. muricata*, with high and low rates being recorded in both summer and winter over the two years that each disease was monitored (Fig 5.3; Appendix 2). Therefore, overall mean rates of disease progression on *A. muricata* were calculated from pooled measurements over the two years and compared between disease types. Average rates of disease progression on *Acropora muricata* were faster on colonies infected with BBD than on colonies infected with SEB (Fig 5.4), both when compared for subsets of months during which measurements overlapped (December 2004 to January 2005, t test: $t = -10.76$, $df = 28$, $p < 0.001$; January to May 2005, t test: $t = -2.74$, $df = 8$, $p = 0.025$; within May 2005, t test: $t = -5.81$, $df = 28$, $p < 0.001$; Appendix 2) and when all months were pooled (t test: $t = -13.6$, $df = 170$, $p < 0.001$; Fig 5.4a). Based on pooled measurements for all species of *Acropora,* BBD progressed across *Acropora* colonies killing coral tissues at an average rate of 7 ± 0.4 mm day^{-1}, which was over

five-fold faster than progression rates of SEB disease fronts (1.26 ± 0.2 mm day^{-1}) (ANOVA: F = 87.3, df = 2, p < 0.001). Linear rates of BBD progression on hemispherical colonies of the *Goniopora* sp. (0.3 ± 0.08 mm day^{-1}) were at least 22 times slower than on the three branching acroporids (*A. yongei*: 9.9 ± 1.2 mm day^{-1}, *A. florida*: 7.4 ± 1 mm day^{-1}, *A. muricata:* 6.8 ± 0.4 mm day^{-1}; F = 20.85, df = 3, p < 0.001; Fig 5.4b), but similar to progression rates recorded for SEB. Although there was a two-fold difference in mean SEB progression rates between the two acroporid and the pocilloporid species examined, means were not statistically different ($ANOVA_{(Species)}$: F = 2.9, df = 2, p = 0.059; Fig 5.4c).

Whole colony mortality: Rates of whole colony mortality were higher for colonies with BBD infections than for colonies with SEB. The likelihood of a whole colony dying over the 2 years of monitoring was two-fold higher if it was infected with BBD than if infected with SEB (84% versus 39% of colonies died respectively; $\chi^2 = 76.94$, df = 1, p < 0.001; Table 5.1). In fact, ~66% of colonies infected with BBD suffered whole colony mortality within only three months. The likelihood of whole colony mortality also varied among species for both diseases (BBD comparison among the five most abundant species: $\chi^2 = 68.72$, df = 4, p < 0.001; SEB: $\chi^2 = 19.33$, df = 2, p < 0.001; Table 5.1). A high and approximately equivalent percentage of the three acroporid species with BBD suffered whole colony mortality (*A. muricata:* 90.6%; *A. yongei:* 87.5% and *A. florida:* 86.6% of colonies; Table 5.1). In contrast, no *Goniopora* colonies with BBD died in the 11-month period they were followed. The likelihood of whole colony mortality resulting from a SEB infection in *A. muricata* (48.2% of colonies died completely) was two-fold higher than for *Pocillopora damicornis* (20% died) and ten-fold higher than for *A. pulchra* (4.4% of colonies died) (Table 5.1). No colonies with

growth anomalies suffered whole colony mortality during this study and growth anomalies remained present for the duration of the study on all tagged colonies.

Table 5.1. Percentage of tagged colonies of *Acropora muricata, Acropora yongei*, *Acropora florida* and *Goniopora* sp. with black band disease (BBD) and colonies of *A. muricata, Acropora pulchra* and *Pocillopora damicornis* with skeletal eroding band (SEB), suffering whole colony mortality, recovering from and maintaining persistent disease infections or developing signs of other diseases, pooled for all species and time-periods and separately for all coral species for each time period. The total number of colonies tagged, the number of months each group of colonies were followed and the dates colonies were first tagged and last revisited are also indicated. * indicates colonies in which impacts of disease for colony growth were monitored and # the colonies in which impacts of disease for reproduction were monitored.

Disease	Species	Date colonies tagged	Date colonies last revisited	# months colonies followed	n	% colonies suffering whole colony mortality	% colonies healthy	% colonies still with disease	% colonies with other disease
BBD	All species pooled				201	83.7	9.5	6.8	
	Acropora muricata	24th July 03	3rd Nov 05	27	60	100			
		3rd Nov 04	11th Nov 05	12	31	96.7	3.23		
		3rd May 05	13th Nov 05	7	40	100			
		4th Sept 05	13th Nov05*#	3	37	78.4	5.4	16.2	
	Acropora yongei	27th Dec 04	7th Nov 04	11	8	87.5	12.5		
	Acropora florida	3rd Nov 03	11th Nov 05	24	14	86.6	7.15	7.15	
	Goniopora sp.	14th Jan 05	13th March06	14	11	0	36.4	63.6	
SEB	All species pooled				178	39.3	31.4	28.1	1.2
	Acropora muricata	1st Nov 03	5th Nov 05	24	56	76.79	24.43	1.79	
		24th Nov 04	10th Jan 05*	3	22	18.18	4.55	72.73	4.55
		6th May 05	10th Nov 05#	7	44	38.64	38.64	20.45	2.27
		9th Sept 05	4th Nov05*#	3	13	7.69	15.38	76.92	
	Acropora pulchra	6th Jan 05	6th Nov 05	11	23	4.4	65.2	30.4	
	Pocillopora damicornis	12th Jan 05	17th Nov 05	11	20	20	50	25	5

Figure 5.3. Temporal variability in daily rates of disease progression recorded from *Acropora muricata* colonies with a) black band disease (BBD), and b) skeletal eroding band (SEB)

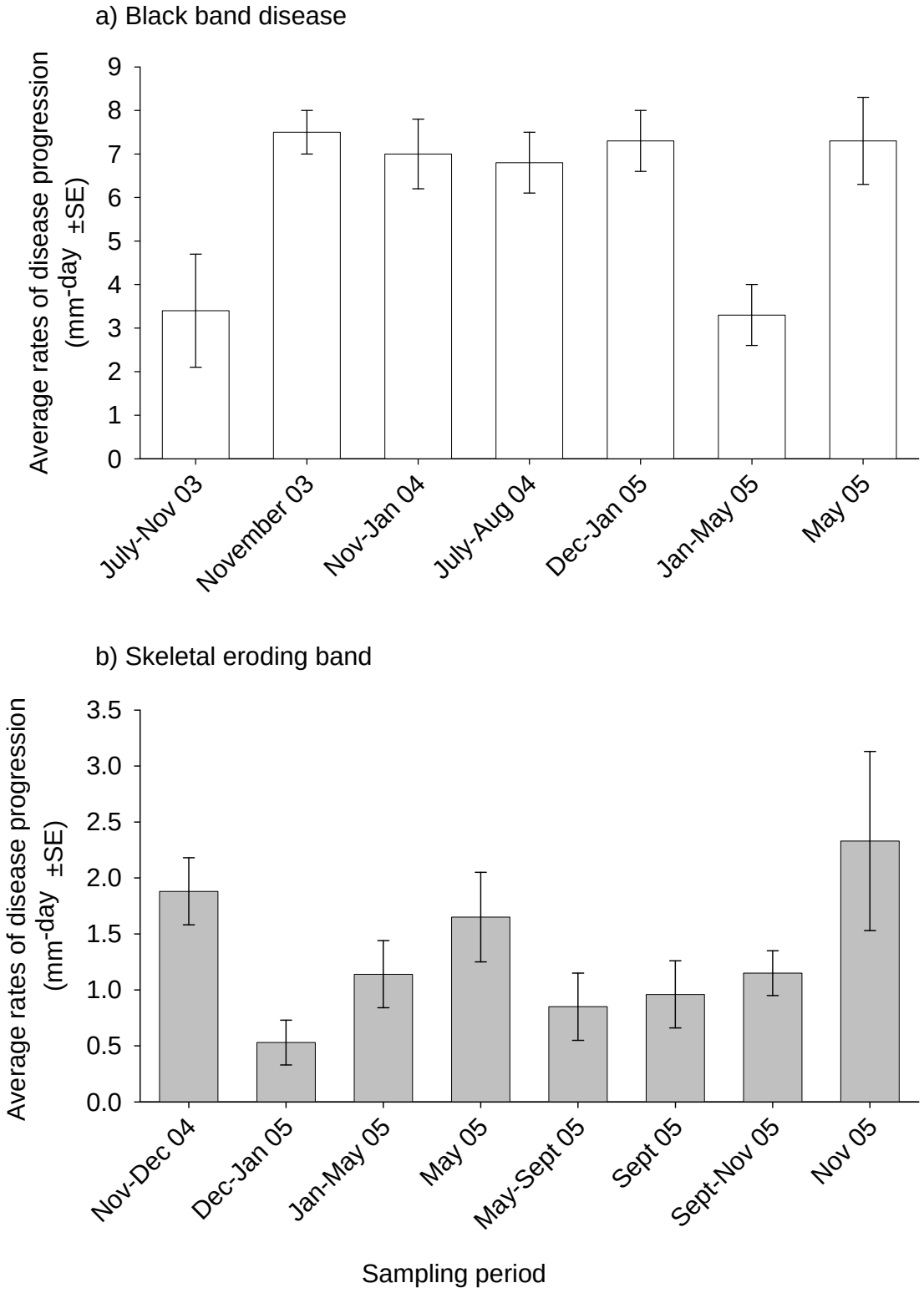
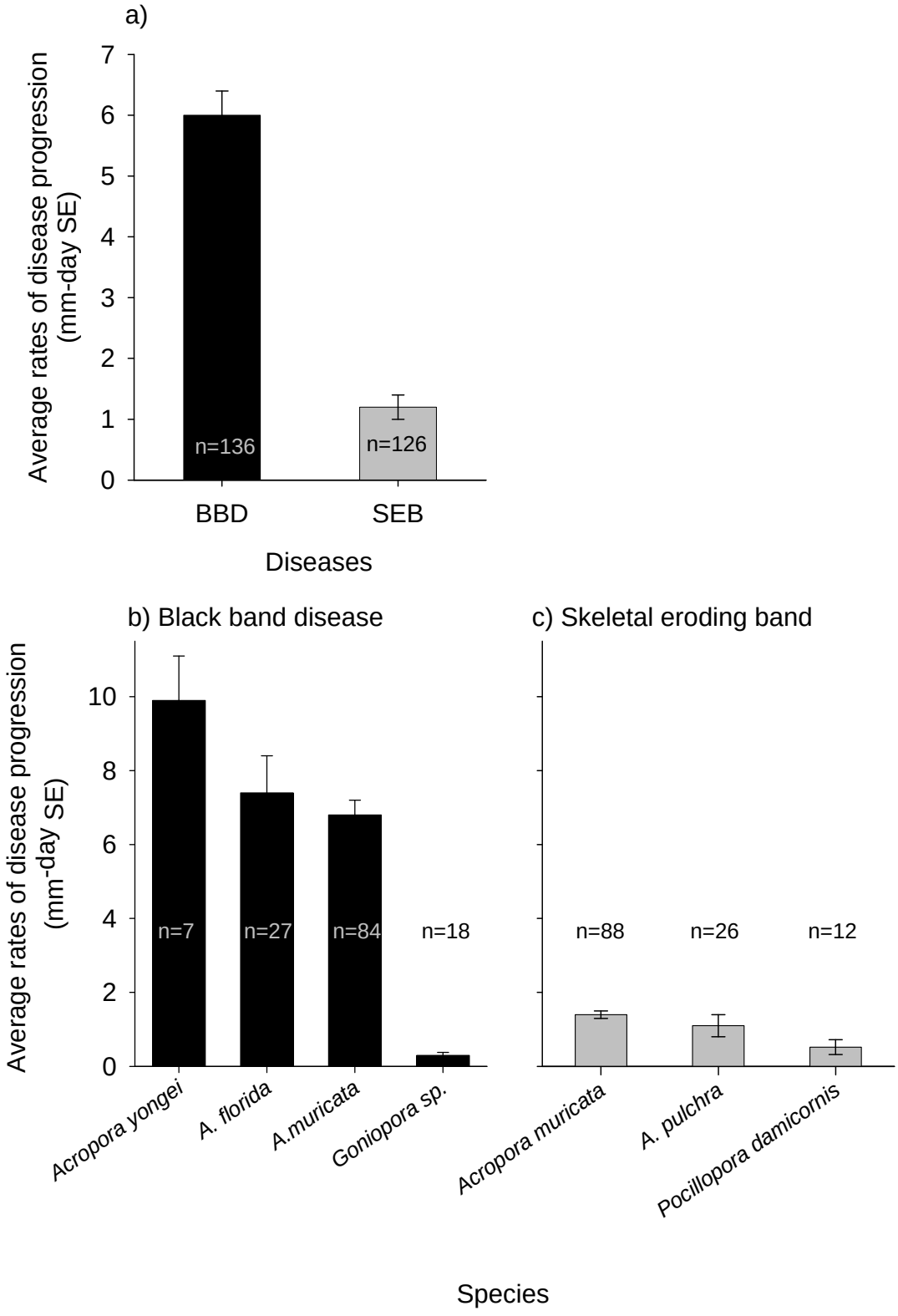


Figure 5.4. Average daily linear rates of tissue loss recorded for, a) black band disease (BBD) and skeletal eroding band (SEB) pooled for all species, and for colonies of, b) *Acropora yongei, A. florida, A. muricata* and *Goniopora* sp. with BBD, and c) *A. muricata, A. pulchra* and *Pocillopora damicornis* colonies with SEB, individually.



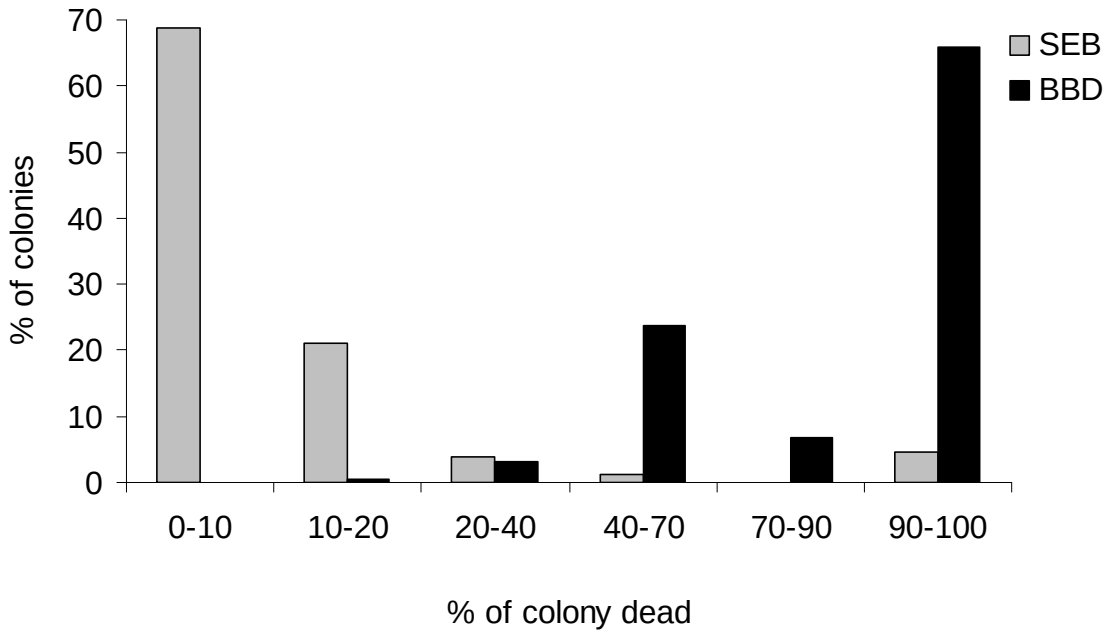
Partial colony mortality: Rates of partial colony mortality, measured as the percent of colony surface area lost in the three months after colonies were tagged, were over six-fold higher for BBD than for SEB. On average, colonies with BBD lost 85.7 ± 1.6 % of their tissue surface area, and over 60% of colonies with BBD lost over 90% of their surface area within three months (Fig 5.5). In contrast, colonies with SEB lost less than 15% of their surface area within three months on average (mean = 12.9 ± 1.7% of surface area), and only ~5% of colonies lost more than 90% of their tissue surface area (Fig 5.5).

The percentage of colonies recovering and appearing healthy at the end of this study was three-fold lower for colonies initially infected with BBD (9.5%) than for colonies initially infected with SEB (31.4%; Table 5.1). Surviving colonies that retained signs of disease at the last visit represented approximately 27% and 7% of the original tagged colonies with BBD and SEB infections, respectively. Less than 1% of colonies initially infected with BBD developed SEB during this study; however none showed signs of SEB on completion of this study. Less than 1% of colonies initially infected with SEB developed BBD, bleaching or had their basal tissues overgrown by algae on completion of this study.

Comparisons among species showed that recovery from BBD was over three-fold higher in *Goniopora* sp. (36%) than in any of the three acroporid species monitored (*A. muricata:* 5.8%, *A. yongei*: 12.5%, *A. florida*: 7.15%). The percentage of colonies recovering from SEB varied almost three-fold between species, with recovery being most likely in *A. pulchra* (65.2% of colonies recovering) and least likely in *A. muricata* (23% of colonies recovering). Half of all *P. damicornis* colonies recovered from their SEB infections and appeared healthy when last revisited. The percentage of colonies in

which signs of SEB persisted was similar for the three species examined (*A. muricata*: 27.4%, *A. pulchra*: 30.4% and *P. damicornis*: 25%).

Figure 5.5. Percentage of colonies with persistent skeletal eroding band (SEB) or black band disease (BBD) infections suffering categories of partial colony morality in the three-month period after colonies were tagged. Colonies healthy in appearance or with signs of other diseases three-months after being tagged were excluded from this figure.



5.4.2. Sub-lethal Impacts of Disease on Coral Growth

Skeletal eroding band: Average rates of colony growth were two-fold lower in colonies of *A. muricata* with SEB (0.32 ± 0.07 mm day^{-1}) than in healthy colonies (0.73 ± 0.09 mm day^{-1}) when growth was measured in late spring-summer (between November 2004 and January 2005) ($p > 0.05$; Table 5.2; Fig 5.6a). The number of newly initiated growing tips (incipient axial corallites) was also two-fold lower in colonies with SEB (3.4 ± 0.5 axials $colony^{-1}$ versus 7 ± 0.8 axials $colony^{-1}$ for healthy colonies; t-test: $t = -2.103$, $df = 39$, $p = 0.04$). Thus, lower rates of growth were correlated with lower numbers of incipient axials in colonies with SEB ($r^2 = 0.52$, $df = 39$, $p < 0.001$). There was no significant difference in either the height or surface area of colonies with SEB versus healthy colonies ($t\text{-test}_{(\text{Colony height})}$: $t = 0.57$, $df = 39$, $p = 0.56$; $t\text{-test}_{(\text{Surface area})}$: $t = 0.729$, $df = 39$, $p = 0.47$), indicating that differences in growth rates between these two health states were not a consequence of differences in colony sizes.

In contrast, when growth rates were measured in spring 2005 (between September and November 2005), they did not differ significantly between healthy (0.77 ± 0.09 mm day^{-1}) and SEB infected colonies of *A. muricata* (0.76 ± 0.08 mm day^{-1}; $p > 0.05$; Table 5.2; Fig 5.6b). Again, no differences in the number of incipient axials, surface area or colony height were found between the healthy versus SEB affected groups of colonies ($t\text{-test}_{(\text{Axials per colony}}$: $t = -0.35$, $df = 12$, $p = 0.7$; $t\text{-test}_{(\text{Surface area})}$: $t = -0.8$, $df = 23$, $p = 0.4$; t-test: $t = 1.2$ $df = 32$, $p = 0.2$; $t\text{-test}_{(\text{Colony height})}$: $t = -0.53$, $df = 23$, $p = 0.6$).

Black band disease: Rates of colony growth were not influenced by the presence of BBD infections, when growth was measured over a 2 month period between September and November in 2005, as demonstrated by the similar growth rates for *A. muricata* with (0.64 ± 0.14 mm day^{-1}) and without BBD (0.77 ± 0.09 mm day^{-1}; $p > 0.05$; Table 5.2; Fig 5.6b). Growth rates could not be compared over other sampling intervals because the majority of tagged colonies died from the disease.

Growth anomalies: The presence of growth anomalies had no effect on growth rates of acroporid corals, as evidenced by the lack of significant difference among rates of linear extension for colonies of *A. intermedia* and *A. muricata* with and without growth anomalies (*A. intermedia:* $ANCOVA_{(Health)}$: $p = 0.89$, *A. muricata:* $ANCOVA_{(Health)}$: $p = 0.46$; Table 5.2; Fig 5.7). Similarly, the number of incipient axials did not differ between colonies with and without growth anomalies in either *A. muricata* or *A. intermedia* (*A. muricata* t-test: $t = -0.448$, $df = 36$, $p = 0.65$; *A. intermedia* t-test: $t = -0.123$, $df = 35$, $p = 0.9$). Both the height and surface area of *A. muricata* colonies with and without growth anomalies were similar ($t\text{-test}_{(Colony\ height)}$: $t = -0.98$, $df = 36$, $p = 0.33$; $t\text{-test}_{(Surface\ area)}$: $t = -0.29$, $df = 36$, $p = 0.77$), confirming that the two groups of colonies were equivalent in measures of colony size. In contrast, *A. intermedia* colonies with growth anomalies were, on average, ~31% larger in surface area (183.37 ± 14.9 cm^2) than healthy colonies of this species (139.55 ± 14 cm^2; t-test: $t = 2.139$, $df = 35$, $p = 0.04$), although the heights of the two groups did not differ significantly (t-test: $t = 0.4$, $df = 35$, $p = 0.36$).

Figure 5.6. Rates of total daily linear skeletal growth (mm^{-day}) recorded from colonies of *Acropora muricata* with skeletal eroding band (SEB), black band disease (BBD) or healthy in appearance in a) late spring-summer (24th November 2004 and the 10th January 2005), and b) spring (9th of September and the 4th of November 2005).

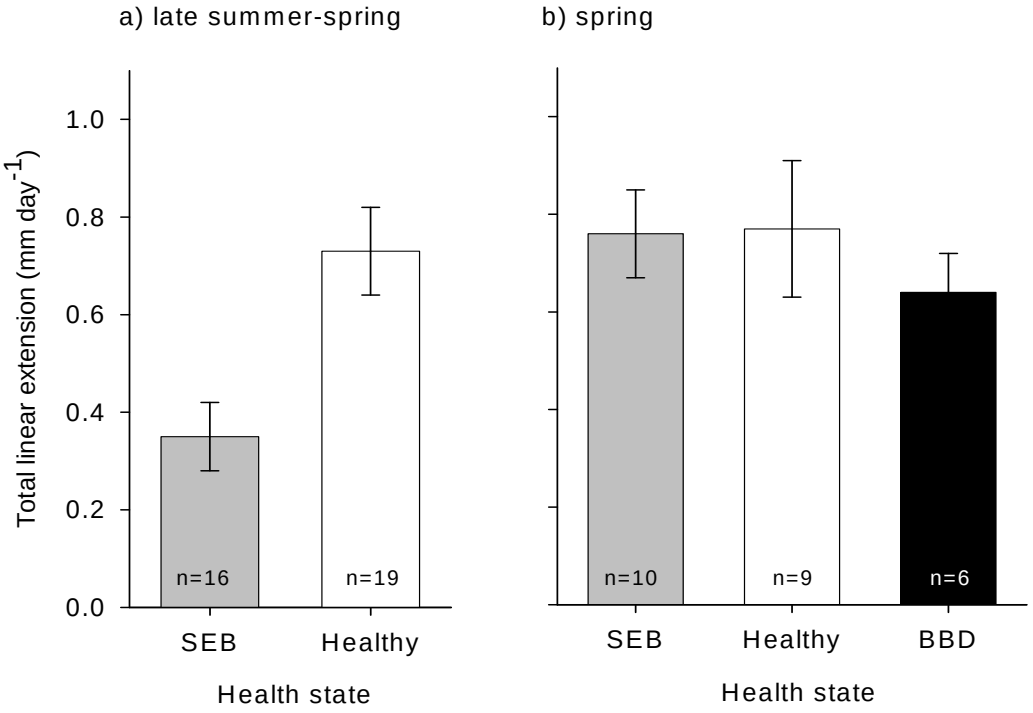


Figure 5.7. Rates of total daily linear skeletal growth (mm^{-day}) measured from a) *Acropora intermedia* colonies with and without growth anomalies at Lizard Island in winter-spring (7th of May to the 1st of November 2005), and b) *Acropora muricata* colonies with and without growth anomalies at North Direction Island in spring (10th September to the 2nd of November 2005).

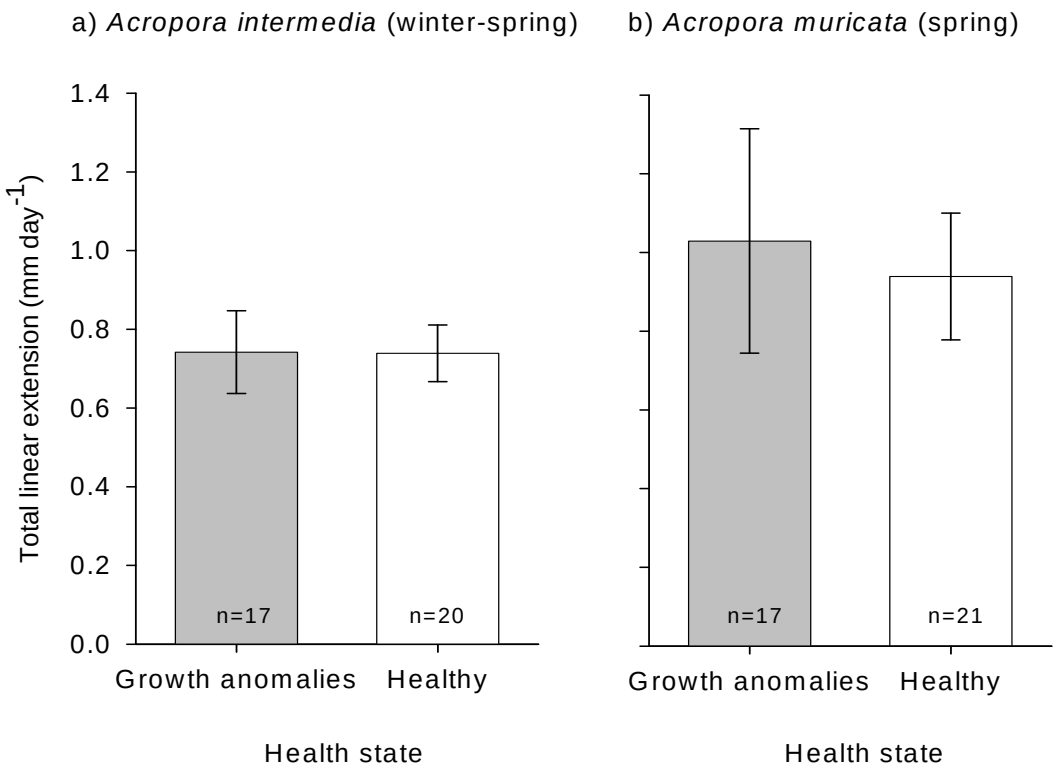


Table 5.2. Univariate analyses of covariance (ANCOVA) testing for differences in linear growth rates among *Acropora muricata* colonies with signs of either skeletal eroding band (SEB), black band disease (BBD) or growth anomalies, and colonies healthy in appearance, where colony surface area is the covariate. * denotes significance at $\alpha = 0.05$.

Disease compared with healthy colonies	Species	Dates between which growth was measured	Source	Type III SS	df	MS	F	p
SEB	*A. muricata*	24th Nov 04-10th Jan 05	Health	1.516	1	1.515	10.088	0.003*
			Colony Size (mm^2)	0.150	1	0.151	1.003	0.323
			Error	4.959	33	0.150		
SEB	*A .muricata*	9th Sept – 4th Nov 05	Health	0.00037	1	0.00037	0.0007	0.977
			Colony Size (mm^2)	0.001	1	0.001	0.002	0.096
			Error	7.59	16	0.47		
BBD	*A. muricata*	9th Sept – 4th Nov 05	Health	0.007	1	0.007	0.014	0.90
			Colony Size (mm^2)	0.55	1	0.55	1.117	0.31
			Error	5.98	12	0.49		
Growth anomalies	*A.intermedia*	7th May – 1st Nov 05	Health	0.0025	1	0.0025	0.017	0.896
			Colony Size (mm^2)	0.0163	1	0.0163	0.111	0.741
			Error	5.0078	34	0.1472		
	A. muricata	10th Sept – 2nd Nov 05	Health	0.2978	1	0.2978	0.5437	0.465
			Colony Size (mm^2)	1.0837	1	1.0837	1.978	0.168
			Error	19.1713	35	0.5477		

5.4.3. Sub-lethal Impacts of Disease on Reproduction

Skeletal eroding band: Based on collections from randomly selected colonies, the percentage of *A. muricata* with oocytes present in the week prior to spawning was similar between colonies with SEB (85%) and those that were healthy in appearance (80%) (χ^2 = 0.672, df = 1, p = 0.4; Fig 5.8a). However, two other measures of reproductive output, the average percentage of polyps containing oocytes and the average number of oocytes produced per polyp, were both lower in colonies with SEB than in healthy colonies. Specifically, in colonies that were reproductive, the average percentage of polyps containing oocytes was ~30% lower in colonies with SEB (68 ± 11.5 of polyps) than in healthy colonies (94.4 ± 3.9% of polyps)(t-test: t = -2.57, df = 24, p = 0.01; Fig 5.9a). Polyp fecundity was also 13% lower in colonies with SEB (5.1 ± 0.1 oocytes polyp^{-1}) than in healthy colonies (5.8 ± 0.2 oocytes polyp^{-1}), though this difference was not statistically significant (p > 0.05; Table 5.3; Fig 5.10a). Significant variation in the number of oocytes produced per polyp between colonies within each health category is likely to have contributed to the absence of overall differences in oocyte numbers between randomly selected diseased and healthy colonies (Table 5.3).

In tagged colonies of *A. muricata,* none of the reproductive parameters measured differed significantly between healthy colonies and those infected with SEB. One half of both healthy and SEB affected colonies contained oocytes (Fig 5.8b). When colonies were reproductive, approximately half of all polyps contained oocytes in both healthy and diseased corals (i.e. 55.2 ± 7.8% of polyps in healthy colonies and 52.5 ± 10% of polyps in colonies with SEB) (t-test: t = -0.367, df = 177, p = 0.7; Fig 5.9b). Finally, polyp fecundity was similar between healthy and diseased corals (4.5 ± 0.09 oocytes polyp^{-1} in healthy colonies versus 4.8 ± 0.1 oocytes polyp^{-1} for infected colonies) (p > 0.05; Fig 5.10b; Table 5.3).

Black band disease: BBD did not significantly impact on reproductive parameters measured for the group of randomly selected colonies of *A. muricata*, though it is likely that they had not been infected for more than three months since none of the tagged colonies survived to reproduce. First, oocytes were present in a statistically similar proportion of healthy (80%) and BBD infected (95%) colonies ($\chi^2 = 2.05$, df = 1, p = 0.15; Fig 5.8a). Secondly, the average percentage of polyps containing oocytes was 10% lower in colonies with BBD (84.2 ± 5.3 % of polyps) than in healthy colonies (94.4 ± 3.9% of polyps), but this difference was not statistically significant (t-test: t= -1.597, df = 26, p = 0.12; Fig 5.9a). Finally, the average number of oocytes produced per polyp did not differ between *A. muricata* colonies with BBD (5.9 ± 0.1 oocytes polyp^{-1}) and healthy colonies (5.8 ± 0.2 oocytes polyp^{-1}; $p < 0.05$; Table 5.4; Fig 5.10a).

Growth anomalies: Oocytes were absent from tumorous tissues of both *A. muricata* and *A. intermedia*. Moreover, oocytes were also absent from healthy tissues adjacent to growth anomalies on colonies of *A. muricata* (n = 19; Fig 5.11a). In contrast, mature pink oocytes indicating imminent spawning were present in ~43% of healthy colonies of *A. muricata* (n = 21; Fig 5.11a). The absence of oocytes from ~50% of healthy colonies of *A. muricata is* consistent with environmental conditions being unsuitable for successful gametogenesis by these colonies or alternatively that spawning can occur over 3 months in this species (B. Willis pers. comm.). Oocytes were absent from all colonies of *A. intermedia,* including both those with (n = 25) and without (n = 20) growth anomalies (Fig 5.11b), excluding one colony. This colony had six growth anomalies, which were up to 28mm in diameter, the maximum number recorded from any one colony of this species in this study, nevertheless it contained oocytes in healthy tissues adjacent to growth anomalies. The absence of oocytes from all but one colony of

A. intermedia is consistent with observations that spawning can occur over 3 months in *A. intermedia* (B. Willis pers. comm.). Alternatively, it is possible that the environment in which these colonies were located was not suitable for the successful production of oocytes.

Figure 5.8. The percentage of, a) randomly selected colonies of *Acropora muricata* with skeletal eroding band (SEB), black band disease (BBD) or healthy in appearance, and b) tagged colonies of *Acropora muricata* with SEB and healthy in appearance, in which oocytes were present in the week prior to spawning in November 2005.

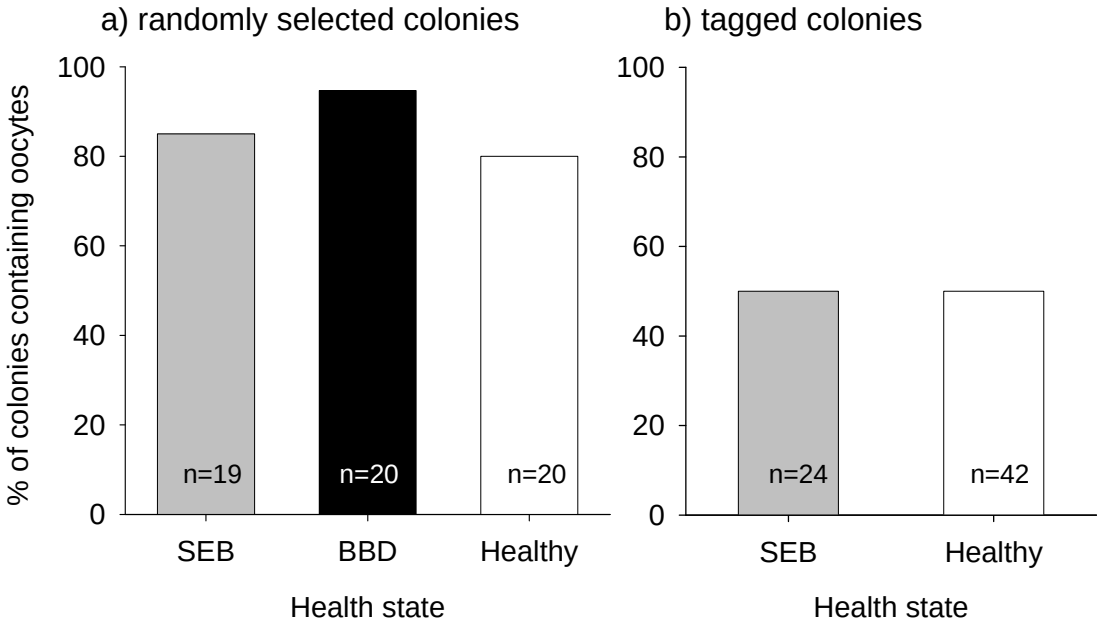


Figure 5.9. The mean percentage (±SE) of polyps containing oocytes in, a) randomly selected colonies of *Acropora muricata* with skeletal eroding band (SEB), black band disease (BBD) and healthy in appearance, and b) tagged colonies of *Acropora muricata* with SEB and healthy in appearance, in the week prior to spawning in November 2005.

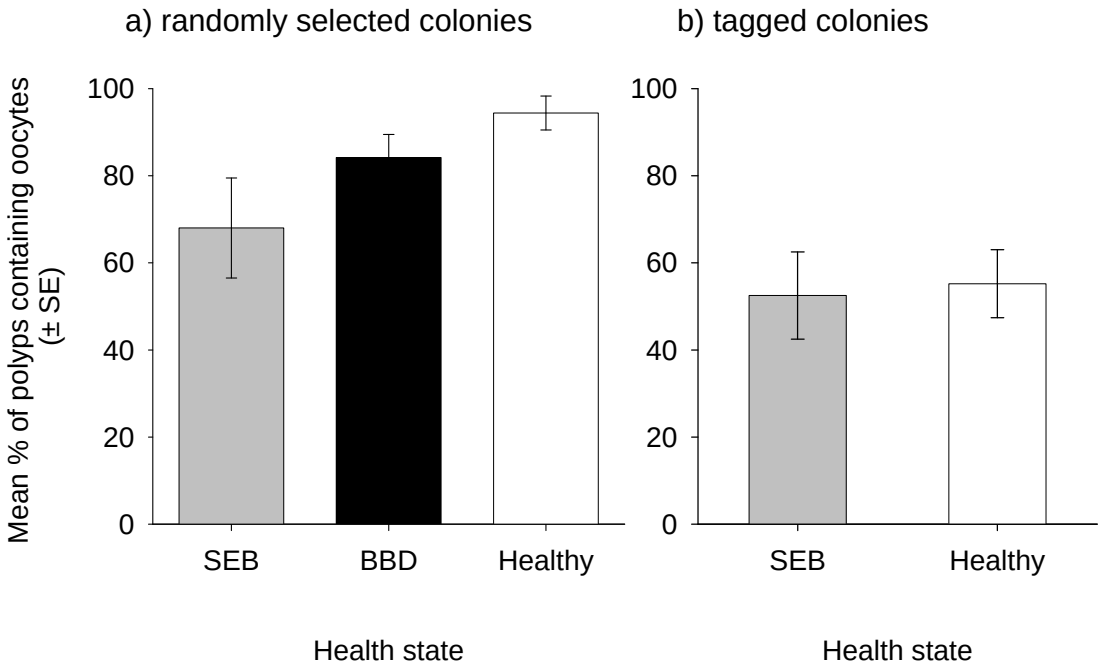


Figure 5.10. The mean number of oocytes produced per polyp (±SE) in, a) randomly selected *Acropora muricata* colonies with skeletal eroding band (SEB), black band disease (BBD) or healthy in appearance, and b) tagged *Acropora muricata* colonies with SEB or healthy in appearance, in the week prior to spawning in November 2005.

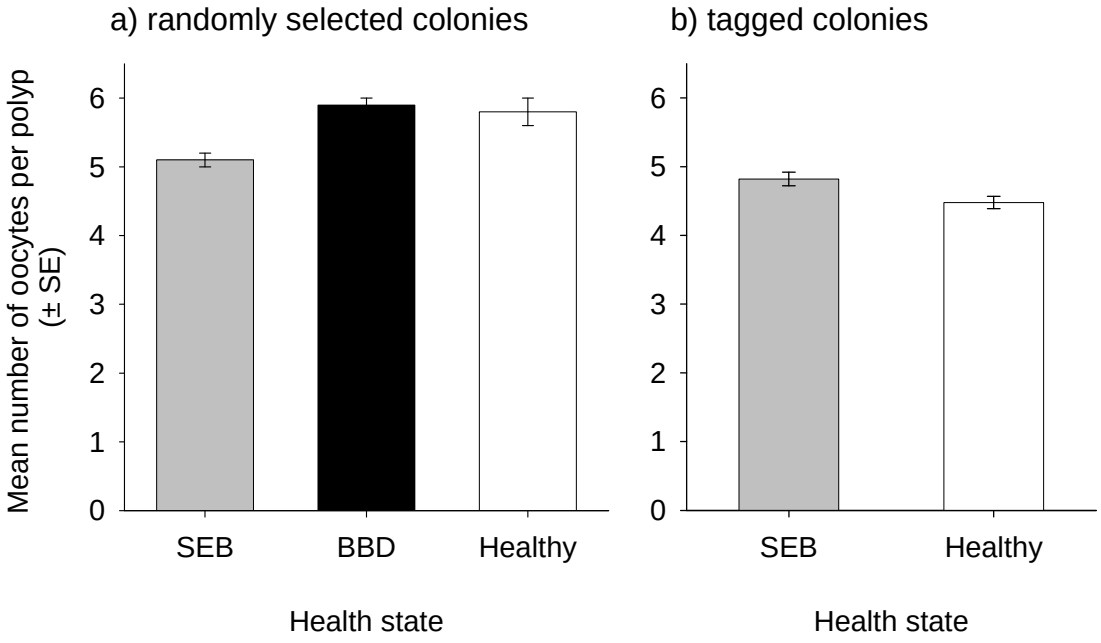


Table 5.3. Univariate analyses of variance (ANOVA) testing for differences in the number of oocytes produced per polyp in randomly selected and tagged colonies of Acropora muricata infected with skeletal eroding band and healthy in appearance. * denotes significance at $\alpha = 0.05$. Lower case letters indicate error terms against which tests were performed. Data were square root transformed to correct for heterogeneity of variances.

Colonies	Source of variation	Hypothesis or error	Type III SS	df	MS	F	p
Randomly selected	Health state	Hypothesis	1.382	1	1.382	2.981	0.099
	Colony(Health state)	Hypothesis	9.735	21	0.464a	5.897	<0.001*
		Error	16.273	207	0.079b		
Tagged	Health state	Hypothesis	0.114	1	0.114	0.418	0.524
	Colony(Health state)	Hypothesis	7.818	24	0.352a	5.779	<0.001*
		Error	10.752	191	0.056b		

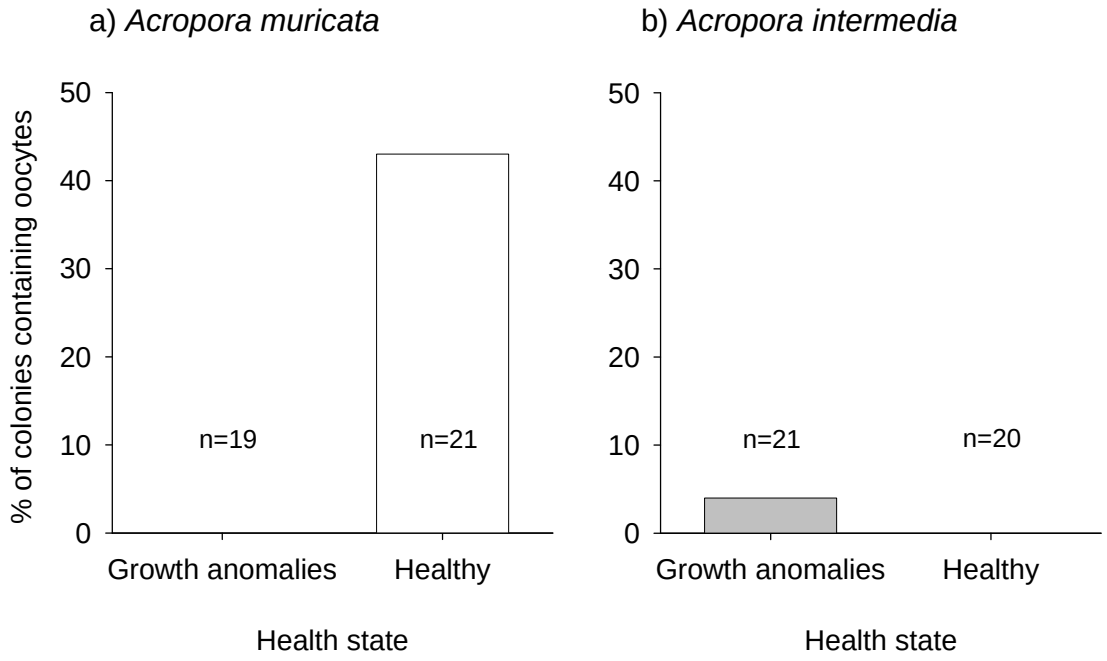
a = MS(Colony(Health state)
b = MS(Error)

Table 5.4. Univariate analyses of variance (ANOVA) testing for differences in the number of oocytes produced per polyp in randomly selected colonies of *Acropora muricata* infected with black band disease and colonies healthy in appearance. * denotes significant variation at α=0.05. Data were square root transformed to correct for heterogeneity of variances. Lower case letters indicate error terms against which tests were performed.

Source of variation	Hypothesis or Error	Type III SS	df	MS	F	p
Health state	Hypothesis	0.931	1	0.921	2.428	0.138
	Error	6.518	17	0.383a		
Colony(Health state)	Hypothesis	0.6518	17	0.383	4.227	<0.001*
	Error	15.512	171	0.91b		

a = MS(Colony(Health state)
b = MS(Error)

Figure 5.11. The percentage of, a) *Acropora muricata* and b) *Acropora intermedia* colonies with and without growth anomalies, containing oocytes in healthy tissues in the week prior to spawning in November 2005. Oocytes were absent from all tumorous tissues in both of species and are therefore not included in this figure.



5.5. DISCUSSION

Here I document chronic and acute impacts of three of the most common coral diseases on the GBR: black band disease (BBD), skeletal eroding band disease (SEB) and growth anomalies (GAs). Although the prevalence of each of these diseases is typically low, each affecting < 2% of corals, increases in the abundance of SEB and white syndrome in the last 10-15 years (Antonius and Lipscomb 2001; Bruno et al. 2007), combined with trends for increasing disease with ocean warming (Bruno et al. 2007) and water quality deterioration (Kuta and Richardson 2002; Bruno et al. 2003), suggest further increases in disease prevalence are likely in the future. Hence an understanding of population level impacts of these diseases is paramount.

5.5.1. Acute Impacts of Disease

Black band disease: This study demonstrates that BBD can cause significant partial mortality in northern GBR populations of three branching species of *Acropora* and one massive species of *Goniopora.* The greater than 85% mean loss of tissue within three months found for the combined group of study species is greater than the highest rates of tissue loss reported for BBD on Caribbean reefs and nearly double some of the rates recorded over four years on Jamaican reefs (see Table 5.5 for comparative rates and references). Rates of partial colony mortality recorded for BBD in northern GBR populations are also greater than those recorded for many other Caribbean and Indo-Pacific diseases, such as white band disease, white plague and yellow band disease (Bruckner and Bruckner 2006) in the Caribbean, and atramentous necrosis and *Porites* ulcerative white spot in the Indo-Pacific (see Table 5.5 for comparative rates and references).

Table 5.5. Rates of progression of disease fronts, rates of partial and whole colony mortality recorded for common coral diseases affecting scleractinian corals on Indo-Pacific and Caribbean Reefs.

Disease	Rate of progression (average or range of rates)	Rate of partial mortality	Rate of whole colony mortality	Reference
Black band	GBR: 4.1-9.9 mm $day^{-1;}$ Caribbean: 0.3-10mm day^{-1}	Virgin Islands: 75% in half of colonies (6 months); Jamaica: 49-68% (4 years)	Virgin Islands: 33% (7 months): Jamaica: 48% (4 years); Florida Keys: 0% (2 years); Venezuela: 0% (1 year)	(Antonius 1981a; Rutzler et al. 1983; Edmunds 1991; Bruckner and Bruckner 1997b; Weil 2004; Borger 2005; Boyett et al. 2007; Rodríguez and Cróquer 2008)
White syndrome (Indo-Pacific only)	Solitary Islands: 0.39 -5.2mm day^{-1}; Southern GBR: 0.52mm^2 day^{-1}	NA	NA	(Dalton and Godwin 2006; Roff et al. 2006)
White band disease	0.8-40mm day^{-1}	88% (20 months)	Virgin Islands: 96% (3 months); Florida Keys: 28% (20-months)	(Antonius 1981a; Gladfelter 1982; Peters et al. 1983; Ritchie and Smith 1997; Williams and Miller 2005)
White plague	<0.2-30mm day^{-1}	Puerto Rico: 60-100% (1 year?)	Florida keys: 26% (11 weeks)	(Dustan 1977; Bruckner and Bruckner 1997a; Richardson et al. 1998; Nugues 2002; Weil 2004)
White pox	2.5cm^2 day^{-1}	NA	NA	(Patterson et al. 2002)
Yellow band	0.1- 0.33mm day^{-1}	32% (4 years)	8% (4 years)	(Cervino et al. 2001; Borger and Steiner 2005; Bruckner and Bruckner 2006)
Dark spot disease	0.09-0.25 mm day^{-1}	NA	NA	(Cervino et al. 2001; Borger 2004)
Caribbean folliculinid infections	~0.3 mm day^{-1}	NA	NA	(Rodríguez et al. 2008)
Atramentous necrosis	NA	30-60% (19 months)	4% (3 months)	(Jones et al. 2004)
Porites ulcerative white spot disease	NA	70-90% in ~half of colonies (17 months)	8% (17 months)	(Raymundo et al. 2003)

Rates of whole colony mortality caused by BBD were also high in northern GBR populations (e.g. 66% of *Acropora* and *Goniopora* colonies died within three months) and much greater than rates of whole colony mortality recorded for BBD on Caribbean reefs (Table 5.5). Differences in colony morphology of coral species susceptible to BBD in the two reef regions partially explain differences in the severity and rates of partial and whole colony mortality. On the GBR, BBD predominately affects branching acroporid species, which comprised 90% of colonies in this study, whereas hemispherical species, including *Diploria, Colpophyllia, Dichocoenia* and *Montastraea,* are most susceptible to BBD on Caribbean reefs (Edmunds 1991; Bruckner et al. 1997; Borger 2005), with only one BBD case ever documented on a Caribbean acroporid colony (Garzón-Ferreira et al. 2001). Greater allocation of resources to growth in branching acroporid species may result in less energy available for investment in disease resistance or maintenance of colony boundaries at lesion sites in comparison to hemispherical corals (Tunnicliffe 1981). This interpretation is supported by the three-fold greater rates of recovery from BBD infections found for *Goniopora* sp. in this study (i.e. 36.4% of *Goniopora* versus a maximum of 12.5% of *Acropora yongei* colonies recovered within three months).

The high rates of partial and whole colony mortality described above, reflect the relatively rapid rates of BBD progression found in the four study populations (i.e. 6.1 ± 0.4 mm day^{-1}; see also Boyett et al. 2007; Table 5.5). This mean rate of BBD progression is within the range of rates recorded in numerous studies of BBD in Caribbean coral populations (summarised in Weil 2004), but greater than rates of disease progression recorded for other coral diseases, such as white syndrome, white plague I and yellow band disease in a diverse range of geographic locations (Table 5.5). However, even the maximum rates of BBD progression recorded in the current study

(i.e. 9.9 mm day^{-1} on *A. yongeii*) are at the lower end of the range of progression rates recorded for the more virulent white diseases affecting Caribbean corals, such as white band disease and white plague II (see Table 5.5 for comparative rates). Nevertheless, even the slowest rate of BBD progression reported here (i.e. 0.3 ± 0.08 mm day^{-1}on *Goniopora*) reduced colony surface areas by ~43% on average over three months, highlighting the significant impact BBD can have on the fitness of Indo-Pacific coral populations.

Skeletal eroding band: This study is the first from any reef region to demonstrate that SEB can cause significant partial mortality. However, the six-fold lower mean rate of tissue loss caused by SEB for the combined group of study species on northern GBR reefs (12.9 ± 1.7 % tissue loss within three months for two branching species of *Acropora* and *Pocillopora damicornis* pooled) in comparison to BBD (85.7 ± 1.6 % tissue loss within three months), indicates that acute effects of this disease are unlikely to pose as great a threat to Indo-Pacific reefs as BBD. Comparisons of rates of partial mortality recorded for SEB and other diseases are made difficult by variation in the time-periods over which rates were recorded (8-48 months; Table 5.5). Nonetheless, rates of partial colony mortality recorded here for SEB are likely to fall within the range of values recorded for slow moving diseases such as atramentous necrosis on reefs in the central GBR (Jones et al. 2004) and yellow band disease on Caribbean reefs (Bruckner and Bruckner 2006) (see Table 5.5 for comparative rates). Similarly, rates of whole colony mortality caused by SEB (e.g. 39% of *Acropora* and *Pocillopora* colonies died within two years) were two-fold lower than those recorded for BBD in northern GBR populations, and much lower than rates recorded for some of the more virulent Caribbean diseases, such as white band disease and white plague (see Table 5.5 for

comparative rates). Nevertheless, in reef regions with high prevalence of SEB, such as the Red Sea where SEB affects at least 50% of the most susceptible genera (Winkler et al. 2004), SEB infections may cause significant mortality in coral populations.

Rates of progression of SEB disease fronts were five- to seven-fold slower than rates recorded for BBD in northern GBR corals (1.26 ± 0.2 mm day^{-1} versus 6 to 9.9 mm day^{-1}; see also Boyett et al. 2007) and were also much slower than rates recorded for a variety of white diseases on the GBR and on Caribbean reefs (see Table 5.5 for comparative rates). Differences in the coral species infected by *Halofolliculina* spp. on the GBR and in the Caribbean may partially explain higher rates of tissue loss associated with folliculinid infections on the GBR (average rate: 1.26mm day^{-1}) compared to the Caribbean (~0.3mm day^{-1} Rodríguez et al. 2008). Prioritisation of energetic resources for growth by fast growing branching acroporid and pocilloporid species may result in less energy being available for investment in disease resistance, in comparison to the slower growing agaricid corals that are typically affected by folliculinid infections in the Caribbean. Alternatively, the *Halofolliculina* sp. infecting Indo-Pacific corals may be more virulent than the Caribbean *Halofolliculina* sp.. Morphometric and molecular comparisons of ciliates from these two regions are required to confirm that they are in fact different species, before further comparisons of their impacts can be made.

Growth anomalies: This study demonstrates that, unlike BBD and SEB, growth anomalies (GAs) do not cause significant partial or whole colony mortality in northern GBR populations of branching *Acropora* species. Nonetheless, GAs may increase the susceptibility of colonies to other factors that cause coral mortality. For example, the likelihood of mortality of small colonies of *Montipora informis* following a bleaching

event was higher for colonies with GAs than for those without them on Japanese reefs (Yamashiro et al. 2000). Given that bleaching may increase the likelihood of the development of GAs (McClanahan et al. 2009), but also the possibility that GAs may increase the vulnerability of corals to disturbances like bleaching events, they represent an additional factor compromising the health and fitness of Indo-Pacific coral populations.

In summary, combined partial and whole colony mortality caused by BBD was at least two-fold higher than mortality caused by SEB in northern GBR coral populations (97% compared to 47% loss of live surface area within approximately two years for BBD and SEB, respectively). These figures are likely to underestimate mortality caused by disease in these populations since rates of partial mortality were only measured over three months. In contrast, GAs had no acute impacts on the three northern GBR populations. The higher rate of recovery of *Goniopora* sp. following BBD infections, in comparison to the three species of *Acropora,* may be attributable to differential allocation of energy to disease resistance mechanisms versus growth for corals with massive versus branching morphologies. This study provides baseline data on the comparative contributions of three common coral diseases to current levels of partial and whole colony mortality in Indo-Pacific coral populations. It also highlights the importance of recording acute disease impacts over time frames sufficient to document rates of whole colony mortality and colony recovery.

5.5.2. Sub-lethal Impacts of Disease

Determining sub-lethal impacts of coral diseases provides insights into resource allocation strategies in corals and increases understanding about how diseases affect the fitness of coral populations. As in any organism, energetic resources available to corals are finite and must be distributed among competing physiological functions, typically through physiological trade-offs involving maintenance, growth and reproduction (Soong and Lang 1992; Stearns 1992). Significant tissue loss in diseased colonies reduces available energetic resources and, in combination with the need for increased investment in disease resistance and lesion repair, means that there is little surplus energy available for investment in growth or reproduction (Soong and Lang 1992; Rinkevich 1996; Kramarsky-Winter 2004). Accordingly, resources that might otherwise be allocated to processes of growth and reproduction are likely to be traded off to repair processes to ensure survival of corals. The use of amoebocytes derived from stem cells for lesion regeneration (Meesters et al. 1997; Oren et al. 1997; Vargas-Ángel et al. 2009; Mydlarz et al. 2009) may also limit the availability of stem cells for production of germ cells in diseased corals. Despite the likelihood of sub-lethal impacts of diseases on coral growth and reproduction, only a few studies have examined the effects of common diseases on coral growth (Cheney 1975; Bak 1983; Aeby 1991) and reproduction (Hunter 1997; Yamashiro et al. 2000; Petes et al. 2003).

Black band disease: The lack of difference in growth rates between colonies of *Acropora muricata* with and without BBD, even after diseased colonies had lost up to 90% of tissue surface area, suggests that this species does not vary its strategy for allocation of resources to growth versus disease resistance mechanisms, at least in response to rapidly progressing BBD infections. However, small sample sizes may have

limited the capacity to detect impacts of BBD infections on coral growth in this study. The lack of difference in polyp fecundity found between colonies of *A. muricata* with and without BBD is also consistent with a fairly rigid energy allocation strategy. However, because data were only collected from colonies that had been infected in the last 2 months of gametogenesis, it is also likely that there were limited options for diverting resources from reproduction to disease resistance mechanisms since oocytes were nearing maturation. Palmer et al. (2008), which found that acroporids produce lower levels of constituents involved in innate immunity in response to wounding than the more disease resistant coral *Porites,* provides corroborative evidence that *Acropora* species may divert comparatively few resources to mechanisms of disease resistance. Alternatively, BBD may interrupt or impair energetic and cellular trade off mechanisms that enable the diversion of resources from growth and reproduction to lesion repair and disease resistance. Thus the impacts of BBD on the fitness of northern GBR populations of both *Acropora* and *Goniopora* were caused primarily by high rates of partial and whole colony mortality, which dramatically reduced the number of polyps available for reproduction, but did not reduce rates of colony growth or the per polyp fecundity of colonies that survived BBD infections.

Skeletal eroding band: This study demonstrates the significant impact SEB infections can have on the growth of *Acropora muricata* colonies. Two-fold lower rates of growth and numbers of newly initiated growing tips in colonies with SEB, compared to colonies without SEB in late spring-summer (November 2004 to January 2005), suggest that SEB infections reduce the availability of energy for allocation to growth in *A. muricata*. It is possible that mobilization of amoebocytes as part of a generalised disease resistance mechanism in this species may limit the availability of stem cells, which are

derived from amoebocytes in the mesoglea of anthozoans (Young 1974; Fadlallah 1983), and hence the availability of material for the budding of new polyps leading to colony growth. However, SEB infections did not reduce rates of growth and numbers of newly initiated branches in colonies of *A. muricata* in spring 2005 (September to November 2005). Differences in the impacts of SEB infections on rates of growth between the two sampling periods, could reflect seasonal variability in the availability of either energetic surpluses for physiological processes not directly linked to colony survival (Fitt et al. 2000) or stem cells. Variation in the consequences of SEB infections for the growth of *A. muricata* colonies could also reflect differences in the density and/or duration of *Halofolliculina* infestations during these two sampling periods. The influence of infection severity and duration could not be examined in this study, since ciliate densities were not quantified and because it is not known how long colonies were infected with SEB before they were tagged. However, the potential for SEB to halve rates of growth of *A. muricata* colonies has serious implications for the fitness of Indo-Pacific coral populations and the continuing capacity of branching *Acropora* spp. to dominate coral assemblages on Indo-Pacific reefs.

In contrast to BBD, SEB infections were found to compromise energy available for investment in gamete production. The absence of oocytes from 30% of polyps and the 13% fewer oocytes produced by fertile polyps in randomly selected, diseased colonies of *A. muricata* (compared to healthy controls) demonstrates that this species prioritises survival at the expense of reproduction in the presence of SEB infections. The chronic nature of SEB infections compared to the acute nature of BBD may account for differences in the impact of these two diseases on the reproduction of *A. muricata*. Reproduction in corals is sensitive to stressors that reduce energy available for processes not directly involved in survival over extended periods of time (Jokiel and

Guinther 1978; Rinkevich and Loya 1989). Large reductions in the reproductive output of gorgonians suffering long-term infections of the fungus *Aspergillus sydowii* also support the hypothesis that energetic resources available for investment in gametogenesis are limited in chronically diseased corals (Petes et al. 2003). Lack of reductions in measures of reproductive output in the tagged group of diseased *A. muricata* may reflect variation in the severity and/or duration of SEB infections in comparison to the random set of diseased corals collected just prior to spawning. Given the possibility that *Halofolliculina* infections affecting Caribbean corals could have similar consequences for the fitness of susceptible populations, studies of the chronic impacts of *Halofolliculina* infections on Caribbean corals are needed. The potential for SEB to reduce both the fecundity of surviving polyps and rates of growth of acroporid colonies has significant fitness consequences for these populations, and limits their resilience and capacity to recover from SEB epizootics.

Growth anomalies: The similar growth rates found for colonies of *Acropora muricata* and *A. intermedia* with and without growth anomalies (GAs) are in contrast to previous studies, which found that the presence of GAs significantly suppressed the growth of *Acropora* spp. on both Caribbean and Indo-Pacific reefs (Cheney 1975; Bak 1983). Seasonal variation in energy available for growth is unlikely to be the cause of this discrepancy, since previous studies documented the suppression of *Acropora* growth rates in the presence of GAs in both summer and winter (Cheney 1975; Bak 1983). It is possible that growth anomalies in the present study were not as well developed or as numerous as those in the other two studies. Alternatively, the position of most growth anomalies at some distance from branch tips in the present study may have reduced their impact on growth as measured by the extension of branch tips. Further studies that link

the growth of branch tips with their proximity to growth anomalies are needed to distinguish these alternative explanations. Overall, however, my results indicate that the impact of growth anomalies on coral growth is not of primary concern for northern GBR populations of *A. muricata* and *A. intermedia.*

The absence of oocytes from tumorous tissues of both *A. muricata* and *A. intermedia* colonies may indicate the absence of functional mesenteries in tissues associated with GAs, as documented in previous studies (Coles and Seapy 1998; Domart-Coulon et al. 2006). The absence of oocytes from healthy tissues adjacent to GAs in all *A. muricata* colonies, but from only 43% of healthy *A. muricata* colonies, suggests that the allocation of resources towards the proliferation of GAs reduces the availability of resources for gametogenesis in acroporid colonies. This interpretation is corroborated by two previous studies, which found that GAs reduced the proportion of polyps producing oocytes in *Montipora informis* colonies in Japan (Yamashiro et al. 2000) and the number of oocytes produced per polyp in colonies of *Porites compressa* in Hawaii (Hunter 1997). Alternatively, it is possible that GAs are more likely to develop in corals already immunosuppressed. This hypothesis is supported by correlations between bleaching intensity and the prevalence of GAs on *Porites* corals on Kenyan reefs (McClanahan et al. 2009). The absence of oocytes from almost half (43%) of healthy *A. muricata* colonies, however indicates that factors other than GAs contributed to the failure of these colonies to produce oocytes. Such absences are consistent with the prioritisation of energy allocation to asexual reproduction rather than sexual reproduction by branching acroporids in some years (A. Baird pers.com). The absence of eggs in healthy colonies of *A. intermedia* is also consistent with the absence of eggs found in some polyps of *A. intermedia* in each of three months over the extended breeding season that appears to be characteristic for this species (B. Willis,

pers. comm.). Nonetheless, results of this study indicate that GAs have the potential to suppress reproduction in *Acropora* spp. on northern GBR reefs, thus reducing the capacity of these populations to recover from declines caused by other disturbances.

Resource allocation strategies may differ with disease type: Variation in the sub-lethal impacts of three common coral diseases on growth and reproduction in this study suggest that *Acropora* species may have the capacity to vary energy allocation strategies in response to different diseases. Differing patterns of resource allocation could be triggered by differing rates of tissue loss associated with the three diseases. Continued investment of resources in colony growth and reproduction in colonies with BBD may represent an attempt to maximise short-term fitness, given rapid rates of tissue loss and the high probability of dying. Maximisation of current reproduction to compensate for loss of future reproductive opportunities has also been documented from studies of diseases and parasites in plants (Minchellla 1985; Shykoff and Kaltz 1998). In contrast, colonies infected by GAs or slower progressing diseases such as SEB appear to divert resources that would normally be invested in growth and reproduction to physiological functions involved in maintenance and repair, such as the upregulation of disease resistance mechanisms, in response to the likelihood that some portion of the colony will survive and even recover. Further studies of intra-colony translocation of photoassimilates in diseased colonies (Roff et al. 2006) would confirm the circumstances under which diseases are recognised as a threat and energetic resources diverted to enhance survival. Greater knowledge of cellular mechanisms of disease resistance of scleractinian corals would also provide insights into the role that the availability of stem cells plays in disease resistance, and the impact that exhaustion of stem supplies might have on rates of growth and reproduction.

Synthesis of chronic and acute impacts of coral diseases: When both partial and whole colony mortality are combined for each of the three diseases investigated in this study, it is clear that BBD infections have much greater fitness consequences for Indo-Pacific *Acropora* populations than SEB. After ~2 years, only 3% of the original surface area of *Acropora* colonies remained alive following BBD infections, whereas more than 50% of the original surface area of colonies remained alive following SEB infections. Even considering further reductions in the growth rates and reproductive output of surviving portions of *A. muricata* colonies, BBD still had a much greater impact on the fitness of these northern GBR populations. Despite the lack of mortality associated with GAs in this study, the potential for failure of gametogenesis in a large proportion of *Acropora* colonies implies that the impacts of GAs on the fitness of Indo-Pacific coral populations could rival those of BBD. The contribution of sub-lethal impacts of GAs to overall fitness reductions highlights the importance of documenting both acute and chronic impacts of diseases to understand the threat that increases in disease, predicted to occur with climate warming (Harvell et al. 2002), pose to the resilience of Indo-Pacific coral populations.

In summary, this study presents important baseline data on the lethal and sub-lethal impacts of three diseases that commonly affect Indo-Pacific corals and thus provides insights into the potential of these diseases to reduce the fitness and resilience of Indo-Pacific coral populations. The contributions of both chronic and acute impacts of disease infections to fitness reductions varied markedly among the three disease types examined, highlighting the necessity to study disease dynamics over time frames sufficient to document rates of whole and partial colony mortality as well as sub-lethal impacts. Variation in the acute (lethal) impacts of diseases, which were greatest for BBD and lowest for GAs, is likely to reflect variation in the effectiveness of disease

resistance mechanisms of corals against the range of causative agents associated with these diseases. Differences in the investment of resources into growth and reproduction by colonies of *A. muricata* when infected by different diseases indicate potential flexibility in resource allocations strategies to maximise contributions to future generations in response to differing levels of disease virulence. Results of this study considerably expand knowledge of both the chronic and acute consequences of common diseases for the fitness of Indo-Pacific coral populations. Such data are particularly pertinent to understanding how escalating disease abundance is likely to affect Indo-Pacific corals.

Chapter 6

General Discussion

Large tabulate *Acropora* colony killed by white syndrome (bottom centre), surrounded by an otherwise healthy coral assemblage (Dip Reef, Central Great Barrier Reef)

6.1. Insights into Factors Influencing Patterns in Disease Prevalence and Host Susceptibilities in Coral Populations on the Great Barrier Reef

Large-scale temporally replicated surveys of diseases affecting anthozoans on reefs spanning 1,000kms of the north-south axis and the width of the continental shelf of the Great Barrier Reef (GBR) (Chapters 2, 3 and 4) have provided invaluable insights into the importance of disease in the natural ecology of GBR coral communities. Disease is ubiquitous and persistent in coral assemblages throughout much, if not all, of the GBR, and common diseases like black band disease (BBD) and skeletal eroding band (SEB) are widely distributed, both in terms of their spatial spread and the host taxa they affect (Chapters 2, 3 and 4). The prevalence of disease on the GBR is typically low, disease affecting only 3% of scleractinian corals (Chapter 4). Nonetheless, the prevalence of BBD and SEB are within the range of values recorded for diseases causing significant coral declines in the wider Caribbean (Chapters 2 and 3). This is the first study to document the prevalence of coral diseases on reefs spanning large areas of the GBR, and it is clear that disease is likely to play a more important role in structuring coral assemblages throughout the GBR than previously recognised by studies considerably smaller in scale.

Spatial patterns in the prevalence of BBD (Chapter 2) and hierarchies in susceptibilities of scleractinian families to disease (Chapter 4) provide important insights into disease drivers on the GBR. Large-scale surveys indicate that cross- and long-shelf gradients in the exposure of coral communities to environmental factors, including turbidity, wave action and water quality, are not the primary factors determining BBD prevalence and hierarchies in disease susceptibilities on the GBR. The greater prevalence of disease in acroporid and pocilloporid corals, but also the vulnerability of these families to a wide variety of pathogens, is in part likely to reflect

the high abundance of pocilloporid and acroporid corals on GBR reefs (Chapter 4), given that density dependence is a key characteristic of infectious diseases (Anderson and May 1979). However, the abundance of families is not the only factor determining hierarchies in susceptibilities to disease since acroporid and pocilloporid corals were amongst those most susceptible to disease in regions where they formed only a minor component of coral assemblages (Chapter 4), as well as in regions where they dominate coral assemblages. Instead, life-history strategies are likely to be an important factor influencing susceptibility to disease. The greater prevalence of disease in three dimensional, fast-growing branching acroporid and pocilloporid corals is consistent with both the greater exposure of these morphologies to water borne pathogens and allocation strategies that prioritise the use of energetic resources for growth rather than upregulation of disease resistance mechanisms (Chapter 4).

Experimental studies provided important insights into the likely importance of injury in the development of SEB in GBR corals, injury enhancing the ability of *Halofolliculina corallasia*, the putative pathogen of SEB, to colonise otherwise healthy corals (Chapter 3). However, the lack of band-like aggregations of ciliates and tissue loss on experimental colonies suggests that, like other wildlife diseases, disease in corals is likely to involve a web of causation, including nutritional and environmental stressors, and potentially multiple micro-organisms (Wobeser 2006). Studies are needed, not only to confirm the absence of microbes other than *Halofolliculina corallasia* in experimental wounds, but also to investigate nutritional and environmental stressors in corals with SEB to determine if folliculinid ciliates are the primary cause of tissue lysis in progressing disease bands on GBR corals. The need for additional stressors to facilitate the development of pathogenic SEB infections is supported by previous studies which have shown that injuries caused by breakage (Bak and Criens

1981), feeding corallivores (Antonius and Riegl 1998; Nugues and Bak 2009) or interactions with macro-algae (Nugues et al. 2004), as well as bleaching (Muller et al. 2008) are likely to facilitate disease development in corals. Complex relationships between host health, pathogens and the environment could underlie the difficulty of identifying the causative agents responsible for many coral diseases (Richardson et al. 2001; Cervino et al. 2004).

6.2. Impacts of Disease on the Fitness of GBR Coral Populations

This is the first study to assess and compare both lethal and sub-lethal impacts of BBD, SEB and growth anomalies on an Indo-Pacific coral population (Chapter 5) and thus provides valuable insights into the consequences of common corals diseases for the fitness and resilience of coral populations in this region. When both partial and whole colony mortality are combined for each of the three diseases, BBD infections have much greater fitness consequences for Indo-Pacific *Acropora* populations than SEB. Only 3% of the original surface area of *Acropora* colonies remained alive following BBD infections, whereas more than 50% of the original surface area of colonies remained alive following SEB infections after ~two-years (Chapter 5). However, SEB infections are seven-fold higher in prevalence in GBR *Acropora* populations than BBD (SEB affected 4.37% and BBD 0.64% of branching *Acropora* colonies; Appendix 1), suggesting that the fitness consequences of advancing SEB infections could rival those of BBD. Reductions in growth rates and the reproductive output of surviving portions of *A. muricata* colonies infected with SEB further increase the fitness consequences of SEB in these northern GBR populations. Such losses are concerning because further increases in disease, predicted to occur with climate warming (Harvell et al. 2002), could seriously jeopardise the fitness and persistence of *Acropora* populations in this

region. This finding highlights the importance of combining information on disease prevalence and both acute and chronic impacts of diseases to understand the threat that disease poses to the resilience of coral populations.

Variation in the sub-lethal impacts of three common coral diseases on growth and reproduction in this study (Chapter 5), suggest that *Acropora* species may have the capacity to vary energy allocation strategies in response to different diseases. Different energy allocation strategies may represent alternative means of maximising fitness given variable rates of tissue loss associated with the three diseases. Maintenance of energy allocations to growth and reproduction in colonies with BBD may represent an attempt to maximise current reproduction in response to rapid tissue loss to compensate for the loss of future opportunities to reproduce, given the likelihood that colonies with BBD will die. In contrast, reductions in rates of growth and reproductive output of *Acropora muricata* colonies infected by more chronic diseases such as GAs or SEB, suggest that resources may be prioritised for use in lesion repair and /or the upregulation of disease resistance mechanisms, given the greater likelihood that some portion of the colony will survive and even recover from these diseases. Variability in sub-lethal impacts among diseases highlights the importance of documenting the chronic impacts of other common Indo-Pacific coral diseases in future studies to understand their implications for the fitness and resilience of Indo-Pacific coral populations.

On a more positive note, a proportion of colonies infected with SEB (~30% of colonies) and BBD (~10% of colonies) recovered from their infections, while others remained unaffected by disease during this study (Chapter 5). These colonies may represent disease resistant genotypes that could naturally become more common within coral populations as susceptible genotypes are killed by disease infections. Proliferation of disease resistant genotypes would then enhance the potential for susceptible coral

populations to recover and persist (Vollmer and Kline 2008), particularly populations of acroporid and pocilloporid corals which may rely predominately on asexual reproduction for recovery on local scales (Wallace 1985). Greater efforts are needed to identify factors contributing to variation in the susceptibilities of genotypes to disease infections to understand how management strategies might enhance the recovery of disease resistant genotypes and hence coral populations following disease epizootics.

6.3. Relevance to Management and the Need for Long-Term, Large-scale Monitoring

The vast abundance of colonies and species comprising GBR coral assemblages, and thus the time-intensive and costly nature of disease prevalence surveys in this region, have limited the geographic scale of surveys of disease prevalence to three or fewer reefs (Loya et al. 1984; Dinsdale 2000; Willis et al. 2004). Consequently, this is the first study to document the prevalence of disease over large-spatial scales of the GBR and it considerably expands knowledge of the ecology of diseases affecting corals in this region. The up to four-fold greater percentage of reefs with BBD in this study (73.7% of reefs: Chapter 2 versus 19% of reefs: Miller 1996) is almost certainly attributable to the greater likelihood of detecting macroscopic signs of disease by searching a comparatively small reef area by SCUBA (Chapter 2). The absence of SEB records from one reef (Middle Reef, central GBR) in two of three years of surveys (Chapter 3) and increases in disease host ranges in successive years of surveys (Chapter 4), highlight the need for temporally and spatially replicated surveys to accurately document the distribution and host ranges of diseases that have low abundance. This study provides important baselines of disease host ranges and prevalence (Chapters 2, 3 and 4) from which future changes in pathogen virulence and host susceptibility with

climate warming might be detected, highlighting not only the important contributions that temporally replicated large-scale spatial surveys make to epidemiological studies of coral disease, but also the importance of continuation of these large-scale surveys of disease prevalence.

Large-scale surveys of disease spanning cross- and long-shelf gradients in levels of anthropogenic pollution suggest that BBD prevalence is relatively unaffected by levels of land based pollutants, the prevalence of BBD being highest on mid-shelf reefs and in the relatively pristine northern Cooktown/Lizard Island sector (Chapter 2). However, the influence of anthropogenic pollution on the severity of BBD infections, as well as the severity and prevalence of other diseases commonly affecting GBR corals, is as yet unclear. It is therefore not possible to conclude that increased inputs of anthropogenic pollutants will not increase disease impacts in GBR coral populations and communities, particularly given increasing evidence of a link between eutrophication and increases in disease prevalence and severity in Caribbean and Indo-Pacific corals (Bruno et al. 2003; Smith et al. 2006a; Dinsdale et al. 2008).

Evidence that diseases are widely distributed and persistent in coral assemblages throughout the GBR, and that their prevalence can reach values similar to those recorded for diseases that have significantly reduced the resilience of Caribbean coral populations (Chapter 2, 3 and 4), indicates that diseased corals on the GBR and in other Indo-Pacific regions should no longer be considered a novelty by reef managers. Dramatic and widespread impacts of disease epizootics in Caribbean coral populations (Gladfelter 1982; Nagelkerken et al. 1997; Precht et al. 2002; Kim and Harvell 2004; Bruckner and Bruckner 2006), combined with predictions of increases in disease epizootics with future deteriorations in water quality and climate change (Harvell et al. 1999; Epstein 2001; Bruno et al. 2003) and a paucity of options for managing disease

epizootics in corals (Bruckner 2002), highlight the urgency with which management strategies that minimise disease increases and mitigate impacts of diseases in coral populations must be developed and implemented.

Large-scale surveys (Chapter 4) and experimental studies (Chapter 3) highlight the important role that factors which compromise coral health, including bleaching, injury from predators, macro-algal overgrowth and cyclones, are likely to play in facilitating disease development in GBR corals. Thus results of this study indicate the need for management strategies that minimise both the impacts of these stressors on coral assemblages, as well as those that drive increases in their occurrence or severity, i.e. climate warming and inputs of nutrients into reefal waters (Agee 1991; Hoegh-Guldberg 1999; Brodie et al. 2005), to limit disease spread in Indo-Pacific coral communities. At present, populations of acroporid and pocilloporid species dominate many Indo-Pacific coral assemblages, however their vulnerability, not only to compounding losses from disease, but also to other major disturbances, including cyclones, bleaching and crown-of thorns outbreaks, on the GBR and in a diversity of Indo-Pacific reef regions (Chapter 4), mark them as highly vulnerable to future catastrophic declines throughout the Indo-Pacific. For this reason, acroporid and pocilloporid species should be the targets of management strategies designed to minimise localised and controllable sources of coral mortality to minimise their loss from Indo-Pacific coral assemblages. Widespread and dramatic losses of acroporid corals from Caribbean reefs, resulting from their vulnerability to multiple disturbances (Gladfelter 1982; Hughes 1994; Precht et al. 2002), should stand as a serious warning to managers of Indo-Pacific reefs of the need to carefully manage coral taxa vulnerable to multiple disturbances.

6.4. Summary and Conclusions

1. Seven diseases commonly affect a wide range of anthozoan taxa throughout much of the GBR. The prevalence of disease in shallow GBR coral assemblages is generally low, disease affecting ~3% of scleractinian colonies per year. Nonetheless the prevalence of diseases, including BBD and SEB, are within the range of values recorded for diseases affecting Caribbean corals. In combination, these results indicate that disease is an integral part of the natural ecology of GBR coral communities.
2. Patterns of highest BBD prevalence on mid-shelf reefs in the more pristine northern Cooktown/Lizard Island sector, indicate that BBD prevalence is relatively unaffected by inputs of terrestrial pollution from mainland catchments.
3. SEB is by far the most prevalent disease affecting shallow corals on the GBR, having been recorded from ~2% of scleractinian colonies. The host range of SEB exceeds that of all other Indo-Pacific and Caribbean diseases.
4. Experimental studies indicate that injury is likely to play an important role in the development of SEB. However, ciliates did not form band-like aggregations associated with tissue lysis on experimental corals, suggesting that *H. corallasia* is not sufficient in itself to cause tissue mortality or that interactions with additional microbial, nutritional or environmental factor may be required before folliculinid infections become pathogenic.
5. Scleractinian corals are twice as susceptible to disease as other anthozoan taxa, and within the order Scleractinia, the families Pocilloporidae and Acroporidae are 2 to 3-fold more susceptible to pooled diseases than other families on upper slopes of back reefs on the GBR.

6. Concordance in family rankings for susceptibility to diseases and to other factors that compromise coral health on the GBR (bleaching, injury from predators and macro-algae overgrowth), but also in a range of reef regions, is consistent with family differences in life history tradeoffs and colony morphology underpinning these susceptibilities. The vulnerability of compromised corals to invasion by pathogens may also in part underlie correlations between the families most susceptible to disease and other factors that compromise coral health.
7. Studies of tagged colonies revealed that the acute impacts of disease (rates of disease progression, partial and whole colony mortality) are higher for BBD than for SEB, in northern coral GBR coral populations. Tissue loss was not associated with growth anomalies in this study.
8. SEB infections have the potential to suppress growth and reproductive output of *Acropora muricata* colonies, while evidence suggests that growth anomalies suppress oogenesis in acroporid colonies. No sub-lethal impacts of BBD infections on colony growth and reproductive output were detected in this study, most probably because most colonies died before reproduction could be assessed. Variation in the sub-lethal impacts of diseases suggests that *Acropora* spp. have the capacity to vary their response, in terms of allocation of resources to physiological processes, in response to different pathogens or rates of tissue loss, to maximise their contribution to future generations.
9. Although the acute impacts of BBD were greater than those of SEB in northern GBR *Acropora* populations, the prevalence of SEB infections were seven-fold higher. In combination with the potential for SEB to decrease rates of growth and reproduction, the comparatively high prevalence of SEB in GBR *Acropora* populations suggests that the fitness consequences of advancing SEB infections

could be significant. Tissue loss was not associated with GAs in this study, however the potential for GAs to suppress oogenesis in a large proportion of *Acropora* colonies reduces the capacity of these populations to recover from declines caused by other disturbances.

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