Dynamics and Drivers of Coral Disease on Indo-Pacific Reefs

Thesis submitted by
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THESIS DEDICATION

I dedicate this thesis to my fiancé, Mike Flavell, and to my son, Benjamin Haapkylä-Flavell.

ABSTRACT

Coral reefs are currently declining worldwide and coral diseases have been recognised as a major contributor to this pattern. This thesis investigated the dynamics and potential environmental drivers of coral diseases on reefs spanning 20 degrees of latitude in the western Pacific: equatorial reefs in the Wakatobi Marine National Park (WMNP), South-East Sulawesi, Indonesia; Magnetic Island reefs in the central Great Barrier Reef (GBR) Marine Park, Australia; and Heron Island reefs in the southern GBR Marine Park.

Surveys conducted in the WMNP revealed that both disease prevalence and the numbers of diseases affecting corals increased between 2005 and 2010. Disease progression rates were comparable to those found in the Caribbean and on the GBR, indicating that diseases may have serious impacts on coral populations in the WMNP. Similar numbers of coral taxa were recorded as diseased in the WMNP and at Heron Island, with species of staghorn *Acropora* being the most susceptible group at both locations. In the WMNP, high sedimentation rates may have increased disease prevalence at the site with the greatest disease prevalence in 2007. At this site, a dramatic decline in coral cover from 75% in 2007 to 18% in 2010 was documented, with six diseases: *Porites* ulcerative white spots syndrome (PUWS), ulcerative white spots (UWS), growth anomalies (GA), skeletal eroding band (SEB), white syndrome (WS) and black band disease (BBD) present in 2010. Highly significant decreases in coral cover between 2005 and 2007 at all sites in the WMNP demonstrate that even reefs in this remote area of the Coral Triangle are experiencing deteriorating coral health mainly due to the over-exploitation of marine resources which is likely to have significant impacts

on this global biodiversity hot spot. Although both the prevalence and number of coral diseases have increased, the overall disease prevalence still remains low in the WMNP.

Disease prevalence was generally higher on Heron Island reefs than in the WMNP. A total of six coral diseases were found at Heron Island with brown band (BrB), UWS and GA being the most abundant. The prevalence of UWS was higher in the Austral summer, whereas, for the first time, a higher prevalence of BrB was detected in the Austral winter. No clear seasonal trend in GA prevalence was detected, but prevalence increased over the 3 years of the study. Disease prevalence on Heron Island reefs was dependent on the coral community composition, with sites having high abundance of staghorn *Acropora* and plate-like *Montipora* experiencing the highest levels of disease prevalence. Diseases were most common at sites with intermediate host coral cover in comparison with sites with high coral cover. A shift in the coral community structure was observed from a community dominated by tabular *Acropora* in 2007 to a community dominated by *Goniastrea*, bushy *Acropora*, *Coscinarea* and *Stylophora* in 2009. Since the surveys were conducted half-yearly, it is not possible to conclusively attribute this shift to disease, highlighting the importance of regular long-term monitoring to detect change in reef ecosystems.

A two-year study of environmental drivers of the coral disease atramentous necrosis (AtN) was conducted at two sites around Magnetic Island, an inshore fringing reef. At the study sites, AtN primarily affects the plating coral *Montipora aequituberculata*. The abundance of AtN was strongly negatively correlated with low salinity and strongly positively correlated with particulate organic carbon. A

weaker positive relationship was observed between AtN abundance and seawater temperature, as recorded 7 days prior to and including the sampling date. An aquarium-based study investigating the impacts of salinity and temperature on AtN rates of progression provided corroborative evidence of the importance of these two environmental parameters in driving disease dynamics in the field. The highest mortality rates caused by AtN were recorded in the high temperature (32°C) and low salinity (20) treatments. Results from both the field and experimental studies highlight the importance of the combined impacts of high temperature and low salinity, conditions that prevail typically in the austral summer, as important environmental drivers of AtN.

The results of this thesis demonstrate the important role that coral diseases have in altering reef ecosystems and the potential they have to lead to phase shifts. Identifying drivers of disease help in implementing more effective management strategies that will aim to protect coral reefs as this ecosystem will be subject to increasing stress levels in the future due to climate change.

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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

Infectious diseases of wildlife are increasingly recognized as key threats to animal populations, but relatively little is understood about their ecology compared with human diseases (Daszak et al., 2000). Because there has been a substantial increase in the number and proportion of human diseases originating from wild populations of terrestrial animals (i.e. zoonotic diseases) in the past few decades e.g. severe acute respiratory syndrome (SARS) and Ebola virus (Jones et al., 2008), there has been an increase in targeted surveillance efforts focusing on activities that bring humans and terrestrial wildlife into close contact (Jones et al., 2008, Wolfe et al., 2007). However, there has been comparatively less effort devoted to understanding diseases of marine populations even thought some research demonstrates the devastating role diseases can have on marine wildlife populations such as the long-spined sea urchin in the Caribbean (Lessios, 1988), phocine distemper virus (Heidejorgensen and Harkonen, 1992), black abalone in California (Lafferty and Kuris, 1993) and elkhorn coral *Acropora palmata* in the Caribbean (Patterson et al., 2002, Lipp et al., 2002). Although diseases that affect marine animals are less well understood than those that affect humans and terrestrial wildlife (Harvell, 2004a), there is no reason to suspect that their impacts on populations are less severe.

Reviews of mass mortalities in marine ecosystems have revealed a number of known and suspected links with pathogens, including Caribbean sea urchin mortality (Lessios, 1988), phocine distemper virus (Heidejorgensen and Harkonen, 1992), pilchard mortalities (Jones et al., 2008), turtle grass *Thalassia testudinum* mortality (Robblee et al., 1991) and disease-related coral mortalities (Harvell et al., 1999, Harvell et al., 2001, Harvell et al., 2002, Bruno et al., 2007).

Understanding the causes of these mortalities is challenging because epidemiological theories developed for terrestrial wildlife may not readily translate to marine ecosystems (Harvell, 2004b, McCallum et al., 2004). For example, diseases appear to spread more rapidly in the comparatively more open marine ecosystem (McCallum et al., 2003), and marine pathogens are more diverse both taxonomically and life history-wise (McCallum et al., 2004). In addition, understanding how large-scale environmental changes, particularly those related to climate change (Intergovernmental Panel on Climate Change (IPCC), 2007), and local-scale changes, such as increases in nutrients in the environment, affect host-pathogen interactions adds further complexity to the task of developing appropriate management strategies (Johnson et al., 2010).

Coral reefs, the most biodiverse ecosystem of the ocean, are estimated to harbour one-third of all described marine species (Reaka-Kudla, 1997, Reaka-Kudla, 2001). Climate change (Veron et al., 2009), terrestrial runoff (De'ath and Fabricius, 2010) and over-exploitation of resources (Jackson et al., 2001) currently threaten this ecosystem, which tens of millions of people depend on for protein and other services (Costanza et al., 1997). Expert opinion, supported by extensive monitoring and assessment data, suggest that the world has lost the goods and services provided by about 19% of the global coral reef area (Wilkinson, 2008). The consequences of this destruction are not limited to the loss of the goods and services that reefs provide, but also extend to the extinction of a significant part of global biodiversity (Veron et al., 2009).

Coral disease was first documented in 1965 (Squires, 1965) and is now thought to be a major cause of coral reef decline (Dustan, 1999, Porter et al.,

2001). Various studies on coral disease exist from the Caribbean (e.g. Rodriguez-Martinez et al., 2001, Weil et al., 2002) whereas comparatively less is known about Indo-Pacific coral diseases. Recent reviews indicate that 27 different diseases affect corals worldwide with 9 diseases affecting Indo-Pacific corals (Willis et al., 2004). There are comparatively few reports of coral disease from Indo-Pacific reefs compared to more than 70% of the records found in the Caribbean (Harvell et al., 2007) which continues to be considered a coral disease hotspot (Green and Bruckner, 2000, Weil, 2004).

The potential of disease outbreaks to significantly change coral reef community structure was shown in the early 1980s, when diseases caused significant mortality of three Caribbean keystone species. Populations of the black sea urchin *Diadema antillarum* were reduced by an unknown pathogen by more than 97% (Lessios et al., 1984, Lessios, 1988, Carpenter, 1990). The decline of *D. antillarum* accelerated the degradation of coral reefs through shifts from coral- to algal-dominated communities (Hughes et al., 1987, Lessios, 1988, Carpenter, 1990, Hughes, 1994, Ostrander et al., 2000). Subsequent surveys of acroporid corals demonstrated that between 1996 and 2001, coral reefs of the Florida Keys lost 37% of their living coral (Porter et al., 2001), and that *Acropora palmata* had declined by 88% (Sutherland and Ritchie, 2004). Coral disease was the primary cause of the reef decline in the Caribbean (Aronson and Precht, 1997, Gladfelter, 1982, Kim and Harvell, 2004). White band disease is believed to have induced a community shift from reef framework-building species of *Acropora* to small, encrusting species over large areas, representing levels of regional mortality that are without precedent in the late Holocene (Aronson and Precht, 1997, Aronson

and Precht, 2001). *A. cervicornis* was the most important reef-building coral in Belize until the mid-1980s after which its population decreased dramatically over 10 years (Aronson and Precht, 2001). Due to the fact that *A. cervicornis* mainly reproduces by asexual fragmentation (Shinn, 1966, Tunnicliffe, 1981, Highsmith, 1982), the prospects for its recovery are poor (Aronson and Precht, 2001) whereas *A. palmata* has a higher rate of sexual recruitment (Stoddart, Highsmith, 1982, Rosesmyth, 1884, Jordandahlgren, 1992) suggesting that there may be potential for recruitment from local or other populations. Due to their asexual reproduction strategy that leads to low genetic variability, *Acropora* spp. may have an increased susceptibility to white band disease (Bak, 1983). If the *Acropora* populations do not recover, the current high abundance of macroalgae that started after the *D. antillarum* die-off (Hughes et al., 1987, Lessios, 1988, Carpenter, 1990, Hughes, 1994, Ostrander et al., 2000) will persist and a shift to brooding corals will be a likely scenario (Aronson and Precht, 2001). The rapid and widespread losses of *A. palmata* and *A. cervicornis* have resulted in a 'threatened' status for both species under the US Threatened Species Act (Anonymous, 2005, Hogarth, 2006) and a 'critically endangered' status under the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (Carpenter et al., 2008).

Mortality of *Acropora* spp. resulted in a cascade of significant ecological changes in the dynamics, function and structure of Caribbean coral reefs at local and geographic scales (Hughes, 1994, Harvell et al., 1999, Aronson and Precht, 2001, Bruckner, 2002, Lirman et al., 2002, Weil et al., 2002, Weil, 2004). Apart from epizootics of white band and white pox diseases on *Acropora* (e.g. Aronson and Precht, 2001, Patterson et al., 2002), a widespread epizootic of a fungal

disease has affected Caribbean populations of the sea fan *Gorgonia ventalina* (Smith et al., 1996, Nagelkerken et al., 1997, Kim et al., 2000, Harvell et al., 2002, Weil et al., 2002, Smith and Weil, 2004). No widespread epizootics have been reported for the Indo-Pacific (Weil et al., 2006), but surveys in Australia (Willis et al., 2004, Page and Willis, 2006, Page and Willis, 2008, Sato et al., 2009, Haapkyla et al., 2010), in the Philippines (Raymundo et al., 2003, Raymundo et al., 2005, Kaczmarsky, 2006), Hawaiian Islands (Aeby, 2005, Williams et al., 2010), U.S. Pacific remote islands (Sandin et al., 2008, Vargas-Angel, 2009) and Indonesia (Haapkyla et al., 2007, Haapkyla et al., 2009) reveal numerous diseases that have resulted in significant mortality in all surveyed locations.

1.1 CORAL DISEASE PREVALENCE IN THE INDO-PACIFIC

Long-term monitoring studies on disease can reveal temporal and spatial disease trends in time (Lafferty et al., 2004). On the Great Barrier Reef (GBR) of Australia, the abundances of coral diseases have been monitored since 1998 by the AIMS long-term monitoring program, and a 20-fold increase in white syndromes (WS), which primarily affect acroporids, was documented between 1998 and 2003 (Willis et al., 2004). Seven diseases are currently recognized to affect corals of the GBR; white syndrome (WS), black band disease (BBD), skeletal eroding band (SEB), brown band syndrome (BrB), *Porites* ulcerative white spot disease (PUWS), growth anomalies (GA) and atramentous necrosis (AtN) (Willis et al., 2004, Page and Willis, 2006, Page and Willis, 2008, Sato et al., 2009, Boyett et al., 2007, Raymundo et al., 2003, Jones et al., 2004b),

although the mean disease prevalence was relatively low (only 3% of scleractinian corals affected by disease) (Page, 2009). Surveys conducted on 8 longitudinal and cross-shelf locations on the northern and southern GBR revealed a total disease prevalence of 9 ± 0.8% (Willis et al., 2004). The most common disease on the GBR was skeletal eroding band (SEB), which affected approximately 2% of 283,486 scleractinians and hydrocorals surveyed on 18 reefs (Page and Willis, 2008). Its host range was large, affecting 12 families and at least 87 scleractinian species, as well as the hydrocoral *Millepora*. Pocilloporidae and Acroporidae were the most susceptible coral families, the former being up to five times more susceptible than other families (Page and Willis, 2008). Black band disease (BBD) was found on 74% of the 19 surveyed GBR reefs but it had a low prevalence of 0.1% and affected mostly branching *Acropora* and the family Pocilloporidae (Page and Willis, 2006). The lethal and sub-lethal impacts of SEB and BBD can be similar to diseases found in the Caribbean such as Caribbean yellow band disease and dark spot syndrome (Foley et al., 2005, Cervino et al., 2001), highlighting the significant role disease may play in structuring GBR coral assemblages (Page, 2009). 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 energy available for growth or reproduction (Soong and Lang, 1992, Rinkevich, 1996, Kramarsky-Winter, 2004). These findings from the GBR show that coral diseases may have a serious impact on Indo-Pacific reefs.

Diseases have also been recorded elsewhere in the Indo-Pacific. For instance in the Philippines, a mean total disease prevalence of 8% (n=8 reefs) was

observed (Raymundo et al., 2005), which is similar to the prevalence observed on the GBR (9 ± 0.8% according to Willis et al., 2004). Two syndromes, *Porites* ulcerative white spot syndrome (PUWS) and growth anomalies (GAs), occurred at high prevalence in 2002 and 2003 (Kaczmarsky, 2006). GAs were detected on 39% of massive *Porites* and PUWS on 54% of massive and branching *Porites*.

Twelve potential new host species for PUWS were identified (*Echinopora lamellosa*, *Goniastrea minuta*, *Heliopora caerulea*, *Porites annae*, *Favia stelligera*, *Favia* sp., *Montipora grisea*, *M. malampaya*, *M. turtlensis*, *M. digitata*, *M.vietnamensis*, and *M. turgescens*) and 5 likely new hosts for BBD (*Coscinaraea columna*, *E. lamellosa*, *G. minuta*, *M. hispida* and *P. solida*) (Kaczmarsky, 2006). Severe impacts of coral disease have been reported from the North-western Hawaiian Islands, where WS led to tissue loss of 10-60% on tabular acroporids (Aeby, 2005). A recent study on WS detected a low prevalence of 0.02 to 0.9 % in Kaneohe Bay, Hawaii, causing infected *Montipora* colonies to lose an average of 3% of live tissue per month (Aeby et al., 2010). Values for total disease prevalence of less than 0.6% were detected in the U.S. Pacific remote islands (Vargas-Angel, 2009) and in South-East Sulawesi, Indonesia (Haapkyla et al., 2007, Haapkyla et al., 2009). These values may represent background levels of coral disease prevalence in relative pristine coral reef environments of the Indo-Pacific.

More extensive data on spatio-temporal dynamics of coral diseases in the Indo-Pacific are needed to better understand the origin, etiology and epizootiology of the most important diseases affecting corals (Richardson et al., 2001, Weil et al., 2002, Sutherland et al., 2004, Weil, 2004). An improved understanding of

coral disease may provide answers as to how to prevent and manage this problem (Weil and Croquer, 2009). Managing diseases in the ocean will require an acknowledgment of the complex interactions that occur between disease and the environment (Lafferty et al., 2004). It remains unclear what is causing the increase in marine and coral diseases and why they are spreading so fast (Weil and Croquer, 2009).

1.2 ENVIRONMENTAL DRIVERS OF CORAL DISEASE

Localized human impacts (e.g. eutrophication, sedimentation) and global climate change are two of the most significant factors currently thought to promote coral disease epizootics, as both factors may compromise coral resistance and enhance virulence of pathogens (Harvell et al., 2002, Bruno et al., 2003, Kaczmarsky et al., 2005). However the lack of baseline data for marine organisms, including corals, makes it difficult to understand the immediate causes that trigger epizootic events and lead to population- or ecosystem-scale impacts, hindering the development of efficient and effective management plans to address this problem (Ward and Lafferty, 2004).

1.2.1 Temperature

Current research supports a connection between a warming climate and the increasing incidence of disease (Harvell et al., 2001, Harvell et al., 2002, Bruno et al., 2007). Anomalously high temperatures can influence the severity and

dynamics of infectious marine diseases by increasing both host susceptibility and pathogen virulence (Harvell et al., 2002, Lafferty and Holt, 2003).

Since ocean temperatures are expected to rise during this century (Intergovernmental Panel on Climate Change (IPCC), 2007) it is likely that coral disease will become more prevalent (Ben-Haim et al., 2003a). The severity of numerous coral diseases increases with elevated temperatures, e.g. aspergillosis (Alker et al., 2001), BBD (Carlton and Richardson, 1995, Edmunds, 1991, Rutzler et al., 1983), yellow band disease (YBD) (Korrubel and Riegl, 1998), white pox (WP) (Patterson et al., 2002), dark spot syndrome (DSS) (Gil-Agudelo and Garzon-Ferreira, 2001), atramentous necrosis (AtN) (Jones et al., 2004b), white plague (Bruckner and Bruckner, 1997a) and white syndrome (WS) (Bruno et al., 2007). Elevated temperature may also increase disease progression rates. BBD progressed approximately twice as fast during the austral summer than in cooler months (Boyett et al., 2007) and the abundance peaked in the austral summer (Sato et al., 2009). Rates of new infections and linear progression rate of lesions were both positively correlated with seasonal fluctuations in seawater temperatures and light, suggesting that the seasonal increases in these environmental parameters promote the virulence of the disease (Sato et al., 2009). Brown band syndrome (BrB) is the fastest progressing coral disease on the GBR (rate up to 2 cm d^{-1}), especially during austral summer (Nash, 2003, Boyett, 2006).

Laboratory studies have demonstrated increased pathogen growth and virulence at higher temperatures, notably for the bacteria *Vibrio shiloi* and *Vibrio coralliilyticus* and for the fungus *Aspergillus sydowii* (Benin et al., 2000, Alker et

al., 2001, Ben-Haim et al., 2003a, Ben-Haim et al., 2003b, Ward et al., 2007). Elevated temperature was shown to increase the activity of anti-fungal compounds produced by the sea fan *Gorgonia ventalina* and the growth rates of *A. sydowii,* potentially before host resistance mechanisms were activated (Ward et al., 2007). Several coral pathogens have optimum growth rates at temperatures above 30ºC. For instance, optimal growth was recorded at 30 to 35ºC for a pathogen identified for white plague type II, *Aurantimonas coralicida* (Remily and Richardson, 2006) and a pathogen documented to cause white pox disease, *Serratia marcescens* (Looney et al., 2010). High temperature triggers the expression of temperature-regulated bacterial virulence genes in the bacterium *Vibrio shiloi*, resulting in bleaching of the Mediterranean coral *Oculina patagonica* (Kushmaro et al., 1998). Seawater temperature was a critical environmental parameter in determining the outcome of infection when the coral *Pocillopora damicornis* was exposed to the bacterium *Vibrio coralliilyticus* at 27 and 29°C, resulting in the lysis of the corals within two weeks at both temperatures (Ben-Haim et al., 2003a). Some evidence also exists that thermal bleaching events in corals can be followed by outbreaks of diseases (Guzman and Guevara, 1998, Muller et al., 2008), and coral mass mortalities caused by diseases have been linked to anomalously high water temperatures (Cerrano et al., 2000, Riegl, 2002, Bruno et al., 2007, Heron et al., 2010). Warm temperatures may lead to a decrease in the resistance of the host coral making it more susceptible to infections (Harvell et al., 1999). Corals have an innate immune system that may contribute to their capacity to resist disease and bleaching (Mydlarz et al., 2008, Palmer et al., 2008, Palmer et al., 2009) and

that the amount of energy invested in immunity is species specific (Stearns, 1983, Rinkevich, 1996).

1.2.2 Nutrients

Many coastal coral reefs are exposed to increasing loads of nutrients, sediments, and pollutants exported from catchments developed for agricultural, industrial and urban uses (Spalding et al., 2001, Smith et al., 2003, Brodie et al., 2011). High levels of turbidity, nutrients, and sedimentation lead to the deterioration of coral reefs at local scales (Fabricius, 2005). Over the past decade there has been considerable concern that near shore fringing reefs of the GBR are becoming degraded due to human influences (Schaffelke et al., 2003). Terrigenous runoff of nutrients and sediments from rapidly expanding land use is considered to be one of the most severe impacts on coastal areas of the GBR World Heritage Area (GESAMP, 1990, Zann, 1995). On the GBR, catchments south of 15 ºS are extensively used for grazing, sugar cane, and horticulture, resulting in five- and ten-fold increased river discharges of sediments and nutrients compared to pre-European settlement circa 1860 (Furnas, 2003). Within the GBR, both background and elevated nutrient levels are closely associated with terrestrial runoff being higher inshore in the austral summer (Furnas, 2003). Most of this run-off is delivered in short-lived flood events during the 5-month summer wet season, forming distinct flood plumes in the coastal zone that sometimes reach far out into the lagoon (Devlin and Schaffelke, 2009).

Enrichment by inorganic nitrogen and phosphorus can affect coral disease dynamics by increasing pathogen virulence, as demonstrated by the higher

prevalence of aspergillosis on reefs with higher concentrations of dissolved inorganic nitrogen (ammonium, nitrate and nitrite) (Kim and Harvell, 2002). A field experiment on nutrient (phosphorous, nitrate and ammonium) enrichment confirmed that a moderate increase in nutrient concentrations can substantially increase the severity of aspergillosis and Caribbean yellow band disease in the (Bruno et al., 2003). Voss and Richardson (2006b) showed that the addition of nutrients doubled the progression rate of BBD both in the field and in the laboratory and that elevated nutrient levels may reduce the coral host's ability to resist infection by pathogenic microorganisms. Reducing nutrient pollution is an important management tool for controlling coral epizootics.

The only study to date attempting to link anthropogenic influence and coral disease on the GBR found no correlation between the prevalence of BBD and terrestrial influences (Page and Willis, 2006). This contrasts with earlier studies where sediment stress and pollution were associated with BBD epizootics (Antonius, 1985, Littler and Littler, 1996, Bruckner and Bruckner, 1997b, Al-Moghrabi, 2001). Higher overall disease prevalence was linked to sewage outfall in the Caribbean (Kaczmarsky et al., 2005) and the highest disease prevalence was detected at close proximity to Dumaguete city in the Philippines correlating disease prevalence with anthropogenic influence (Kaczmarsky, 2006).

1.2.3 Sedimentation

Sedimentation is a severe disturbance for coral reefs (Fabricius, 2005). It has been associated with profound changes in coral population structure, such as altered size frequencies, declining mean colony sizes, altered growth forms, and reduced growth and survival (Rogers, 1990). Responses to sedimentation differ

substantially between species and also between sediment types (Fabricius, 2005). Grain size, as well as the organic and nutrient content of sediments affects the impact sediments have on corals (Weber et al., 2006). Nutrient rich sediments may cause anoxia and produce hydrogen sulphide on the surface of the coral (Weber et al., 2006). Coral mucus, rich in nutrients, produced during sediment stress can stimulate bacterial production (Ducklow and Mitchell, 1979, Meikle et al., 1988). On the other hand, sedimentation can protect corals from mortality caused by high temperature and high light conditions that lead to bleaching (Anthony et al., 2007). This may be due to particles in the water providing corals with more food under highly turbulent conditions by facilitating tissue growth and lipid levels (Anthony and Fabricius, 2000, Anthony et al., 2002). Smothering by sedimentation or sediment-trapping macroalgae is the main factor affecting recruitment and the survival of early life stages in corals (Fabricius, 2005). Significantly higher sedimentation rates found at sites with BBD compared to sites with no disease indicate that sedimentation may be an important environmental driver of coral diseases (Voss and Richardson, 2006a). By stressing corals, sediments may make corals more susceptible to infection by microbial pathogens and may also act as disease reservoirs (Voss and Richardson, 2006b). Terrigenous sediment stress and pollution has also been linked to BBD in the Pacific and in the Red Sea (Antonius, 1985, Littler and Littler, 1996, Al-Moghrabi, 2001). In Jamaica, excessive sedimentation associated with floods and run-off was linked to a BBD epizootic (Bruckner and Bruckner, 1997b).

Most of the riverine sediment on the GBR is initially deposited close to river mouths, but over time, the finer particles are re-suspended and carried along

the coast (Lembeck and Woolfe, 2000). The amount of fine sediment and nutrients transported by runoff each year into the GBR is directly related to the volume of freshwater discharge which is highest on the central section of the GBR (Furnas, 2003). So far, no studies investigating potential links between sedimentation and coral disease have been undertaken on the GBR.

1.3 SPECIFIC AIMS OF THIS STUDY

Despite increasing research effort on coral diseases in the Indo-Pacific, the environmental variables that control, trigger or promote these diseases are poorly understood. Consequently my overall objectives for this study were to document patterns of spatio-temporal disease dynamics and their environmental drivers within Indo-Pacific coral populations spanning a range of latitudes and contrasting environmental regimes. Research for this PhD study was carried out at three geographical locations: Wakatobi Marine National Park in South-East Sulawesi, Indonesia, an equatorial, high diversity region where the role of coral diseases has not been investigated; Heron Island, in the southern Capricorn-Bunker sector of the GBR, representing the most southerly development of coral reefs on the GBR; and Magnetic Island, an inshore reef in the central GBR, which is exposed to both high summer temperatures and high terrestrial inputs in rainy seasons.

My specific aims, corresponding to studies presented in chapters two to five, were as follows.

1. **To observe spatio-temporal patterns of coral disease dynamics in the Wakatobi Marine National Park (WMNP), Indonesia.**

   Little is known about coral diseases in the Coral Triangle, a global marine biodiversity hotspot. Understanding patterns of disease prevalence, disease progression rates, tissue mortality and the susceptibility of different taxa to disease will improve the understanding of how coral diseases may alter coral reef communities in a high diversity region.

2. **To describe inter-annual, spatio-temporal patterns of coral disease dynamics in relation to temperature, coral cover and coral community structure on Heron Island in the southern GBR.**

   Understanding temporal variation in disease dynamics in relation to temperature, coral cover and coral community structure will help to assess the impact diseases may have on coral resilience in the face of climate change on a comparatively high latitude reef.

3. **To document seasonal dynamics of the coral disease atramentous necrosis (AtN) in relation to nine seasonally varying environmental parameters to identify potential environmental drivers of AtN within populations of the coral *Montipora aequituberculata* on an inshore GBR reef.**

   Documenting seasonal patterns in AtN dynamics in relation to parameters associated with water quality on the GBR will enhance current understanding of how diseases shape coral communities according to the

prevailing environmental conditions. It will provide information that will contribute to predicting disease outbreaks and consequently enable the development of better management of coral reefs by elucidating parameters that increase disease incidence.

4. **To experimentally investigate the relationship between temperature and salinity in the progression of atramentous necrosis affecting *Montipora aequituberculata*.**

   An experimental approach will help to tease apart the effects of environmental factors correlated with disease prevalence in the field study and identify which are the most important driver(s) of AtN.

Chapter 2

Spatio-temporal coral disease dynamics in the Wakatobi Marine National Park, South-East Sulawesi, Indonesia

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2.1 ABSTRACT

In the present study we investigated inter-annual coral disease dynamics, *in situ* disease progression rates, and disease associated coral tissue mortality in the Wakatobi Marine National Park (WMNP) situated in the coral triangle in South-East Sulawesi, Indonesia. In 2005, only two known syndromes were recorded within the sampling area: white syndrome (WS; 0.42% prevalence) and growth anomalies (GA; 0.15% prevalence), whilst in 2007 four diseases were recorded over the same surveyed area: WS (0.19%), *Porites* ulcerative white spot disease (PUWS; 0.08%), GA (0.05%) and black band disease (BBD; 0.02%). Total disease prevalence decreased from 0.57% in 2005 to 0.33% in 2007. In addition to prevalence surveys, in situ progression rates of four diseases were investigated in 2007: BBD on *Pachyseris foliosa, P. rugosa* and *Diploastrea heliopora*, WS on *Acropora clathrata*, brown band (BrB) and skeletal eroding band (SEB) diseases on *Acropora pulchra*. BrB and WS had the highest progression rates, 1.2 ± 0.36 cm day^{-1} and 1.1 ± 0.07 cm day^{-1} respectively, indicating that diseases may have a significant impact on local *Acropora* populations. BBD had the lowest progression rate (0.39 ± 0.14 cm day^{-1}). WS caused the most severe recorded total tissue mortality; 53 923 cm^2 over a period of 36 days. Sedimentation and coral cover were studied and a highly significant drop in coral cover was observed. This study provides the first documentation of spatio-temporal coral disease dynamics from Indonesia. Despite low total disease prevalence, progression rates comparable to the ones observed in the Caribbean and Australia indicate that diseases may threaten the reef framework in some locations and add to the

degradation of coral reefs in a region already at high risk from anthropogenic impacts.

2.2 INTRODUCTION

Coral diseases have been recognised as one of the main factors contributing to the global deterioration of coral reefs (Weil et al., 2006). Coral diseases may contribute to coral-algal phase-shifts through mortality of key reef-building corals and consequent changes to the reef framework (Nugues, 2002). Coral disease prevalence studies can reveal trends in disease over time as well as predict possible changes to coral communities by identifying the coral taxa most affected by disease (Lafferty et al., 2004). Measuring rates of disease spread and tissue loss help in understanding the impacts diseases have on coral populations (Willis et al., 2004). Diseases have been well studied in the Caribbean which is considered a 'disease hot spot' due to the fast emergence and high virulence of coral reef diseases/syndromes, their widespread geographic distribution, wide host range, and frequent epizootic events with significant coral mortalities (Epstein et al., 1998, Hayes and Goreau, 1998, Green and Bruckner, 2000, Weil et al., 2002, Weil, 2004). However, much less is known about coral diseases in the Indo-Pacific region (Weil et al., 2006).

Examples of disease outbreaks from the Caribbean are numerous. One of the most devastating diseases has been white band disease that was first reported by Gladfelter (Gladfelter, 1982). The large-scale die-off of key reef-building corals *Acropora palmata* (now IUCN red-listed) and *Acropora cervicornis* has been attributed to the combined impacts of white band disease, white pox (Patterson et al., 2002) and hurricane damage (Woodley, 1989, Hughes, 1994).

Another example of a coral disease outbreak in the Caribbean is the rapidly spreading disease white plague II which has destroyed 75% of the key Caribbean reef-building coral *Dichocoenia stokesi* in 7 years and shifted the population structure in a way which suggests that the remaining population is no longer reproducing (Richardson and Voss, 2005).

Despite the fact that no major disease outbreaks have yet been reported from the Indo-Pacific, an increasing number of coral diseases have now been observed at several locations: Australia (Willis et al., 2004), the Philippines (Raymundo et al., 2003), Hawaii (Aeby, 2005), U.S. Pacific remote islands (Vargas-Angel, 2009) and Indonesia (Haapkyla et al., 2007). Diseases have been monitored on the Great Barrier Reef (GBR) since 1998 revealing a 20-fold increase in white syndrome (WS) between 1998 and 2003 (Willis et al., 2004). The overall disease prevalence was 8.97 ± 0.79% in northern Cooktown/Lizard Island and southern Capricorn Bunker sectors of the GBR in 2003 (Willis et al., 2004). Raymundo et al. (2005) reported a total disease prevalence of 8.3 ± 1.2% (n = 8 reefs) in the Philippines, and prevalence of *Porites* ulcerative white spot (PUWS) and growth anomalies (GA) were 53.7% and 39.1% respectively in 2002-2003 (Kaczmarsky, 2006). These studies indicate that infectious pathogens may be a common component of Indo-Pacific coral communities, and may play a greater role in structuring these communities than previously thought (Willis et al., 2004).

Spatio-temporal dynamics of coral diseases are often driven by environmental factors. Anomalously high temperature and other environmental stressors can influence the severity and dynamics of infectious coral diseases by increasing host susceptibility and pathogen virulence (Harvell et al., 2002,

Lafferty and Holt, 2003). The frequency of temperature anomalies, which is predicted to increase in most tropical oceans, can therefore increase the susceptibility of corals to disease, leading to outbreaks where corals are abundant (Bruno et al., 2007). Other environmental factors that can increase disease susceptibility include sedimentation (Voss and Richardson, 2006b), turbidity (Bruckner and Bruckner, 1997b) and nutrients (Bruno et al., 2003).

Little is known about the impacts of coral diseases within the coral triangle region of SE Asia which is regarded as a global marine biodiversity hotspot (Roberts et al., 2002). Wilkinson (2008) reported that coral reefs in Indonesia have continued to show an overall decline in condition since 2004, however the role of disease in this decline remains poorly understood. Haapkylä et al. (2007) documented the occurrence of coral disease in the Wakatobi Marine National Park (WMNP). In the present study we describe spatio-temporal coral disease dynamics and investigate the impact of disease on coral assemblages by recording the *in situ* progression rates and tissue mortality caused by four coral diseases in the WMNP.

2.3 METHODS

2.3.1 Study site

The WMNP is the second largest marine national park in Indonesia and covers an area of 1.39 million hectares. It is situated in the Tukangbesi Island region between the Banda and Flores Seas, South-East Sulawesi (3°- 6°S and 120°45' - 124°06'E) (

Figure 2-1). Indonesian coral reefs are among the most diverse in the world (Allen, 2008). The WMNP is situated in a Global Biodiversity Hotspot with 396 species of hermatypic scleractinian corals belonging to 68 genera and 15 families (Turak, 2003). 10 species of non-scleractinian or ahermatypic hard coral species and 28 soft coral genera are also found in the park (Pet-Soede and Erdmann, 2004). Coral reef habitats of the WMNP are mostly in a healthy state, but incidences of declining coral cover and reduced reef predators related to increased fishing pressure are cause for concern (Unsworth et al., 2007, McMellor, 2007).

Five sites located around the islands of Hoga and Kaledupa were surveyed in 2005 and 2007 (

Figure 2-1). These sites represent a typical Indonesian fringing reef. Sampela is the only site situated close to a local village. The fringing reefs at these sites range in depth from <1 m to approximately 35 m and are situated between 500 m and 1 km offshore. Sampling was conducted between 29 June and 16 September 2005 and between 30 June and 4 September 2007.

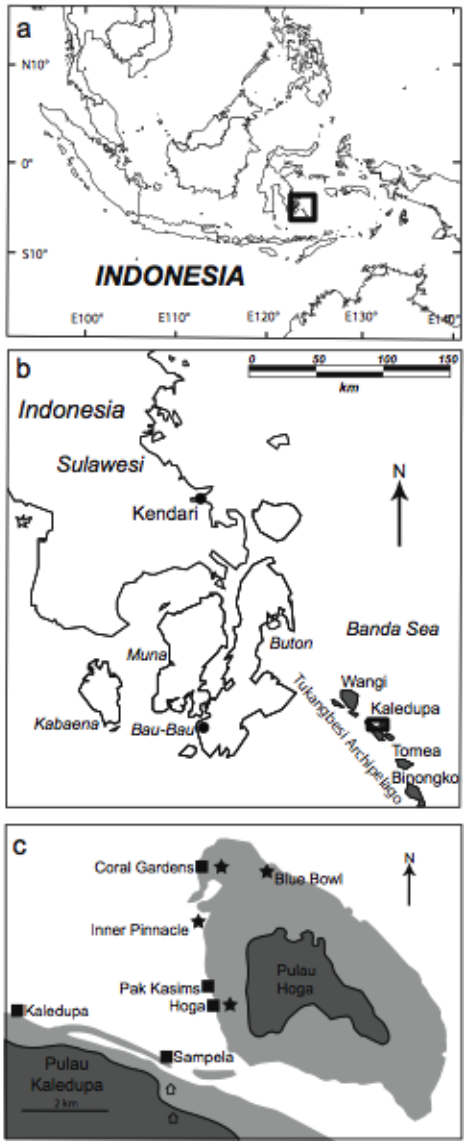


Figure 2-1. Map of survey sites. a) South-East Sulawesi marked with a rectangle; b) WMNP includes Tukangbesi Archipelago: Wangi, Kaledupa, Hoga (rectangle near Kaledupa), Tomea and Binongko Islands; c) survey sites used in 2005 and 2007 around Hoga Island. Rectangles mark disease prevalence survey sites and stars sites where disease progression rates and tissue mortality were observed. Areas with light shading represent reef flats around Hoga and Kaledupa Islands.

2.3.2 Disease prevalence study

Surveys were conducted using belt transects (English et al., 1997) covering an area of 4 x 20 m (2 m on each side of the transect line) in both years. Three

replicate transects were laid in three reef zones: flat (1-3 m depth), crest (3-7 m depth) and slope (8-12 m depth) and each transect was treated as one replicate in the analysis. A total of 45 transects were surveyed in both years. Transects followed the depth contour of the reef. The first transect was located randomly to satisfy assumptions about the independence of data for statistical analysis. The additional transects were located at randomly derived distances from the first transect, but always at a distance > 20 m to ensure independence and to detect site-specific trends and variances. Each coral colony within the belt was counted and recorded as healthy or diseased to genus or family levels according to disease survey methods described by Willis et al. (2004). Prevalence of each disease was calculated by dividing the number of diseased colonies by the total number of coral colonies. Due to the prevalence of disease among different coral taxa, this was believed to be the appropriate method. This method has been previously used in Indo-Pacific disease studies (Page and Willis, 2008, Raymundo et al., 2005) and (Vargas-Angel, 2009). Means and standard errors were calculated from all three transects at each reef zone at each site. Coral cover was estimated by the same observer by using the 0.5 m point intercept transect method in 2005 and the line intercept transect method in 2007 (English et al., 1997) over a 20 m line transect. Coral cover was measured at the five sites surveyed in both years and in the Blue Bowl in 2007.

2.3.3 Disease identification

A disease is defined as any deviation or alteration from the normal structure or function of any body part or organ manifested by a characteristic set of clinical

signs of known or unknown cause (Dorland, 1982). A lesion represents any functional and morphologic change in tissues during disease (Work and Aeby, 2006).

Coral diseases were identified by the presence and characteristics of lesions. Photographs of diseased corals were taken and identified using the Australian Institute of Marine Science (AIMS) coral disease identification cards and photographs compiled by Willis et al. (2004). Photographs were taken of all disease categories and used as a reference in order to keep identification of the diseases consistent. Lesions that did not correspond to any of the disease categories were classified as "undescribed" and abnormally pigmented lesions on corals were classified as "pigmentation responses". Samples of brown band disease (BrB) and skeletal eroding band (SEB) were collected and examined microscopically to verify the presence of ciliates that characterize these diseases.

2.3.4 Disease progression rates

The progression rates of black band disease (BBD), skeletal eroding band (SEB), brown band (BrB) and white syndrome (WS) were investigated by taking photographs from the same angle and including a flexible measuring tape. The diseases were studied at five sites: Blue Bowl, Coral Gardens, Pak Kasims, Inner Pinnacle and Hoga (

Figure 2-1). The observation time varied between 5 and 38 days depending on the disease. Progression rates of SEB and BrB on branching *Acropora pulchra* were investigated on randomly selected branches of separate coral colonies (SEB n=15, BrB n=4) (Table 2.3). A cable tie was secured onto the exposed skeleton a

short distance behind the disease front to avoid interfering with disease progression. The distance from the cable tie to the nearest live tissue at each observation time was measured from photographs using the software Canvas^{TM}X (System version 10.5.5). The difference between the last and the first measurement was used as a measure of linear disease progression and divided by the number of days between measurements to calculate a daily progression rate. Using the software Canvas^{TM}X (System version 10.5.5), three independent measurements extending from a stable reference point on the intersection between healthy and diseased coral tissue/freshly exposed skeleton were recorded for each colony with BBD (foliaceous *Pachyseris foliosa* n=19, hemispherical *Diploastrea heliopora* n=1, laminar *Pachyseris rugosa* n=1) and WS (laminar *Acropora clathrata* n=6) after each observation time (Table 2.3). The mean rate of disease spread between survey times was determined for each colony by calculating the difference between the respective measurements for each survey date (i.e. subtracting the length of measurement 1 in the image from July 4th from measurement 1 on July 10th) and averaging the three resultant differences. The average measurements from each colony were divided with the number of days between survey dates to calculate an average daily disease progression rate. Finally, the mean rate-of-spread of disease for each affected species between each successive survey period was calculated using the data from all individual colonies within each species.

2.3.5 Tissue loss due to coral disease

To determine the tissue loss due to BBD and WS, the surface area of dead coral tissue was measured for each survey date by using the software Canvas^{TM}X (System version 10.5.5). The average tissue losses were calculated as described above for the disease progression. The surface area of *D. heliopora* colony was estimated assuming a hemispherical colony shape: $A = 4\pi r^2/2$ where A = surface area, r = radius

The one colony of *D. heliopora* that we measured tissue loss on had a radius of 50 cm. The surface area of *A. pulchra*s impacted by SEB and BrB was calculated by using the formula for cylinder area: $A = 2\pi(R) \times h$ where A = surface area, R = radius and h = height (i.e. dead coral tissue)

The diameter of *A. pulchra* varies between 7-15 mm (Wallace, 1978). We used the mean of the smallest diameter (3.5 mm) and the largest diameter (7.5 mm) to obtain a range of tissue loss caused by disease.

2.3.6 Environmental parameters

Sedimentation rates were assessed using four standard sediment traps (English et al., 1997) which were deployed at each depth within all sites in the two-year study and at Blue Bowl for a 10-day period in 2007. Sediment and water were filtered, dried and weighed with rates expressed as milligrams dry weight cm^{-2} day^{-1}.

2.3.7 Statistical Analyses

Three-way permutational ANOVAs (Anderson, 2001, McArdle and Anderson, 2001) of disease prevalence, WS and GA prevalence and coral cover were performed using Permanova (version 1.6). Sedimentation values were log10-transformed and a 2-way nested ANOVA was conducted by using MINITAB (version 13.20). Tukey tests were used for post-hoc multiple comparisons. We used $\alpha = 0.05$ for all tests. The distribution of coral diseases within each coral taxa in each site in each year were compared using non-metric multi-dimensional scaling (MDS) based on Bray-Curtis similarity measures. Difference between years, transects and reef zones were tested using ANOSIM, which is a non-parametric permutation procedure. After identification of which transects and years differed the most (ANOSIM pairwise test output), SIMPER analysis was run on the data matrix. SIMPER decomposes Bray-Curtis dissimilarities between all pairs of samples to identify those species that contribute most to differences (Clarke and Warwick, 2001). All multivariate analyses were conducted using PRIMER (version 6.1.10).

2.4 RESULTS

A total of 12271 colonies were encountered in 2005 and 12752 colonies in 2007 in an area of 3600 m^2 (45 belt transects of 4x20 m). These colonies represented 32 coral taxa. Total disease prevalence dropped significantly from 0.57% in 2005 to 0.33% in 2007 ($F_{1,60}$= 4.84, P<0.05) (

Figure 2-2, Table 2.1). In 2005 the most frequent type of lesion was the undescribed category (9.7% of all colonies with lesions that did not correspond to any of the disease categories), while pigmentation responses were most frequent in 2007 (3.42% of all colonies had non-normally pigmented lesions). Neither the undescribed category nor the pigmentation responses were included in the disease prevalence calculations since they are not considered to be coral diseases.

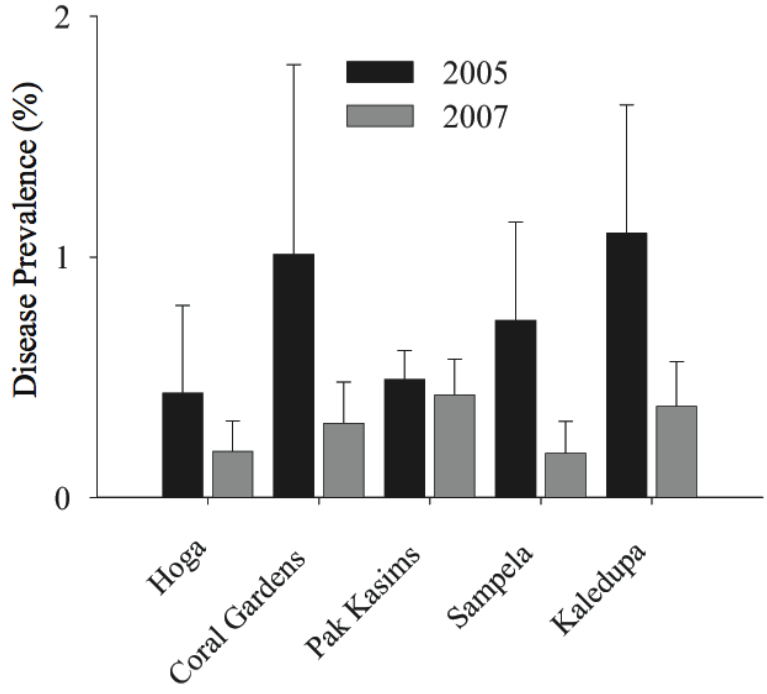


Figure 2-2. Mean disease prevalence (± SE) for five sites (Kaledupa, Sampela, Hoga, Pak Kasims and Coral Gardens) in 2005 and 2007 in the WMNP.

In 2005, only two known syndromes occurred within the sampling area: WS (0.42%) and GA (0.15%) (Haapkyla et al., 2007). In 2007, the prevalence of these two syndromes was lower: 0.19% for WS and 0.05% for GA. In addition, two other syndromes were identified in the sampling area in 2007: PUWS (0.08%) and BBD (0.02%).

There was a significant interaction between reef zone and overall disease prevalence ($F_{20.60}$=2.35, P<0.01) (Table 2.1) with a significant difference between Hoga crests and slopes in 2005, and Sampela flats and slopes in 2007. The prevalence of WS was significantly lower in 2007 ($F_{1,60}$=10.02, P<0.01). There was a significant reef zone interaction with the presence of WS ($F_{20.60}$= 2.49, P<0.01) (Table 2.1) with a significant difference between Coral Gardens flat and slope and Hoga crest and slope in 2005, and between Hoga crest and flat and Sampela crest and flat in 2007. No significant differences were found between years or reef zones for the prevalence of growth anomalies (Table 2.1).

Table 2.1. Three-way nested permutational multivariate analysis of variance (PERMANOVA) for disease prevalence, white syndrome, growth anomalies and coral cover in the WMNP between 2005 and 2007 at five sites (Hoga, Sampela, Pak Kasims, Kaledupa and Coral Gardens), over three habitats (reef flat, slope and crest). Significant differences are taken as those with a Monte Carlo permutational p-value < 0.05.

		Total Disease Prevalence		**White Syndrome**		**Growth anomalies**		**Coral cover**	
Source	d.f.	F	*P*-value (MC)	F	*P*-value (MC)	F	*P*-value (MC)	F	*P*-value (MC)
Year*	1	4.84	<0.05	10.02	<0.01	0.78	NS	81.48	<0.0001
Site (Year)*	8	1.61	NS	1.53	NS	1.96	NS	6.89	<0.0001
Reef zone (Year x Site)*	20	2.35	<0.01	2.49	<0.01	1.22	NS	5.62	<0.0001
Residual	60								
Total	89								

Non-metric Multi-Dimensional-Scaling (nMDS) overlaying years and sites (Figure 2-3) together with ANOSIM showed a significant difference in the distribution of diseases within the coral assemblage between years ($R=0.23$, $p<0.001$) and zones ($R=0.17$, $p<0.001$), and a weak significant difference between sites ($R=0.07$, $P<0.01$). The difference between zones is present at all sites ($p<0.001$), whilst the difference ($P<0.05$) between sites is only present between Hoga-Coral Gardens, Hoga-Sampela, Coral Gardens-Pak Kasims, Coral Gardens-Sampela. SIMPER analysis found that the overall dissimilarity between the two years was mostly the result of differences in the abundance of disease on *Porites* massive (5.34% contribution), M*ontipora* (4.97% contribution) and Dendrophyllids (4.68% contribution) (Table 2.2).

Table 2.2. SIMPER analysis (Primer v.6.1.5) to determine four most similar and dissimilar (decreasing similarity and dissimilarity from top to bottom) coral taxa between 2005 and 2007 in terms of abundance.

Coral taxa	**Average Similarity 2005**	**Coral taxa**	**Average Similarity 2007**	**Coral taxa**	**Average Dissimilarity 2005/2007**
Porites massive	28.37	*Porites* massive	38.29	*Porites* massive	5.34
Montipora	4.83	*Montipora*	2.80	*Montipora*	4.97
Favia / Favites / Montastrea	3.52	Dendrophyllids	2.46	Dendrophyllids	4.68
Agariciidae	2.49	*Porites* branching	1.07	Favia / Favites / Montastrea	3.95

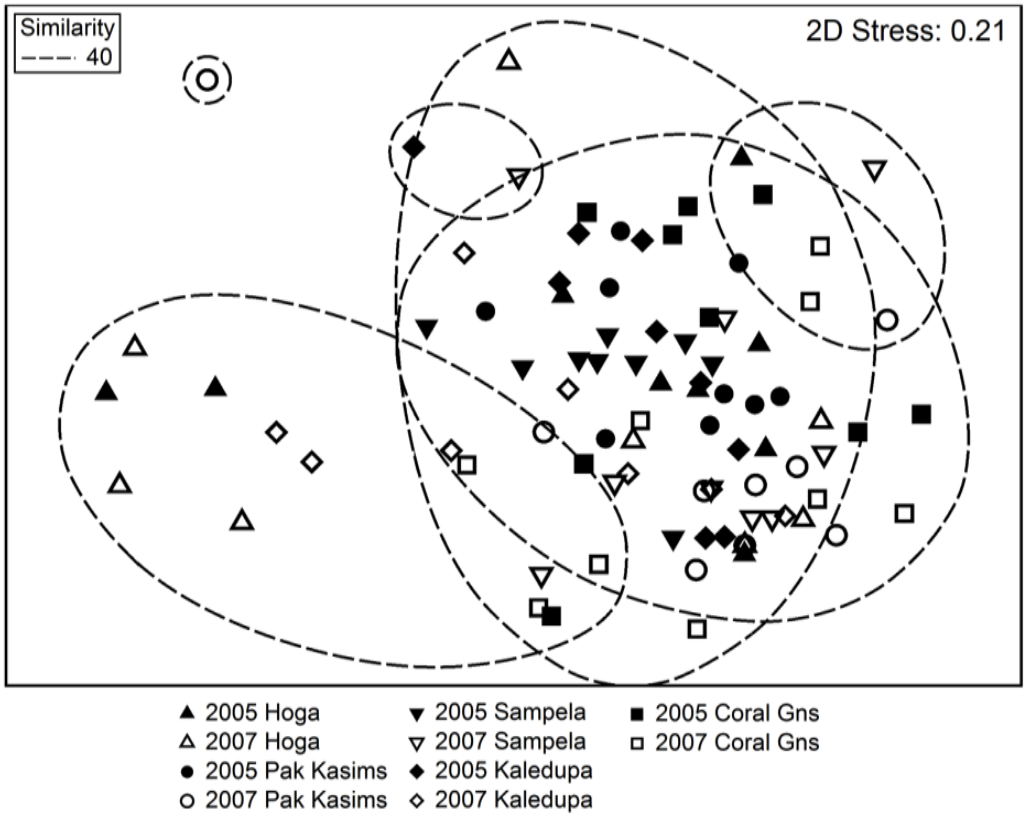


Figure 2-3. Non-metric multi-dimensional scaling (MDS) with superimposed Bray-Curtis similarity clusters (oval shapes) at the 40% similarity level illustrates the distribution of coral diseases within coral assemblages within each site in 2005 and 2007. Sampling used five sites: Kaledupa, Sampela, Hoga, Pak Kasims and Coral Gardens.

In 2005, 13 coral taxa were diseased compared to only 5 in 2007 (

Figure 2-4). *Montipora* was the most common coral genus in both years and suffered very little from disease. WS was most prevalent on massive *Porites* in 2005 and on Acroporids in 2007. PUWS was observed for the first time in 2007 (

Figure 2-4).

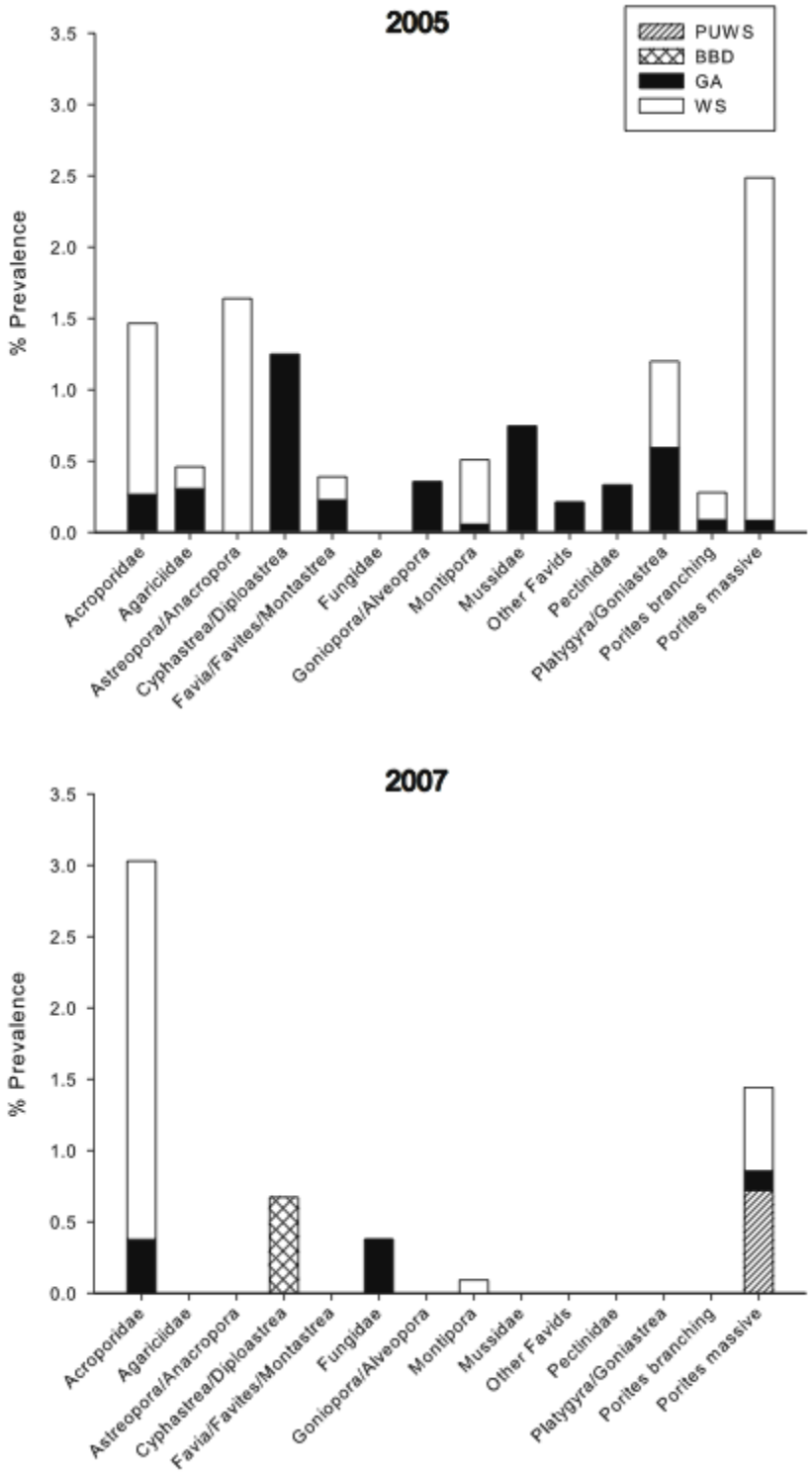


Figure 2-4. Prevalence of four disease categories: white syndrome (WS), growth anomalies (GA), Porites ulcerative white spot syndrome (PUWS) and black band disease (BBD) in scleractinian taxa in 2005 and 2007. Prevalence (per taxa) is calculated relative to the total number of colonies examined in the respective taxa in each year.

Diseases were more common on flats and crests in 2005, but no clear link was found between disease prevalence and depth in 2007 (Figure 2-5).

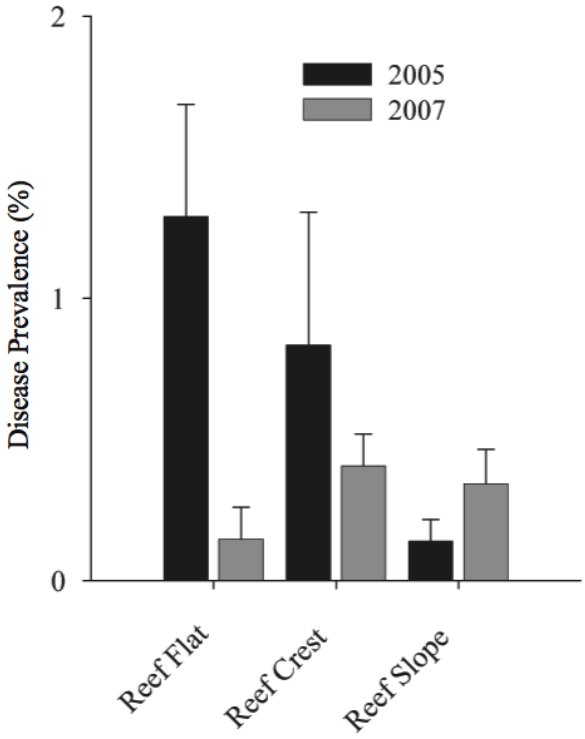


Figure 2-5. Mean distribution of diseases per reef zone (± SE) for five sites (Kaledupa, Sampela, Hoga, Pak Kasims and Coral Gardens) in 2005 and 2007 in the WMNP.

The in situ disease dynamics study of four diseases revealed that BrB and WS had the fastest progression rates (1.2 ± 0.36 cm day^{-1}, n=4) and (1.1 ± 0.07 cm day^{-1}, n=6) respectively (Table 2.3). BBD lesions progressed at the slowest rate (0.39 ± 0.14 cm day^{-1}, n=21) (Table 2.3). WS caused the greatest total tissue mortality; a total of 53 923 cm^2 over a period of 36 days and BBD the second most severe mortality; a total of 16 783 cm^2 over a period of 38 days.

Table 2.3. Summary of linear progression rates of coral diseases. In bold the results from the present study.

Disease	Location	Coral host	Mean progression rate	Reference
BBD	**Blue Bowl, WMNP, Indonesia**	***Pachyseris foliosa* (n=19)**	**0.13 ± 0.02 cm/day**	**Haapkylä et al. (present study)**
BBD	**Inner Pinnacle, WMNP, Indonesia**	***Pachyseris rugosa* (n=1)**	**0.63 cm/day**	**Haapkylä et al. (present study)**
BBD	**Pak Kasims, WMNP, Indonesia**	***Diploastrea heliopora* (n=1)**	**0.42 cm/day**	**Haapkylä et al. (present study)**
BBD	GBR, Australia	*Acropora muricata*	0.41-0.99 cm/day	(Boyett et al., 2007)
BBD	W-Caribbean	Up to 21 species	0.33-1 cm/day	Summarized in (Weil, 2004)
WS	Pak Kasims, WMNP, Indonesia	*A. clathrata* (n=1)	1.16 cm/day	Haapkylä et al. (present study)
WS	**Coral Gardens, WMNP, Indonesia**	***A. clathrata* (n=5)**	**1.03 ± 0.28 cm/day**	**Haapkylä et al. (present study)**
WS	Solitary Islands, Australia	*A. solitaryensis*	0.039-0.52 cm/day	(Dalton and Smith, 2006)
White plague I	Florida	Up to 21 species	0.31 cm/day	Summarized in (Weil, 2004)
White plague II	W-Caribbean	Up to 39 species	2 cm/day	(Richardson et al., 1998)
Caribbean Yellow band	Curacao, Caribbean	*Montastraea annularis*	0.6 cm/month	(Cervino et al., 2001)
Dark spot syndrome	Curacao, Caribbean	*Siderastrea siderea, Stephanocoenia michelinii*	4 cm/month	(Cervino et al., 2001)
SEB	**Hoga, WMNP, Indonesia**	***A. pulchra* (n=15)**	**0.5 ± 0.1 cm/day**	**Haapkylä et al. (present study)**
SEB	GBR, Australia	*A. muricata*	0.03-0.33 cm/day	(Page and Willis, 2008)
BrB	**Hoga, WMNP, Indonesia**	***A. pulchra* (n=4)**	**1.2 ± 0.36 cm/day**	**Haapkylä et al. (present study)**
BrB	GBR, Australia	*A. muricata*	max. 2.1 ± 0.35 cm/day	(Boyett, 2006)

A highly significant decrease in coral cover was observed between years ($F_{1,60}$ =81.48, P<0.0001), in addition to coral cover differing significantly between study sites ($F_{8,60}$=6.89, P<0.0001) and reef zones ($F_{20,60}$=5.62, P<0.0001) (Table 2.1, Figure 2-6). In 2005, all sites were significantly different except for Pak Kasims and Coral Gardens which had similar coral cover. There were no significant differences between sites in 2007. Blue Bowl, considered a pristine site

and studied for the first time in 2007, had by far the highest coral cover (74.7%), it was not included in any of the PERMANOVAs which included only the 5 sites of the disease prevalence study. Amongst these sites, Hoga had the highest coral cover in both years (44.78% in 2005 and 24.46% in 2007) whereas Sampela had the lowest cover in 2005 (12.33%) and Kaledupa the lowest in 2007 (8.8%) (Figure 2-6).

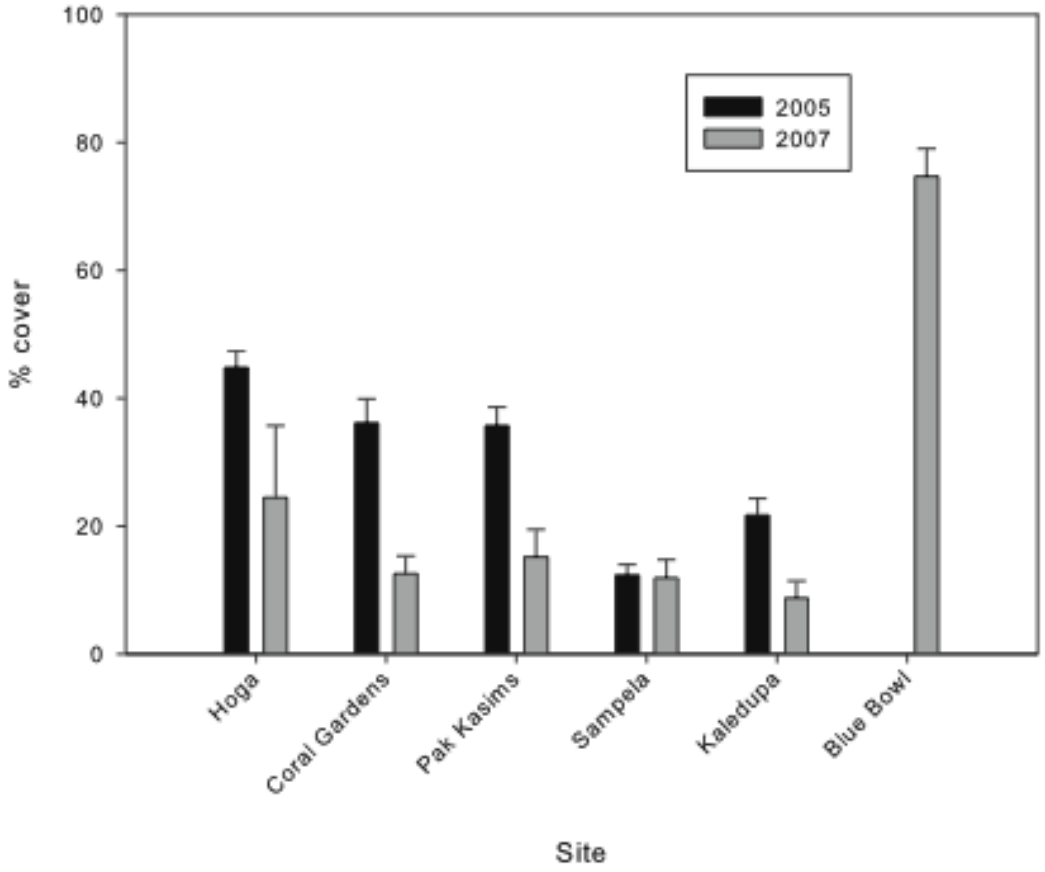


Figure 2-6. Mean coral cover (± SE) for six sites (Kaledupa, Sampela, Hoga, Pak Kasims, Coral Gardens and Blue Bowl) in 2005 and 2007 in the WMNP. Blue Bowl was not part of the PERMANOVA.

Sedimentation was significantly different between sites ($F_{5,38}$=27.00, P<0.0001) and reef zones ($F_{12,38}$=2.04, P<0.05). Blue Bowl (more than 15 mg cm^{-2} day^{-1}) was significantly different from all the other sites except for Sampela

(11.5 mg cm^{-2} day^{-1} on the slope). All the other sites had a sedimentation rate of less than 5 mg cm^{-2} day^{-1} (Figure 2-7).

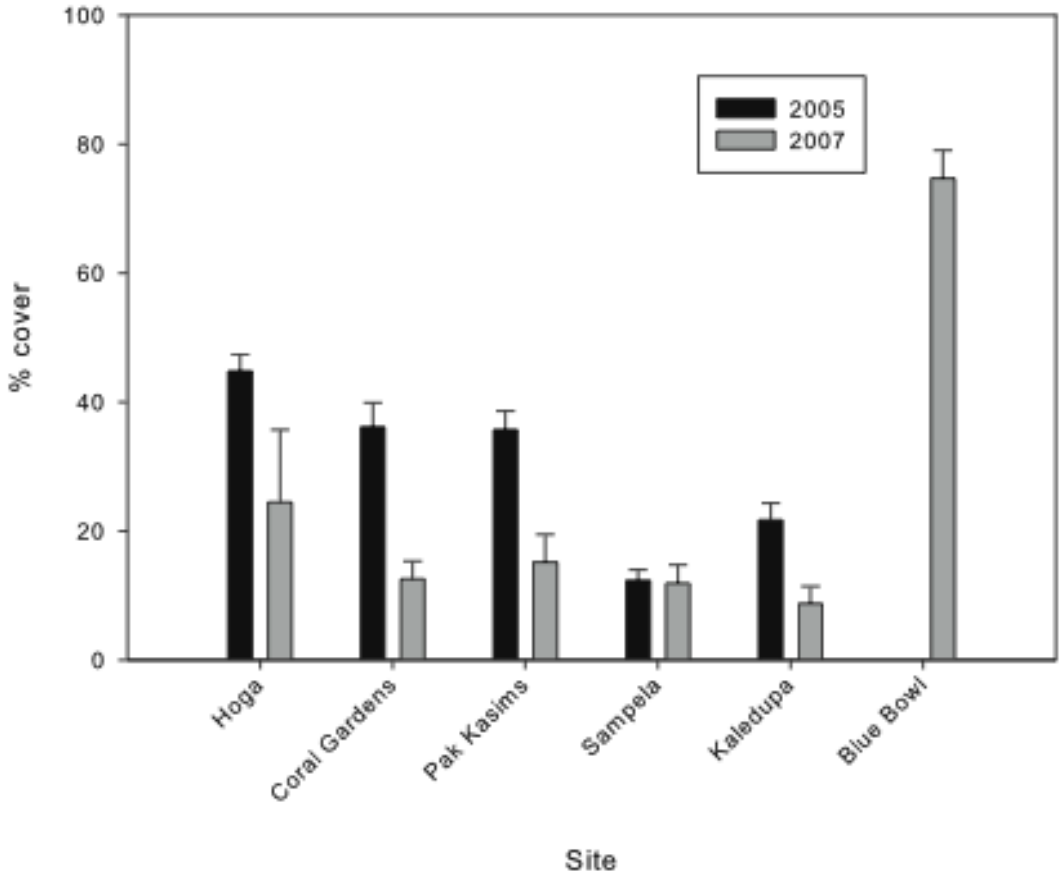


Figure 2-7. Mean sedimentation rate (± SE) for six sites (Kaledupa, Sampela, Hoga, Pak Kasims, Coral Gardens and Blue Bowl) in 2005 and 2007 in the WMNP.

2.5 DISCUSSION

Our study represents the first description of spatio-temporal coral disease dynamics, disease progression and tissue mortality in Indonesia. The annual variability of diseases was high. There was a significant decrease in disease prevalence from 0.57% in 2005 to 0.33% in 2007. The number of diseased coral taxa decreased from 13 in 2005 to only 5 in 2007. The overall disease prevalence in the WMNP is low and similar to the 0.21% overall prevalence recently found in the remote Pacific islands (Vargas-Angel, 2009). Despite the observed decrease in prevalence, two new syndromes were observed in our 2007 surveys: *Porites* ulcerative white spot disease (PUWS) and black band disease (BBD). PUWS has previously been detected in the Philippines where it had a high prevalence (Raymundo et al., 2003, Raymundo et al., 2005).

Of the diseases found in the present study, PUWS, brown band disease (BrB), skeletal eroding band (SEB) and white syndrome (WS) (which is a collective term for Indo-Pacific white diseases), are found only in the Indo-Pacific (Raymundo et al., 2003, Willis et al., 2004), whereas growth anomalies (GA) and BBD are both found globally (Sutherland et al., 2004).

This study reveals for the first time the presence of brown band disease (BrB) in the Wakatobi. SEB was also found at sites outside the area surveyed for disease prevalence in 2005 (Haapkyla et al., 2007). BrB and SEB are both characterised by dense aggregations of ciliates on the surface of the coral: SEB harbouring *Halofolliculina corallasia* (Antonius and Lipscomb, 2001) and BrB a ciliate belonging to the class Oligohymenophorea, subclass Scuticociliata (Bourne et al., 2008). The prevalence of BrB on the GBR is less than 1% and more

common on the southern GBR (Willis et al., 2004). SEB was the most prevalent coral disease on the GBR in 2004-2006 accounting for 40-60% of disease cases recorded in each year (Page and Willis, 2008). Moreover, it was found affecting 38% of corals in the Red Sea (Winkler et al., 2004). Another ciliate of the genus *Halofolliculina* was found from the Caribbean (Croquer et al., 2006a), although it appears to be a different species to the one found in the Indo-Pacific (Croquer et al., 2006b). Therefore SEB is considered an Indo-Pacific disease and its Caribbean variation is called Caribbean ciliate infection (Rodriguez et al., 2009).

Our results show a significantly lower prevalence of WS in 2007 but no significant difference in GA prevalence. In 2007, diseases were less common in shallow reef zones than in 2005. Only one previous study on depth and disease prevalence exists from the Indo-Pacific where Raymundo et al. (Raymundo et al., 2003) found that PUWS prevalence was not depth dependent in the Philippines. In the Caribbean white plague-infected corals were most common between 8 and 18 m depth (Dustan, 1977). A similar depth pattern was found in Venezuela (Croquer et al., 2003).

There was a highly significant drop in coral cover at all sites in the WMNP between 2005 and 2007 (Figure 2-3, Table 2.1). Kaledupa, considered a pristine site in 2005, had the lowest coral cover of all sites (8.8%) whereas Blue Bowl, dominated by foliaceous corals, was studied for the first time in 2007 and had the highest coral cover (74.7%). The overall decrease in coral cover may be due predominantly to anthropogenic exploitation of marine resources such as overfishing and intensive harvesting of invertebrates at low tide (McMellor, 2007,

McMellor, 2008). Regular monitoring of coral disease could reveal the potential influence of localized coral disease outbreaks in declining coral cover.

The major driver of change in the distribution of disease in the coral assemblage between 2005 and 2007 is, according to the SIMPER analysis (Table 2.2), the differences in the abundance of diseases on massive *Porites* (5.34% contribution), M*ontipora* (4.97% contribution) and Dendrophyllids (4.68% contribution). *Porites* and *Montipora* are the two major coral genera in the area. Massive *Porites* was the only taxa showing signs of three syndromes (PUWS, GA and WS). It is a dominant component of Indo-Pacific reefs. Diseases could potentially have larger impacts on massive *Porites* and the reef structure because of its slow growth rates (around 1 cm a year) (Patzold, 1984). In 2007, acroporids represented only 4.6 % of the total number of corals but they were found to be the most diseased coral taxa (3% of all acroporids were diseased). Acroporids are subject to a number of coral diseases such as BBD (Page and Willis, 2006), BrB (Willis et al., 2004), SEB (Page and Willis, 2008) on the GBR and white band (Aronson and Precht, 2001) and white pox (Patterson et al., 2002) diseases in the Caribbean. However, fast growth rates of *Acropora* may compensate for the mortality. Yap & Gomez (Yap and Gomez, 1985) recorded a growth rate of 0.3-2.3 cm per month for *A. pulchra* in the Philippines.

A positive relationship between host density and disease prevalence has been clearly demonstrated in many host-pathogen systems (Lafferty, 2004, Altizer et al., 2003, Rudolf and Antonovics, 2005, Anderson and May, 1979) and is considered a hallmark of the infectious process (Lafferty and Gerber, 2002). Host density is most often associated with greater rates of horizontal transmission (Holt

and Pickering, 1985, Getz and Pickering, 1983, Altizer and Augustine, 1997), leading to localized increase in prevalence. In addition, host density can be positively related to the density of disease vectors (Rosenberg and Falkovitz, 2004, Williams and Miller, 2005). High coral cover has been linked to a high prevalence of WS on the Great Barrier Reef of Australia (Bruno et al., 2007). High cover of *Pachyseris foliosa* in the Blue Bowl may have facilitated the spread of BBD infections.

Sedimentation may also be a driver of coral disease. Voss and Richardson (2006b) observed a link between high sedimentation rate and BBD in the Caribbean and proposed that sediments may act as vectors of coral disease. Sedimentation rates of <10 mg cm^{-2} day^{-1} are typical for reefs not subject to human disturbance (Rogers, 1990). The rates obtained in our study were within this range except for the Blue Bowl, a pristine site, where the rate exceeded 15 mg cm^{-2} day^{-1} (over 40 mg cm^{-2} day^{-1}on the flat). The bowl-like topography of the Blue Bowl and the close proximity of a large sandy reef flat may have enhanced the accumulation of sediments at the site. High sedimentation rate in the Blue Bowl may have contributed to the occurrence of BBD on the slope at this site.

Warmer water temperatures have been linked to higher disease prevalence and progression rates in diseases such as WS (Bruno et al., 2007, Willis et al., 2004) and BBD (Boyett et al., 2007). Prevalence, progression rates and tissue mortality due to coral disease may have been higher at the study sites in the warmer wet season when water temperatures exceed 30 ºC. As a consequence, further studies in the wet season are needed to evaluate seasonal ranges in disease prevalence and dynamics in the WMNP.

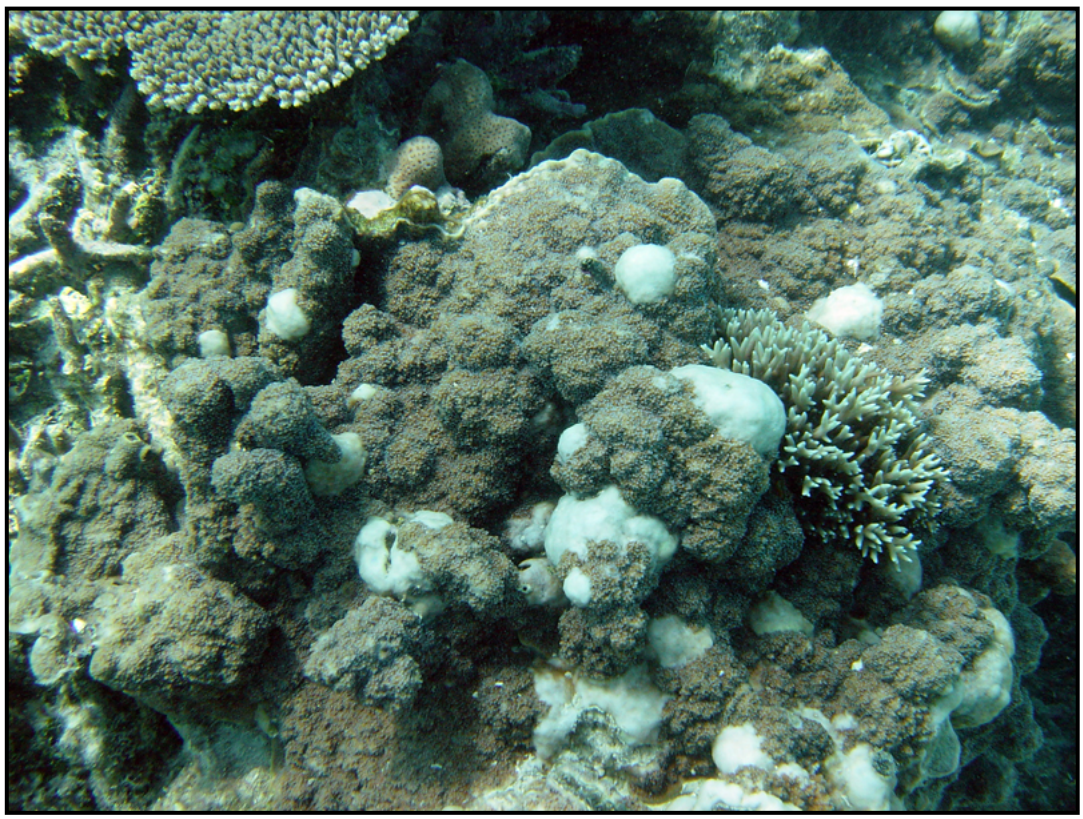
Our study of disease progression rates and tissue mortality accentuates the fact that conclusions about impacts of coral disease should not solely be based on the results obtained from transect monitoring. SEB, WS and BBD all had faster progression rates in the WMNP than those reported from the GBR (Table 2.3). In our study BrB (1.2 ±0.36 cm day^{-1}) had the highest progression rate and WS (1.1 ± 0.07 cm day^{-1}) the second highest. The fastest progression rates in the Caribbean were recorded for white plague type II infections with similar progression rates to BrB on the GBR where the progression rate of BrB was up to 2.1 cm day^{-1} in the austral summer (Boyett et al., 2007).

In this study, WS caused more severe total tissue loss (53 923 cm^2) than BBD (16 783 cm^2). BrB was observed only for 5 days compared to 36 days for WS and 38 days for BBD which resulted in less tissue mortality through time despite the fast progression rate of BrB. Coral diseases may have important impact locally in re-structuring reefs by impacting key reef-building corals.

In conclusion, despite a low overall disease prevalence, we documented fast coral disease progression rates and high tissue mortality rates for coral diseases in the WMNP; our research suggests that coral diseases may contribute to the decline of coral cover in this region. Further effort should be dedicated to understanding coral disease dynamics in Indonesia to better inform management and conservation approaches for reef ecosystems in this hotspot of biodiversity.

Chapter 3

Spatiotemporal patterns of coral disease prevalence on Heron Island, Great Barrier Reef, Australia



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3.1 ABSTRACT

Despite increasing research effort on coral diseases, little is known about factors driving disease dynamics on the Great Barrier Reef (GBR). This is the first study to investigate the temporal patterns of coral disease prevalence and potential drivers of disease around Heron Island in the southern Capricorn Bunker sector of the GBR. Surveys were conducted in two austral summers and three winters between November 2007 and August 2009 on six sites around the island. Six diseases were detected: brown band syndrome (BrB), growth anomalies (GA), ulcerative white spots (UWS), white syndrome (WS), skeletal eroding band disease (SEB) and black band disease (BBD). The lowest overall mean disease prevalence was 1.87 ± 0.75% (mean ± SE) in November 2007 and the highest 4.22 ± 1.72% in August 2008. There was evidence of seasonality for two diseases: BrB and UWS. This is the first study to report a higher prevalence of BrB in the winter. BrB had a prevalence of 3.29 ± 0.58% in August 2008 and 1.53 ± 0.28% in August 2009 while UWS was the most common syndrome in the summer with a prevalence of 1.12 ± 0.31% in November 2007 and 2.67 ± 0.52% prevalence in January 2008. The prevalence of GAs and SEB did not depend on the season, although the prevalence of GAs increased throughout the study period. WS had a slightly higher prevalence in the summer but its overall prevalence was low (<0.5%). Sites with high abundance of staghorn *Acropora* and *Montipora* were characterised by the highest disease prevalence (12% of *Acropora* and 3.3% of *Montipora* species were diseased respectively). These results highlight the correlations between coral disease prevalence, seasonally varying environmental parameters and coral community composition. Given that diseases are likely to

reduce the resilience of corals, seasonal patterns in disease prevalence deserve further research.

3.2 INTRODUCTION

Coral reefs are increasingly threatened worldwide particularly by impacts associated with climate change (Veron et al., 2009), changes in water quality from terrestrial runoff (De'ath and Fabricius, 2010), and over-exploitation (Jackson et al., 2001). In the last three decades, coral diseases have become a significant cause of coral mortality and habitat loss especially in the Caribbean (Aronson et al., 1998). The Caribbean is considered a disease "hot spot" because of the rapid emergence and high virulence of coral reef diseases, the widespread geographic distributions and host ranges of some of these diseases, and frequency of epizootic events that have caused significant coral mortality (Epstein et al., 1998, Hayes and Goreau, 1998, Green and Bruckner, 2000, Weil et al., 2002, Weil, 2004). Recent reports from the Indo-Pacific also reveal the potential for serious impacts of coral diseases on reef-building corals of the world's most diverse coral reef ecosystems (Raymundo et al., 2003, Willis et al., 2004, Aeby, 2005, Haapkyla et al., 2007, Haapkyla et al., 2009, Page and Willis, 2008, Sato et al., 2009, Vargas-Angel, 2009).

Despite global research efforts, the ecological drivers of coral disease, and the long-term consequences of disease for coral communities remains poorly understood (Richardson, 1998, Harvell et al., 2002). Anomalously high temperatures and other environmental stressors can influence the severity and dynamics of infectious coral diseases by increasing host susceptibility and pathogen virulence (Harvell et al., 2002, Lafferty and Holt, 2003). The frequency of temperature anomalies, which is predicted to increase in most tropical oceans, can therefore increase the susceptibility of coral communities to disease outbreaks

(Bruno et al., 2007). Other environmental factors that can increase disease susceptibility include sedimentation (Voss and Richardson, 2006b), turbidity (Bruckner and Bruckner, 1997b) and nutrients (Bruno et al., 2003).

The high variability in disease prevalence, even at small spatial scales within reefs, and the characteristic patchy distribution of epizootic events further complicate our interpretation of biological and/or environmental causes (Weil and Croquer, 2009). Such high variability in prevalence and its patchy distribution in space within reefs and countries might be a consequence of (1) differences in susceptibility associated with differences in coral community composition and the spatial distribution of coral colonies, (2) variability in environmental conditions, and (3) variability in pathogen sources and vectors (Weil and Croquer, 2009). Repeated coral disease prevalence studies at the same site can reveal trends in disease over time as well as predict possible changes to coral communities by identifying the coral taxa most affected by disease (Lafferty et al., 2004).

Monitoring of coral disease has revealed at least eight diseases that commonly occur on reefs stretching along more than 1200 of the 2000 km length of the Great Barrier Reef (GBR) (Willis et al., 2004), which is the largest coral reef tract under management worldwide. Between 1998 and 2003, there was a 20-fold increase in white syndrome (WS) particularly on reefs in the southern GBR (Willis et al., 2004). The latest report from the Australian Institute of Marine Science's long-term monitoring program reveals that trends in coral disease abundance are temporally variable (Sweatman et al., 2008). The abundance of WS has declined since the peak in 2003, but remained at intermediate levels in 2006 and 2007, although these levels were still 7-fold higher than in 1999 when

monitoring started (Sweatman et al., 2008). Available evidence indicates that the overall status of coral reefs on the GBR is relatively good, but is likely to be declining, especially in inshore areas (GBRMPA, 2009). However, the picture is not clear-cut, with reefs in different regions showing enormous differences in trends, including both increases and declines in coral cover. In part, this may be because coral reefs are naturally very dynamic habitats, with ongoing cycles of disturbance and recovery (GBRMPA, 2009). However, recent studies of coral disease dynamics on the GBR also suggest that diseases may play a greater role in structuring these communities than previously thought (Jones et al., 2004b, Willis et al., 2004, Boyett et al., 2007, Anthony et al., 2008, Page and Willis, 2008, Sato et al., 2009).

Given uncertainties regarding the dynamics and causes of variability in coral disease prevalence, we investigated correlates of temporal dynamics in coral diseases around Heron Island, in the southern Capricorn Bunker sector of the Great Barrier Reef (GBR), Australia. The relationship between disease prevalence and susceptible coral taxa was documented for the first time in this area.

3.3 METHODS

3.3.1 Study site

Six sites were located around the periphery of the reef at Heron Island which is a typical platform reef situated in the southern Capricorn Bunker sector of the GBR (23º25.800'S 151º59.940'E). Three sites were located on the northern leeward side and three sites on the southern exposed side of the reef (Figure 3-1). Transects were laid parallel to depth contours at 5 m depth and surveyed on five occasions between November 2007 and August 2009, including two surveys in the austral summer and three in the austral winter. These sites represent a typical fringing reef on the GBR.

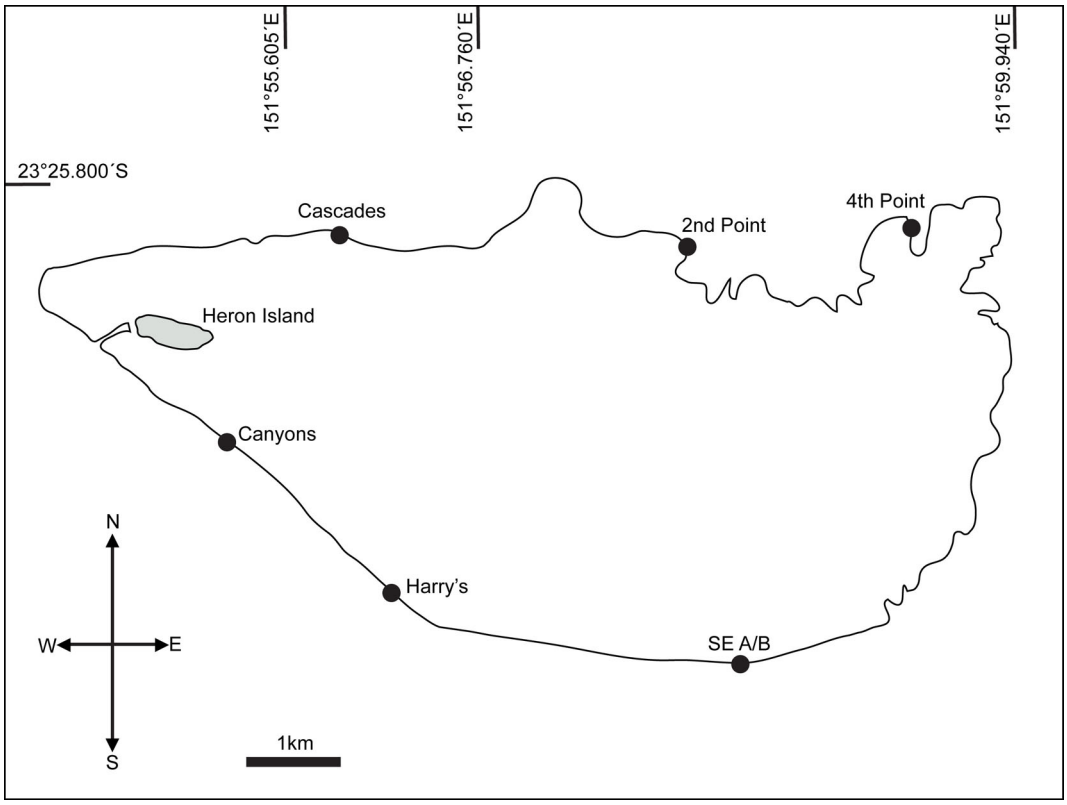


Figure 3-1. Map of six survey sites around Heron Island. The solid line represents the limit of the reef flat.

3.3.2 Disease identification

A disease is defined as "any deviation or alteration from the normal structure or function of any body part or organ manifested by a characteristic set of clinical signs of known or unknown cause" (Dorland, 1982) with a lesion representing any functional or morphological change in tissues associated with disease (Work and Aeby, 2006). Coral diseases recorded in this study were identified by macroscopic characteristics of lesions according to photographs and descriptions in Beeden et al. (Beeden et al., 2008) and Willis et al. (Willis et al., 2004). Photographs were taken of all diseases recorded and used as a reference in order to maintain consistency of disease identification through time. Samples of brown band syndrome (BrB) and skeletal eroding band (SEB) were collected and examined microscopically to verify the presence of ciliates that characterize these diseases (see photos in Willis et al., 2004, Beeden et al., 2008).

3.3.3 Disease surveys

Surveys were conducted using belt transects (English et al., 1997) covering an area of 1m x 15 m (1 m on the crest side of the transect line). Transects were permanently marked with metal stakes and plastic cattle tags. Eight replicate transects were laid on the slope (5 m depth) at each site and each transect was treated as one replicate in the analyses. Due to weather constraints, a total of 43 transects were completed during the first survey in 2007, whereas 48 transects were completed in subsequent surveys. A gap of at least 5 m was left between transects to ensure independence and to detect site-specific trends and variances. Each coral colony within the belt was counted, identified to genus level or growth form for corals within the speciose genus *Acropora*, and recorded as healthy or

diseased (according to disease survey methods described by Willis et al., 2004). Prevalence of each disease was calculated by dividing the number of diseased colonies by the total number of coral colonies. This method has been previously used in Indo-Pacific disease studies (e.g. Raymundo et al., 2005, Page and Willis, 2008, Vargas-Angel, 2009). Taxon-specific disease prevalences were also calculated for a subset of coral hosts and diseases in cases where there was evidence of particularly high disease prevalence among specific coral taxa. Taxon-specific prevalence was calculated as the number of cases of a specific disease or syndrome divided by the number of appropriate hosts encountered. Means and standard errors were calculated for each site based on the eight transects. Coral and algal cover were estimated by using the line intercept transect method (English et al., 1997). Daily average temperatures from the Heron Island reef slope (7 m depth) were provided by the Australian Institute of Marine Science.

3.3.4 Statistical Analyses

Multivariate approaches were used to examine differences in disease assemblages (i.e. the types of diseases recorded in surveys) and coral community composition among sites, years and seasons. Multivariate analyses were conducted using PRIMER version 6.1.10 with PERMANOVA+ extension (Anderson, 2001). Disease assemblages at each site – based on mean prevalences of white syndrome, growth anomalies, ulcerative white spot, skeletal eroding band and brown band – were ordinated in non-metric multidimensional scaling (nMDS) and principal coordinates analysis (PCO) space to visualise differences among sites and between seasons. Similarities in the spatial arrangement of samples

between 2D nMDS and PCO ordinations give weight to the reliability of these ordinations. Black band disease (BBD) prevalence was not included in multivariate analyses as this disease was only observed on one colony throughout the entire duration of the study. Disease prevalence was zero at the survey site SE A/B in January 2008; hence this observation was also excluded from multivariate analyses. PERMANOVA was used to test one design for the disease assemblage data: site × year and one design for the coral community data: site × month. PERMANOVA is robust to unbalanced designs such as the one in this study (Anderson, 2001). All disease data were square root transformed, and analyses were conducted using resemblance matrices of Bray-Curtis similarity. A SIMPER analysis was run on the data matrix. SIMPER decomposes Bray-Curtis dissimilarities between all pairs of samples to identify those diseases that contributed most to seasonality in disease prevalence (Clarke and Warwick, 2001).

To examine seasonality in the prevalence of particular diseases (brown band and growth anomalies) univariate permutational ANOVAs were used in PRIMER and non-parametric one-way ANOVAs in SAS (version 9.1). Non-parametric approaches were used because of non-correctable skew in univariate disease prevalence data. The presence of zeros necessitated using Euclidean distance as the resemblance measure for permutational ANOVAs. One-way ANOVAs (SAS version 9.1) were used to examine differences in taxon-specific disease prevalences among sites. Significant differences in taxon-specific prevalences among sites were considered as evidence of density-independent effects (i.e.

disease prevalence is not a simple function of the probability of encountering an appropriate host).

PERMANOVA is sensitive to differences in dispersion as well as distances in multivariate location (Anderson, 2001). Averages across replicate transects were therefore used in our analyses of coral community composition, which ensured no differences in dispersion between sites and years, and enabled us to attribute any significant PERMANOVA effects to differences in multivariate location only. Coral community composition was ordinated in PCO space and compared correlations between PCO axes and coral species were compared with correlations between PCO axes and disease prevalences to identify coral community 'types' associated with particular diseases. Differences in coral community composition between years were ordinated using canonical analysis of principal coordinates (CAP) (Anderson, 2001). Differences in coral community composition between seasons were not examined. Coral community data were standardised by total colony counts and square root transformed for all analyses. Resemblance matrices of Bray-Curtis similarity were used.

3.4 RESULTS

A total of 36,030 observations of coral colony health were made over the 5 surveys between November 2007 and August 2009. Each survey covered an area of 720 m^2 (48 belt transects of 1 x 15 m), except for the first survey in 2007, which covered an area of 645 m^2. Overall, 39 coral genera and 8 growth forms of *Acropora* were recorded. Combining all disease types, the lowest total mean disease prevalence was 1.87± 0.75% (mean ± SE) in November 2007 and highest 4.22 ± 1.72% in August 2008. Comparisons of disease assemblages among survey sites indicated that 2nd and 4th Point, Canyons and Harry's all had greater disease prevalence than Cascades and SE A/B, particularly for ulcerative white spots (UWS) and growth anomalies (GA) (Figure 3-2). Disease assemblages were quite variable over the five surveys at sites with the lowest disease prevalence (Cascades, Harry's and SE A/B), whereas disease assemblages were less variable through time for sites with the highest disease prevalence (Canyons, 2nd Point and 4th Point) (Figure 3-2).

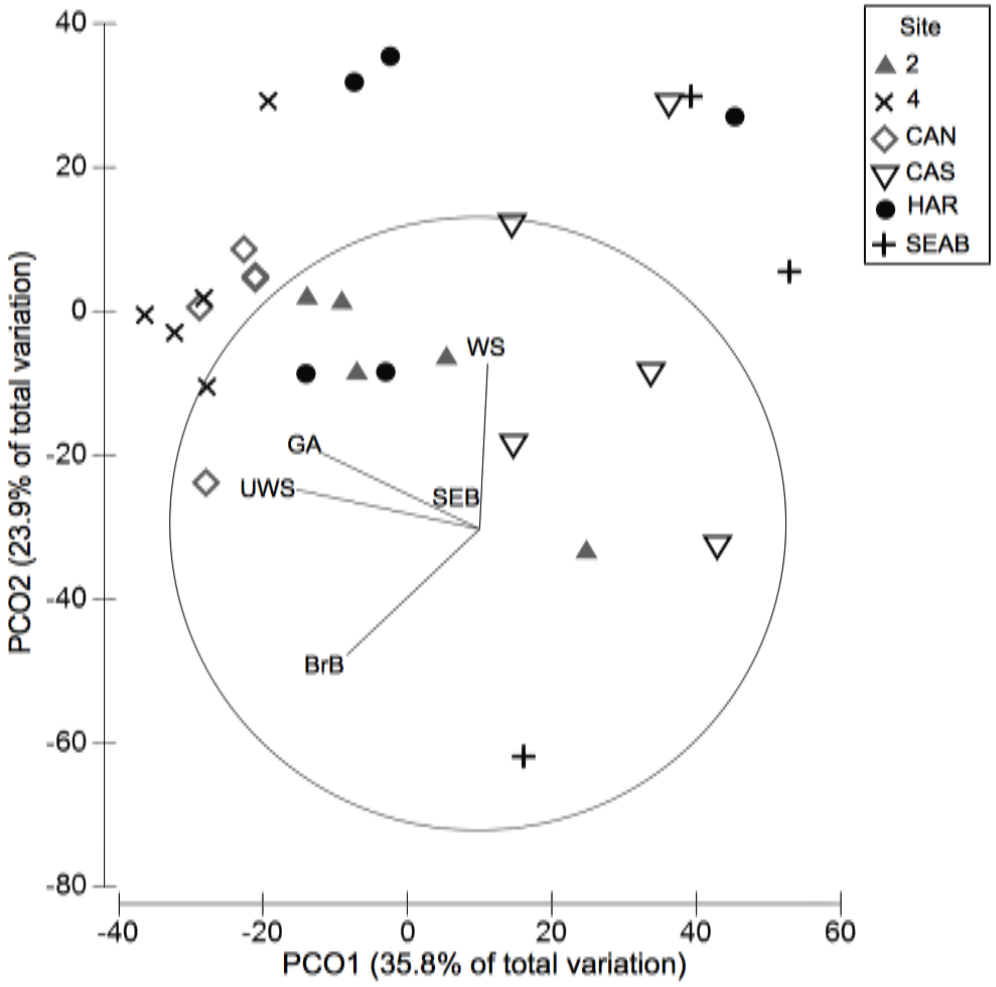


Figure 3-2. Principal coordinates ordination of coral disease assemblages – i.e. average percent prevalence of white syndrome (WS), growth anomalies (GA), ulcerative white spot (UWS), skeletal eroding band (SEB) and brown band syndrome (BrB) across replicate transects – at the six survey sites (2=2nd Point, 4=4th Point, CAN=Canyons, CAS=Cascades, HAR=Harry's, SEAB=SE A/B). Vector overlays indicate multiple correlations between ordination axes and the prevalence of individual diseases. PCO1 and PCO2 together capture 59.7% of the total variation in disease assemblages. GAs and UWS are more prevalent at 2nd Point, 4th Point and Canyons.

There was evidence for seasonality in BrB and UWS prevalence across all sites (Figure 3-3a). BrB was the most commonly occurring disease in the winter (3.29 ± 0.58% prevalence in August 2008 and 1.53 ± 0.28% in August 2009), whereas UWS was the most common disease in summer (1.12 ± 0.31% prevalence in November 2007 and 2.67 ± 0.52% prevalence in January 2008)

(Figure 3-3b). WS and SEB did not appear to have a clear seasonal pattern in their prevalence (Figure 3-3a). GAs seemed to be more common in January but correlations of GAs with seasonal changes in temperature were not well defined (Figure 3-3a). GAs were present in each survey, although their prevalence more than doubled from 0.38 ± 0.1% in 2007 to 0.82 ± 0.14 % by August 2009. SEB and WS both occurred at low prevalence in each survey (<0.5%) and BBD (black band disease) was encountered only once.

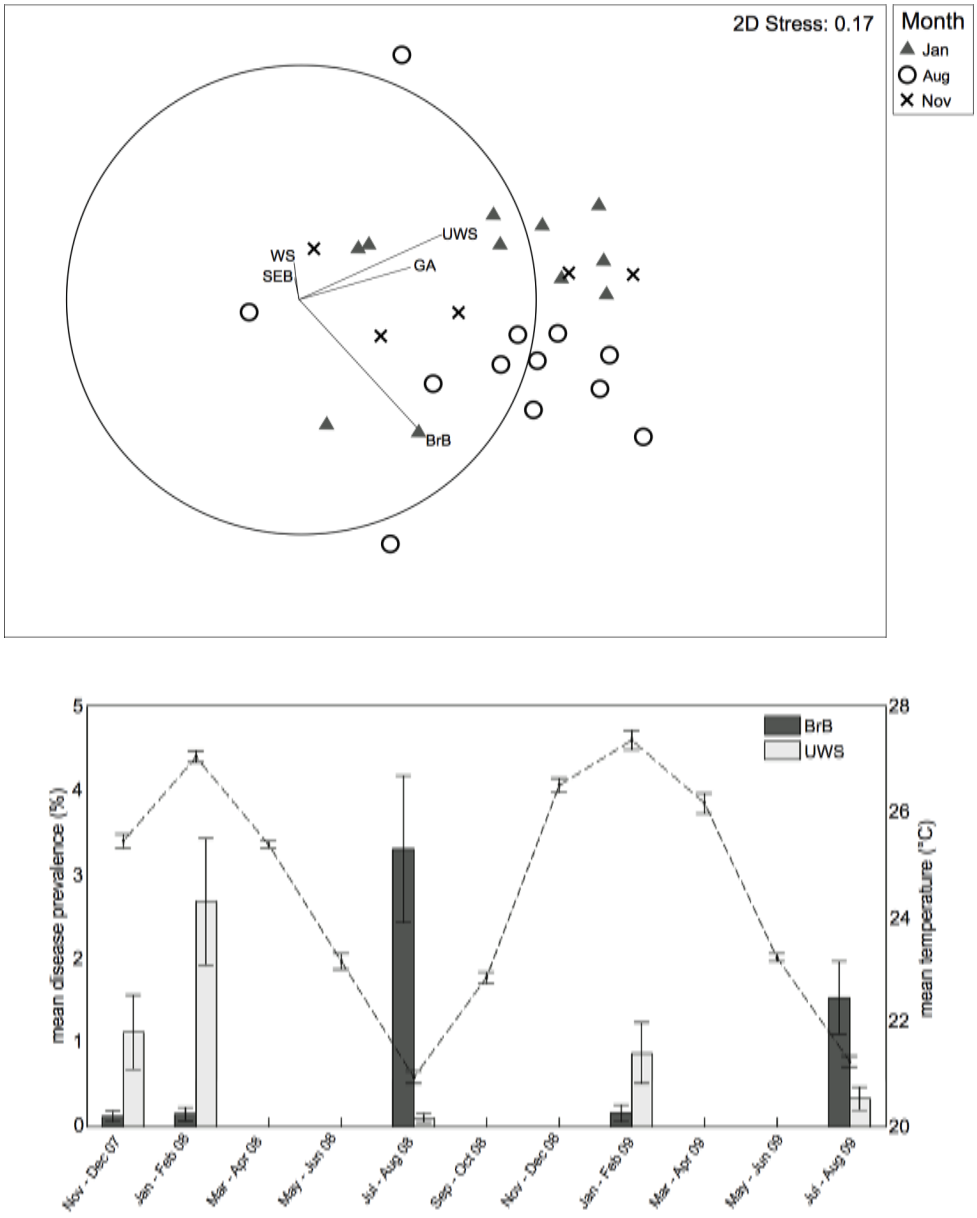


Figure 3-3. a) Non-metric multidimensional scaling (nMDS) ordination of disease assemblages in November (2007), January (2008 and 2009) and August (2008 and 2009) at Heron Island survey sites (averaged across replicate transects). Vector overlays indicate multiple correlations between ordination axes and the prevalence of individual diseases. BrB is more prevalent in August, while UWS and GA are generally more prevalent in January. b) Comparison of mean prevalence of brown band (BrB) and ulcerative white spot syndrome (UWS) across all sites (histograms) with bi-monthly mean temperatures and standard errors for Heron Island (dotted line). BrB is most prevalent in low-temperature months, whereas there is some evidence of the reverse trend (greater prevalence in high-temperature months) for UWS.

The average temperature for the wet season (December to March) was slightly higher in 2008-2009 (27.11°C as opposed to 26.48°C in 2007-2008). Temperature was also more variable in the wet season of 2009. However, the study period did not differ from the seasonal temperature pattern generally encountered at Heron Island. We had insufficient replicates to detect meaningful correlations between disease prevalences and temperature.

The results indicated that disease assemblages were significantly different between sites (PERMANOVA; $F_{5,11}=3.55$, $p<0.001$) and between months (PERMANOVA; $F_{2,11}=2.53$, $p<0.05$). These patterns were consistent across the five surveys (i.e. no interaction effect between site and month). BrB prevalence differed significantly among sites (PERMANOVA; $F_{5,11}=4.54$, $p<0.05$) and months (PERMANOVA; $F_{2,11}=10.04$, $p<0.005$), being greatest at the Canyons site and in the winter month of August 2008 (9.07 ± 10.18). UWS prevalence also differed significantly between months when data were analysed using a non-parametric 1-way ANOVA ($F_{2,11}=6.8$, $p<0.005$), but not when analysed using PERMANOVA. (Incongruous results from these two tests suggest that conclusions regarding differences in UWS prevalence between months may not be reliable). SIMPER analysis confirmed the presence of seasonal patterns in disease prevalence, again with UWS and WS driving similarities for November and January surveys (41% and 29% contributions to similarity for UWS and WS respectively), and BrB driving similarities within groups for August surveys (50% contribution to similarity).

Significant differences in average coral community composition were detected between sites ($F_{5,11}=14.13$, $p<0.0001$) and between years ($F_{2,11}=5.1$,

$p<0.001$) and patterns were consistent across survey years (i.e. no interaction effect between site and year). Multivariate ordination of coral community composition indicated that 4th Point was dominated by species in the genera *Montipora* and *Isopora*, while 2nd Point and Canyons were dominated by staghorn species of *Acropora* (and to some extent, corymbose species of *Acropora*) (Figure 3-4a). Other sites had more varied assemblages characterised by *Favia*, *Favites*, *Porites* and *Seriatopora* (Figure 3-4a).

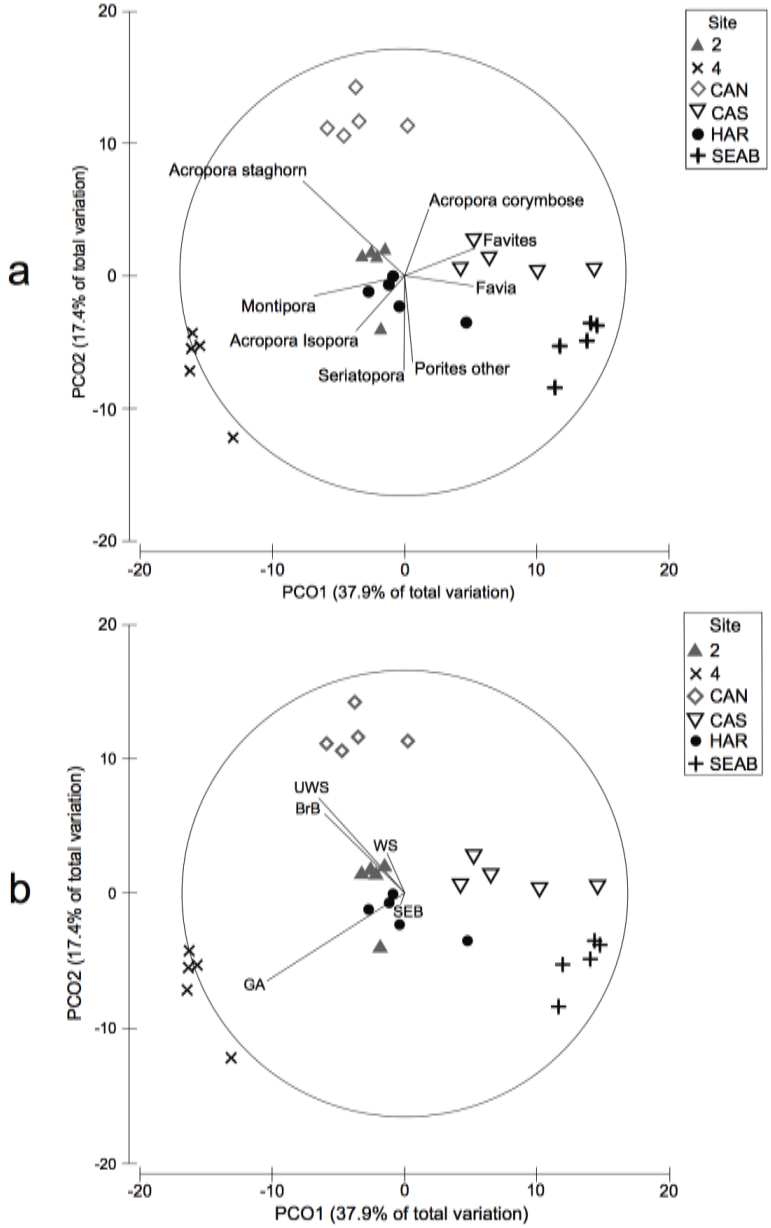


Figure 3-4. a) Principal coordinates (PCO) ordination of coral community structure at the six study sites (based on average colony counts across replicate transects). Vector overlays indicate multiple correlations between ordination axes and individual coral taxa (only correlations >0.3 are shown). Canyons is characterised by a high number of staghorn *Acropora* colonies, while 4th Point is dominated by *Montipora* and *Isopora* corals. Colony counts of *Favia* and *Favites* are highest at the Cascades site. PCO1 and PCO2 axes together capture 55.3% of the total variation in coral community composition. b) Principal coordinates (PCO) ordination of coral community structure with disease assemblage correlations overlaid at the six study sites. PCO 1 and PCO 2 axes together capture 55.3% of the total variation in coral community composition. UWS and BrB are more common at staghorn *Acropora* dominated sites 2nd Point and Canyons. GAs are more common at the *Montipora*-dominated 4th Point.

Multivariate correlations between the prevalence of individual diseases and coral community composition (Figure 3-4b) indicate that the *Montipora*-dominated 4th Point community had higher prevalence of GAs, whereas communities dominated by staghorn species of *Acropora* had higher prevalences of UWS, BrB and WS. Diseases were uncommon at Cascades and SE A/B (Figure 3-2, Figure 3-4b), which were dominated by the favid genera *Favites* and *Favia,* as well as poritid species (Figure 3-4a). Analyses of taxon-specific disease prevalences revealed significant differences between sites in the prevalence of BrB on species of *Acropora* ($F_{5,42}$=4.0812, $p<0.01$) and of GAs on *Montipora* ($F_{5,42}$=11.8, $p<0.0001$). In these cases, disease prevalence was not a simple function of the probability of encountering an appropriate coral host; there was evidence of higher taxon-specific disease prevalence at sites that had an intermediate cover of *Acropora* (highest prevalence 12.5% ± 1.9% (mean ± SE) at a site with 264 *Acropora* colonies per 15 m^2) and *Montipora* (highest prevalence 14.4% ± 1.7% at a site with 139 *Montipora* colonies per 15 m^2). There were no detectable differences in taxon-specific prevalence between sites for UWS or WS on species of *Acropora.* Constrained ordination of coral community composition (Figure 3-5) indicates that there was a significant difference in coral community composition between years which was correlated with a decrease in the abundance of tabular *Acropora* across the three years of the survey period, and a concurrent increase in the abundance of bushy *Acropora*, *Goniastrea*, *Coscinarea* and *Stylophora*.

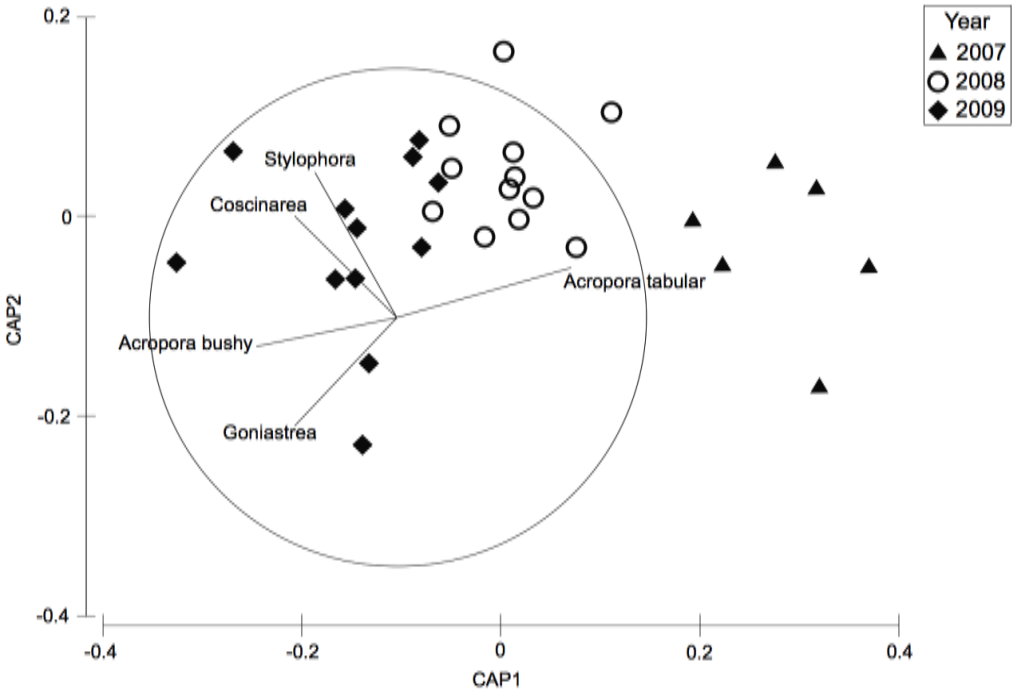


Figure 3-5. Canonical analysis of principal coordinates (CAP) for average coral community composition at the six survey sites. This analysis was constrained by year to emphasise temporal changes in community composition over the survey period. Vector overlays indicate Pearson's correlations between the ordination axes and individual coral taxa (only correlations >0.6 are shown). There is a decrease in the number of tabulate colonies of Acropora from 2007 to 2009, and an increase in the number of colonies of bushy Acropora, *Goniastrea*, *Coscinarea* and *Stylophora*.

Diseases affected a total of 14 coral taxa out of 39 (Figure 3-6). Disease prevalence was greatest for the genera *Acropora* and *Montipora* when data were pooled across all survey years, with 12% of *Acroporas* and 3.3% of *Montiporas* diseased overall. BrB and SEB affected only the genus *Acropora* and UWS was most commonly found on staghorn species of *Acropora* although also on massive *Porites*, *Goniastrea* and *Montipora*. GAs affected 3 genera (*Montipora*, *Acropora* and *Fungia*) but 57.8% of all GAs were found on species of *Montipora* (Figure 3-6).

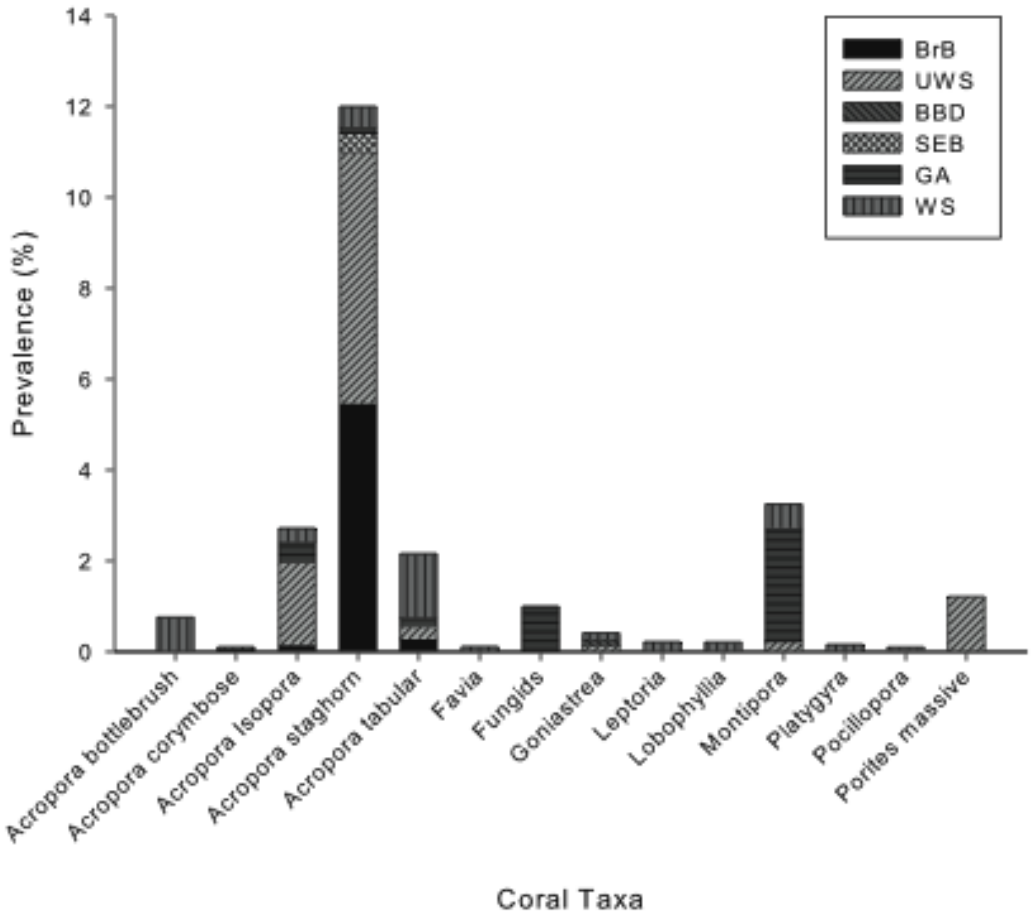


Figure 3-6. Prevalence of 6 diseases: white syndromes (WS), growth anomalies (GA), skeletal eroding band (SEB), black band disease (BBD), ulcerative white spot syndrome (UWS) and brown band syndrome (BrB) in scleractinian taxa. Prevalence (per taxa) was calculated relative to the total number of colonies examined in the respective taxa in all surveys. Disease prevalence is highest in staghorn *Acropora* (12% of all corals diseased), with UWS being the most prevalent disease (5.5%). *Montipora* has the second highest disease prevalence (3.3% of all corals diseased) with GA being the most prevalent disease (2.5%).

Coral cover was greater than 20% at all sites. The highest cover (52.65%) was recorded at 4th Point in 2008 and at Harry's in 2009 (51.55%). Algal cover was greater than 20% at 4th Point and less than10% at all the other sites. There was reasonable correlation between coral cover and disease prevalence (Pearson's correlation coefficient, r = 0.6347, $p<0.1$), but low correlation between algal cover and coral disease prevalence (r = 0.5456, $p<0.25$).

3.5 DISCUSSION

This study is the first to document temporal dynamics and correlates of coral disease on a reef in the southern Great Barrier Reef. Overall prevalence of coral disease at Heron Island (minimum: 1.87% ± 0.75%, maximum: 4.22 ± 1.72%) ranged from being more than four-fold greater than values reported for Indo-Pacific reefs in Indonesia (<1% in Sulawesi: Haapkyla et al., 2007, Haapkyla et al., 2009) and the central Pacific (<1%: Williams et al., 2008, Vargas-Angel, 2009), to being two-fold lower than values reported for the Philippines (8.3 ± 1.2%, Raymundo et al., 2005) and the GBR in the summer of 2003 (8.97±0.79%, Willis et al., 2004). In the Caribbean, disease prevalence values as high as 60% have been recorded (Porter et al., 2001), although a recent study by Cróquer and Weil (Croquer and Weil, 2009) recorded values similar to those found in this study (4.2%) over a large area in the Caribbean. Although records of disease prevalence do not reveal factors driving disease abundance, they highlight the ongoing threat diseases represent to coral reef ecosystems worldwide.

Correlations between the overall prevalence of coral disease at Heron Island sites and season (i.e. summer/winter months) are consistent with patterns found in other studies, with one exception. Previous studies have consistently found a higher prevalence of coral disease during summer on reefs in both the Caribbean (Bruckner and Bruckner, 1997b, Porter et al., 2001, Kuta and Richardson, 2002) and on the GBR (Willis et al., 2004). In particular, higher disease prevalence and progression rates have been recorded on the GBR in summer for BrB (Nash, 2003, Boyett, 2006), WS (Bruno et al., 2007, Willis et al., 2004) and BBD (Boyett

et al., 2007, Sato et al., 2009). In contrast, a higher prevalence of BrB was found in winter months in this study. Page et al. (Page et al., 2009) detected a positive correlation between BrB prevalence and coral bleaching, suggesting that BrB prevalence is positively correlated to high temperatures. However, such a correlation with increasing temperature could reflect either increased virulence of BrB pathogen(s) or reduced disease resistance of the coral host. Lack of difference in the rate of BrB progression in three temperature treatments during short-term experimental studies (Boyett, 2006) suggest that temperature-mediated effects on BrB prevalence may be attributed primarily to increased host susceptibility when coral health is compromised. Thus the 9-fold higher BrB prevalence found in winter in this study could reflect compromised coral health at colder temperatures that facilitate the onset of a ciliate infection.

Decreased BrB prevalence in summer could also be explained by epidemiological dynamics such as the removal of susceptible individuals over time through death or induced disease resistance, leading to a waning of the outbreak, despite increasing temperatures (Sokolow, 2009). This pattern has been recorded for aspergillosis in the Florida Keys (Kim and Harvell, 2004). However, comparisons of BrB prevalence in this study with previous records at Heron Is. (<1%, Willis et al., 2004) indicate that, if anything, prevalence of this disease has continued to rise on this reef. The effects of BrB on a reef may be devastating, given that progression rates of up to 2.1 cm/day have been recorded (Boyett, 2006), thus this disease has the potential to significantly alter coral community structure at these sites.

In the present study, GAs were typically more common in January, although correlations between GAs and seasonal changes in temperature were not well defined (Figure 3-3a). Two-fold increases in the mean prevalence of GAs, from 0.38 ± 0.1% (mean ± SE) in November 2007 to 0.82 ±0.14 % (mean ± SE) in August 2009, highlight a possible longer-term trend that may be a cause for concern, particularly as GA prevalence was less than 0.1% at Heron Is. sites in 2003 (Willis et al., 2004). The cause of growth anomalies has not been unequivocally determined, although previous experimental studies have shown transmission of GAs in *Porites* without identifying the causative agent (Kaczmarsky and Richardson, 2007). McClanahan et al. (McClanahan et al., 2009) suggest that the main catalytic factor is anomalously warm water and environmental factors associated with coral bleaching (Maina et al., 2008), as well as possibly the presence of micro boring organisms (McClanahan et al., 2009). The low correlation found between temperature and GA prevalence in this study could reflect insufficient replication within seasons to derive meaningful correlations. Given that warming trends are likely to continue with climate change, growth anomalies and other temperature-mediated diseases are expected to increase (Harvell et al., 2007). In high densities, GAs may reduce UV absorption rates (Coles and Seapy, 1998), lipid storage capacity (Yamashiro et al., 2001) and linear growth rates of colonies (Bak, 1983). More monitoring and experimental work on GAs is needed to better understand their environmental drivers in light of the impacts they are likely to have on coral health.

Sokolow (Sokolow, 2009) points out that demonstrating seasonal fluctuations in prevalence does not conclusively demonstrate a link between

temperature and coral disease since other variables such as rainfall, light levels, water clarity, run-off, ocean circulation, and nutrients can also fluctuate seasonally, along with temperature (Delcroix and Henin, 1991, Lima et al., 1996, Poulos et al., 1997). For corals, there may be complex interactions between extrinsic forcing (due to temperature and environmental effects) and intrinsic dynamics (due to the interplay of epidemiological variables such as susceptibility and transmission) (Sokolow, 2009). Although temperature was the only environmental parameter investigated in the present study, it is highly unlikely that a factor like water quality would be driving disease around Heron Island since there is no source of freshwater input onto the reef and raw sewage is treated on the island and the purified water recycled. Our study lacked the statistical power to detect temperature effects on disease prevalence, and we have not investigated correlations between disease prevalence and seasonal variability in light levels, water clarity or patterns of ocean circulation. Average annual sea surface temperatures on the GBR are likely to rise over the coming decades by as much as 1 to 3 ºC by 2100 and more in the winter on the southern GBR where Heron Island is situated (GBRMPA, 2009). Ongoing studies of the links between temperature and disease are therefore needed.

In 2009, cyclone Hamish, a category five cyclone, caused extensive damage in the southern part of the GBR. However, the impact of cyclone Hamish on Heron Island could not be detected from the results of this study; despite a slight decrease in the coral cover at three of the study sites, cover remained greater than 20%. Diseases may have contributed to slight declines in coral cover at these sites, but more regular monitoring would be required to detect localized or short-

term disease outbreaks and distinguish these from other sources of mortality. The reasonable correlation found between coral cover and disease prevalence in the present study highlights the role that host density may play in the spread of coral diseases. Bruno et al. (Bruno et al., 2007) found that high (>50%) coral cover was linked to a high abundance of WS on the Great Barrier Reef. High host density is most often associated with greater rates of horizontal transmission (Holt and Pickering, 1985, Getz and Pickering, 1983, Altizer and Augustine, 1997), leading to localized increase in prevalence. In addition, host density can be positively related to the density of disease vectors (Rosenberg and Falkovitz, 2004, Williams and Miller, 2005).

Correlations between coral community structure and disease prevalence found in the present study highlight the vulnerability of species in the family Acroporidae to disease. UWS and BrB most commonly affected staghorn species of *Acropora,* a key reef-building group of corals (12% of corals affected by these two diseases combined). Similarly, GAs were most common on *Montipora* corals (3.3% diseased), another genus in this family. The greater prevalence of these diseases at sites characterised by these genera (Figure 3-4a and Figure 3-4b) may lead to significant re-structuring of reefs on local scales. The observation that taxon-specific prevalence for these diseases was highest at sites with intermediate numbers of host colonies is consistent with density-independent effects, but warrants further investigation beyond the scope of this study. Different coral diseases do show complex associations with a range of environmental variables and these associations can vary between diseases (Williams et al., 2010). In an earlier large-scale survey on the GBR, the Acroporidae and Pocilloporidae were

the families most affected by disease (Willis et al., 2004). On the GBR, acroporids are affected by WS (Willis et al., 2004), BBD (Page and Willis, 2006), BrB (Willis et al., 2004) and SEB (Page and Willis, 2008), and in the Caribbean they are affected by white band (Aronson and Precht, 2001) and white pox (Patterson et al., 2002) diseases. It is possible that corals with fast growth rates have poorer disease resistance strategies as a consequence of life histories that channel resources into growth for space monopolisation rather than into maintenance activities. In contrast, massive corals, which tend to be more committed to confrontational strategies (Jackson, 1979), may have evolved greater disease resistance (Willis et al., 2004, Palmer et al., 2010). More extensive testing of patterns in host susceptibility among coral families is required before life history patterns in disease resistance can be identified.

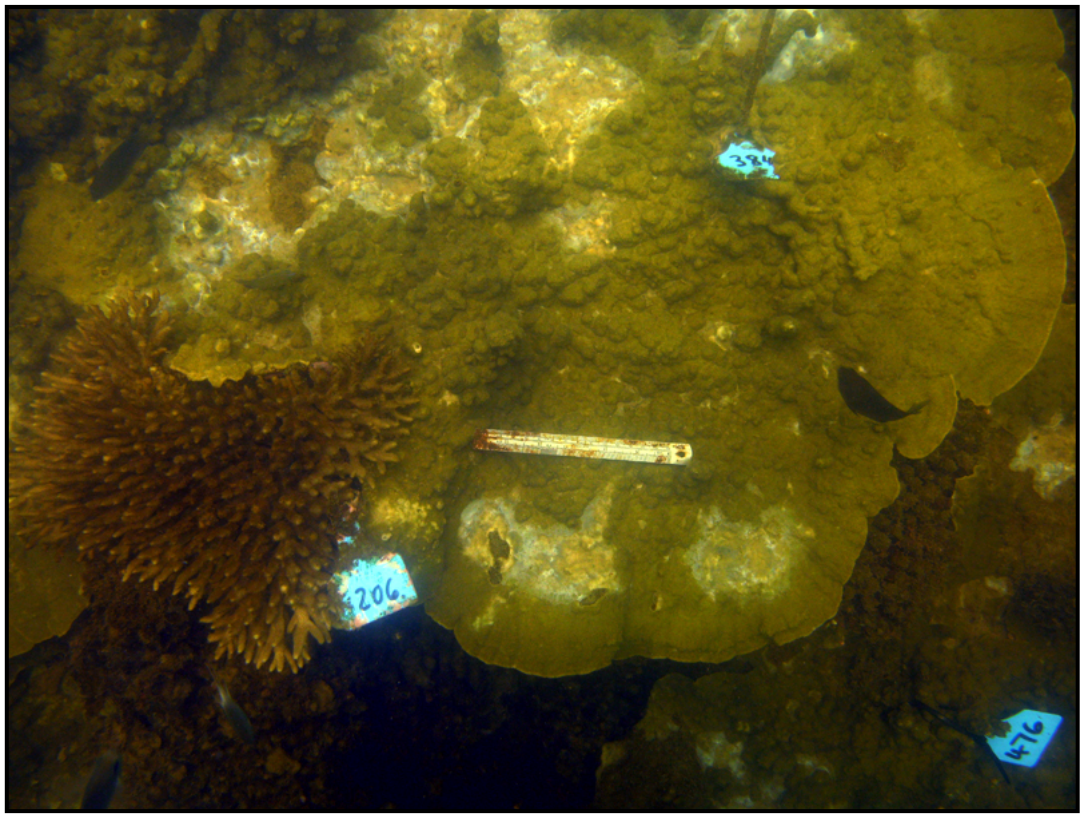
During the study period, a shift in coral community structure was observed at Heron Island sites, from dominance of tabular species of *Acropora* in 2007, to dominance of *Goniastrea*, bushy *Acropora*, *Coscinarea* and *Stylophora* species in 2009 (Figure 3-5). This shift may have lead to a structurally less complex and diverse coral community, which could have implications for the biodiversity of the reef. The reason for this is unknown, but a disease outbreak, bleaching event or storm damage between survey dates cannot be ruled out. More frequent monitoring would enable a better understanding of the underlying reasons of the observed community change.

In conclusion, our results demonstrate that coral diseases are correlated with seasonality and coral community composition around Heron Island. Although seasonality was not detected for some diseases, this may be because disease

prevalence was not high enough to detect patterns and thus seasonality in these diseases might be present at locations with higher disease abundance. The difficulty in detecting temporal patterns in coral disease highlights the need for long-term disease surveys such as the present study. Revealing seasonal patterns of coral disease is a step towards understanding interactions between seasonal disturbances (e.g. bleaching and storm events) and disease. The results from this study suggest that some coral taxa are more vulnerable to disease. This may lead to weaker reef structure and to a less diverse coral community. Together, seasonally occurring and chronic diseases reduce coral resilience to major disturbances. Reduced resilience increases the probability of coral-algal phase shifts and functional collapse of reef systems (Bellwood et al., 2004, Hughes et al., 2007). Further research should be dedicated to understanding environmental drivers of coral disease on both small and large spatial scales on the GBR to better manage the world's largest coral reef.

Chapter 4

Seasonal rainfall and runoff promote coral disease on an inshore reef



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4.1 ABSTRACT

Declining water quality coupled with the effects of climate change are rapidly increasing coral diseases on reefs worldwide, although links between coral diseases and environmental parameters remain poorly understood. This is the first study to document a correlation between coral disease and water quality on an inshore reef. The temporal dynamics of the coral disease atramentous necrosis (AtN) was investigated over two years within inshore populations of *Montipora aequituberculata* in the central Great Barrier Reef, in relation to rainfall, salinity, temperature, water column chlorophyll *a*, suspended solids, sedimentation, dissolved organic carbon, and particulate nitrogen, phosphorus and organic carbon. Overall, mean AtN prevalence was 10-fold greater during summer wet seasons than winter dry seasons. A 2.5-fold greater mean disease abundance was detected during the summer of 2009 (44 ± SE 6.7 diseased colonies per $25m^2$), when rainfall was 1.6-fold greater than in the summer of 2008. Two water quality parameters explained 67% of the variance in monthly disease prevalence in a Partial Least Squares regression analysis; disease abundance was negatively correlated with salinity (R=-0.6) but positively correlated with water column particulate organic carbon concentration (R=0.32). Seasonal temperature patterns were also positively correlated with disease abundance, but explained only a small portion of the variance. The results suggest that rainfall and associated runoff may facilitate seasonal disease outbreaks, potentially by reducing host fitness or by increasing pathogen virulence due to higher availability of nutrients and organic matter. In the future, rainfall and seawater temperatures are likely to

increase due to climate change which may lead to decreased health of inshore reefs.

4.2 INTRODUCTION

Disease has emerged as a significant threat to wildlife populations in recent decades (e.g. Daszak et al., 2000, Dobson and Foufopoulos, 2001). A recent review highlights the substantial role that environmental nutrient enrichment has played in contributing to patterns of emerging human and wildlife diseases and the urgent need for studies to understand linkages, particularly in light of ongoing intensification of global nutrient cycles (Johnson et al., 2010). The current understanding of marine diseases is poor in comparison to knowledge of human, agricultural and terrestrial wildlife diseases (Harvell, 2004b). It appears that epidemiological theories developed for terrestrial diseases may not translate well to marine ecosystems (Harvell, 2004b, McCallum et al., 2004). For example, diseases appear to spread more rapidly in comparatively open oceanic ecosystems (McCallum et al., 2003) and marine pathogens are more diverse taxonomically and in their life histories (McCallum et al., 2004). Thus, marine case studies that advance understanding of potential links between nutrient enrichment and marine diseases are critical if management tools for the long-term conservation of marine wildlife are to be effective.

Coral reefs are increasingly threatened by changes in water quality from terrestrial runoff (De'ath and Fabricius, 2010), climate change (Hoegh-Guldberg et al., 2007, Veron et al., 2009) and over-exploitation (Jackson et al., 2001, Unsworth and Cullen, 2010). Coral bleaching and disease have emerged as dominant drivers of coral population declines on coral reefs, particularly as oceans have warmed in the past few decades (Harvell et al., 2001). Current research supports a connection between a warming climate and increasing incidence of

disease in corals (Harvell et al., 2001, Harvell et al., 2002, Bruno et al., 2007). For example, warm temperatures and high coral cover have been linked to increased abundance of white syndromes on the Great Barrier Reef (GBR) (Bruno et al., 2007) and progression rates of black band disease were higher in the austral summer (Boyett et al., 2007, Sato et al., 2009). However, links to most other anthropogenic disturbances are less clear (Bruckner, 2002).

Although the mechanisms are unknown, outbreaks of disease on some coral reefs have been correlated with increases in nutrient runoff (Kim and Harvell, 2002, Sutherland et al., 2004). In the Philippines, a higher prevalence of growth anomalies and *Porites* ulcerative white spot disease was found near a sewage outfall (Kaczmarsky, 2006), and white pox has also been linked to sewage inputs in the Caribbean (Patterson et al., 2002). Field experiments in the Caribbean have demonstrated that moderate increases in dissolved inorganic nutrient concentrations can substantially increase the severity of aspergillosis and Caribbean yellow band disease (Bruno et al., 2003) and the prevalence of aspergillosis (Kim and Harvell, 2002). In other studies, nutrient exposure resulted in increased progression rates of black band disease, with nutrients thought to reduce the coral host's ability to counteract infection by pathogenic micro-organisms (Voss and Richardson, 2006b). Experiments on the impacts of organic carbon on micro biota suggested that the mechanism may be indirect with elevated nutrients increasing the production of organic carbon (through primary production), which in turn leads to an increased growth rate of microbes living in the corals' mucus layer and a disruption of the balance between corals and their associated micro biota (Kline et al., 2006).

Terrestrial runoff to the inshore GBR is mainly delivered in short-lived flood events during the 5-month summer wet season (Furnas, 2003), often forming distinct flood plumes in the coastal zone that sometimes reach far out into the GBR lagoon (Devlin and Schaffelke, 2009). Elevated concentrations of nutrients, suspended sediments and pesticides, caused by changes in land use over the past 200 years of European settlement, are now potentially affecting the health of coastal and inshore ecosystems (Furnas, 2003, Brodie and Mitchell, 2005, Fabricius, 2005, Schaffelke et al., 2005). In particular, sediment loads to the GBR have increased four to five-fold in this period (Maughan et al., 2008), and five to ten-fold in some catchments (McCulloch et al., 2003). Moreover, the area of the GBR affected by sediment inputs is increasing substantially as a result of changing land management practices, to the point where fine terrestrial sediment is reaching mid-shelf reefs for the first time in their geological history (Maughan et al., 2008). Sediments settling on corals may increase disease prevalence indirectly through increased stress and energy expenditure required to remove sediments (Fabricius, 2005), which could make them more susceptible to infections by microbial pathogens, and/or directly if sediments act as disease reservoirs (Voss and Richardson, 2006b).

Atramentous necrosis (AtN) is one of the few coral diseases with high prevalence values on coastal GBR reefs (B. Willis and C. Page, pers. comm. 2008). AtN was first observed in December 2001 on Magnetic Island, an inshore reef of the Central GBR (Jones et al., 2004b), although subsequently also observed on reefs in both the northern and southern GBR (B. Willis and C. Page, pers. comm. 2008). In March 2002, a peak in AtN causing significant mortality

within Magnetic Island populations of the plate-like coral *Montipora aequituberculata* was observed during a thermal mass-bleaching event (Jones et al., 2004b). However, increased prevalence of AtN was documented in spring (temperature <24.5ºC), well before typical summer temperatures were reached (Anthony et al., 2008), suggesting that temperature may not be the only environmental factor driving the occurrence of this disease.

AtN progresses through four distinct stages: Stage 1 lesions are small (1-2 cm diameter) areas of bleached but intact tissue; Stage 2 lesions are white skeleton devoid of tissue; Stage 3 lesions are covered with a white bacterial film; and in Stage 4, a black, sulphurous deposit accumulates under the white film (Anthony et al., 2008) likely the result of opportunistic secondary microbial community (Bourne, 2005).

This is the first study to investigate a possible connection between the seasonal dynamics of a coral disease and parameters associated with water quality on the GBR. The aims of the present study were to (i) document seasonal dynamics of AtN and nine seasonally varying environmental parameters, and (ii) analyse relationships between disease prevalence and these parameters to identify potential environmental drivers of AtN within populations of the coral *Montipora aequituberculata* on an inshore GBR reef.

4.3 METHODS

A research permit for this study was provided by the Great Barrier Reef Marine Park Authority (GBRMPA).

4.3.1 Study site and assessment of disease dynamics

The study sites were located in two adjacent bays, Nelly Bay and Geoffrey Bay, on the south-eastern side of Magnetic Island (19°S, 147°E), which is situated within the inner shelf region of the Great Barrier Reef. Both bays have fringing coral reefs and are similar in shape, physical structure, and hydrodynamic setting (Larcombe et al., 1995). The study was conducted between December 2007 and December 2009. Sampling was conducted every 2 weeks in the austral summer (Nov-Apr) and once a month in the winter (May-Oct). Increased sampling frequency in summer was based on the hypothesis that AtN increases with warm water temperatures (Jones et al., 2004b, Anthony et al., 2008). Disease dynamics were assessed in three permanent 5x5 m quadrats at 3-5 m depth at each site. Coral colonies demonstrating signs of AtN (33) were tagged with numbered plastic tags attached to cable ties. Visual surveys were able to clearly distinguish the four stages in the development of AtN lesions described above, although the last two stages were combined because they generally occur simultaneously. Thus, in the present study, the disease stages were referred to as: AtN1 (=stage 1), AtN2 (=stage 2), AtN3 (=stages 3 and 4), and S (=disease progression stopped).

The diseased corals were all colonies of *Montipora aequituberculata,* which was the most prevalent species of *Montipora* in the quadrats. New disease cases (disease incidence) were counted and tagged in each plot during each survey. New

AtN infections, in addition to both lesion progression and cessation, were monitored on individual colonies to elucidate spatiotemporal patterns in disease dynamics. Due to logistical constraints, Geoffrey Bay was not sampled in February 2008.

4.3.2 Environmental parameters

At each sampling occasion, two replicate water samples were collected in 1-L plastic bottles 1 m above the coral and on opposite sides of the quadrats for the analysis of concentrations of: dissolved organic carbon (DOC), chlorophyll *a* (chl-*a)*, particulate organic carbon (POC), particulate nitrogen (PN), particulate phosphorus (PP) and suspended solids (SS). Due to logistical reasons, only two replicate water samples were used. Three physical variables were also measured, i.e. salinity, temperature and sedimentation.

DOC samples were filtered immediately through a 0.45µm syringe filter (Sartorius MiniSart N) into acid-washed, screw-cap plastic test tubes. Samples were acidified by adding 100 µl of AR-grade hydrochloric acid (32%) and stored at 4°C until analysis. The concentrations were measured by high temperature combustion (680 °C), using a Shimadzu Total Organic Carbon TOC-5000A carbon analyser. Prior to analysis, CO_2 remaining in the sample water was removed by sparging with O_2 carrier gas (Schaffelke, 2009).

For the chl-*a* analysis, a 100 ml sub-sample was filtered immediately onto a 25 mm pre-combusted glass fibre filter (Whatman GF/F). Filters were wrapped in pre-combusted aluminium foil envelopes and stored at -18°C until analysis. Chl-*a* concentrations were measured fluorometrically using a Turner Designs 10AU fluorometer after grinding the filters in 90% acetone (Furnas et al., 1995).

For analyses of POC, PN and PP, sub-samples of 250 ml were filtered onto 25 mm pre-combusted glass fibre filters (Whatman GF/F) and stored at -18°C. PN was determined by high temperature combustion using an ANTEK 9000 NS Nitrogen Analyser (Devlin and Schaffelke, 2009). PP was determined spectrophotometrically as inorganic P (PO_4), (Parsons et al., 1984) after digestion in 5% potassium persulphate (Schaffelke, 2009). POC was determined by high temperature combustion (950°C) using a Shimadzu Total Organic Carbon TOC-V carbon analyser fitted with a Solid Sample Module SSM-5000A after acidification with concentrated phosphoric acid (Schaffelke, 2009). Inorganic C on the filters (e.g. $CaCO_3$) was removed by acidification of the sample with 2M hydrochloric acid, the filter introduced into the sample oven (950°C), purged of atmospheric CO_2 and the remaining organic carbon combusted in an oxygen stream and quantified by an infrared gas analyser.

Sub-samples for suspended solids (SS) were collected by filtering 1000 mL of water onto pre-weighed, 0.4µm, polycarbonate filters (47 mm diameter, GE Water & Process Technologies), and SS concentrations were determined gravimetrically from the weight difference between loaded and unloaded filters after drying overnight at 60°C (Schaffelke, 2009).

Salinity was measured at each sampling occasion with a hand-held refractometer (r^2 Mini, Reichert GmbH, Germany). Temperature was measured using a temperature logger (ODYSSEY data recording systems, Christchurch, New Zealand) attached underneath a sediment trap in both Nelly and Geoffrey bays. It was retrieved and downloaded approximately every 2 months. Temperature data from sensors were combined with data collected by the

Australian Institute of Marine Science (AIMS) sea surface temperature monitoring program in the same two bays (data available at http://www.aims.gov.au). Maximum temperatures were calculated for the periods of 7 and 14 days up to and including the sampling date.

Two sediment traps (40 cm high with a diameter of 10 cm) were deployed 10 m apart close to the permanent 5x5 m quadrats at each site. Traps were collected at every second sampling occasion in the winter and on each occasion in the summer. After decanting the seawater, the sediment was carefully transferred from the trap into a polycarbonate sample jar using a wash bottle with seawater. Salt in the samples was removed by adding distilled water, gently mixing the sediment and discarding the supernatant after the sediment had settled for a short time. This was repeated three times. Sediment samples were dried at 60 ºC for at least 3 days prior to determining their dry weight. The ash-free dry weight (AFDW) of the sediment was determined after combusting the sample at 450ºC in a muffle furnace for 24 hours. The AFDW was used as a coarse measure of the organic content of the sediment.

Rainfall data for Townsville were obtained from the Australian Bureau of Meteorology web site (http://www.bom.gov.au).

4.3.3 Statistical analyses

Relationships between environmental parameters measured at the field sites were explored using a principal coordinates analysis (PCA) in PRIMER version 6.1.10 (Clarke and Warwick, 2001). PCA results were summarized in a bi-plot

containing the distribution of environmental parameters in two-dimensional space and their correlations with the PCA axes.

To investigate potential relationships between environmental parameters and AtN prevalence in more detail, a Partial Least Squares (PLS) regression model was developed in Minitab. This technique is an extension of multiple regression analysis, in which the effects of linear combinations of several predictors on a response variable (or multiple response variables) are analysed in a stepwise manner to remove descriptive variables that do not contribute to the model. PLS regression is particularly suited to cases in which the matrix of predictors has more variables than observations, or when there is multi-collinearity among variables (Carrascal et al., 2009). This technique was first used in analytical chemistry and has been applied to analyses of ecological data since the late 1990s (Carrascal et al., 2009) and in recent publications (Rasheed and Unsworth, 2011). Monthly disease prevalence data were analysed against maximum temperatures for the periods of 7 and 14 days up to and including the sampling date, assuming a time lag in the corals' response to changing environmental parameters. When observations were missing for temperature, sedimentation, POC, PN, PP, chl-a, DOC and SS, mean values of data before and after the missing data point were used to fill data gaps to be able to run the PLS regression. The PLS analysis calculates an analysis of variance table analogous to conventional multiple regression analysis, providing an overall assessment of the probability of statistical significance of the calculated PLS model. The PLS analysis also calculated a predicted residual sum of squares (PRESS) following cross-validation. This allowed for the calculation of a predicted Global R^2 value in

addition to a conventional Global R^2, hence determining the predictive power of the observed relationship. A predicted Global R^2 value lower than the conventional Global R^2 indicates that the model is dependent upon only a few observations and does not have good predictive power.

4.4 RESULTS

4.4.1 Dynamics of atramentous necrosis

At the two study sites (Nelly and Geoffrey Bays, Magnetic Island), a total of 379 colonies of *Montipora aequituberculata* showing signs of atramentous necrosis (AtN) were tagged during the two-year study. The mean number of diseased corals was clearly higher in the wet season than in the dry season (Figure 4-1).

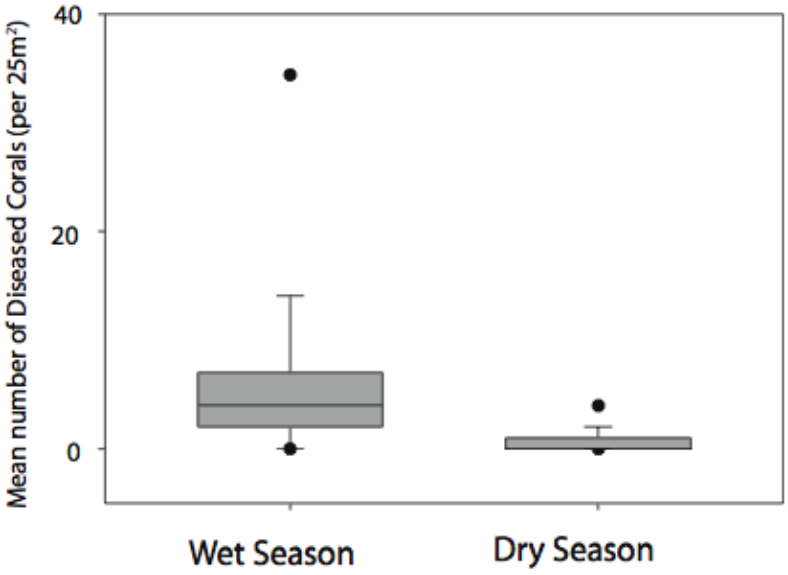


Figure 4-1. A box plot illustrating the distribution of the mean numbers of diseased corals between two seasons (wet season=Nov-March, dry season=April-Oct). Vertical bars illustrate standard deviations and horizontal bars medians. Black dots represent the 95 percentiles.

Highest values of both the mean number of AtN cases and new disease cases (incidence) were measured in the end of February in both 2008 and 2009 (Figure 4-2a, b), although the disease peak was four-fold greater in 2009. In 2009, the mean (±SE) number of diseased colonies was 44 ± 6.67 colonies per 25 m^2 in

Geoffrey Bay (GB) and 40 ± 5.46 colonies per 25 m^2 in Nelly Bay (NB), whereas in 2008, 11 ± 5.51 colonies were infected per 25 m^2 in NB (Figure 4-2a). The mean (±SE) incidence (i.e. number of new infections) was also higher in 2009, with 35 ± 4.04 new infections per 25 m^2 in GB and 19 ± 4.18 per 25 m^2 in NB, compared to only 6 ± 4.51 new cases in NB in 2008 (Figure 4-2b). Disease abundance decreased to 0-2 cases per 150 m^2 in winter and re-appeared from November onwards in both years with 2nd and 3rd stages of AtN (AN2 and AN3, respectively) being most common between January and March (Figure 4-3).

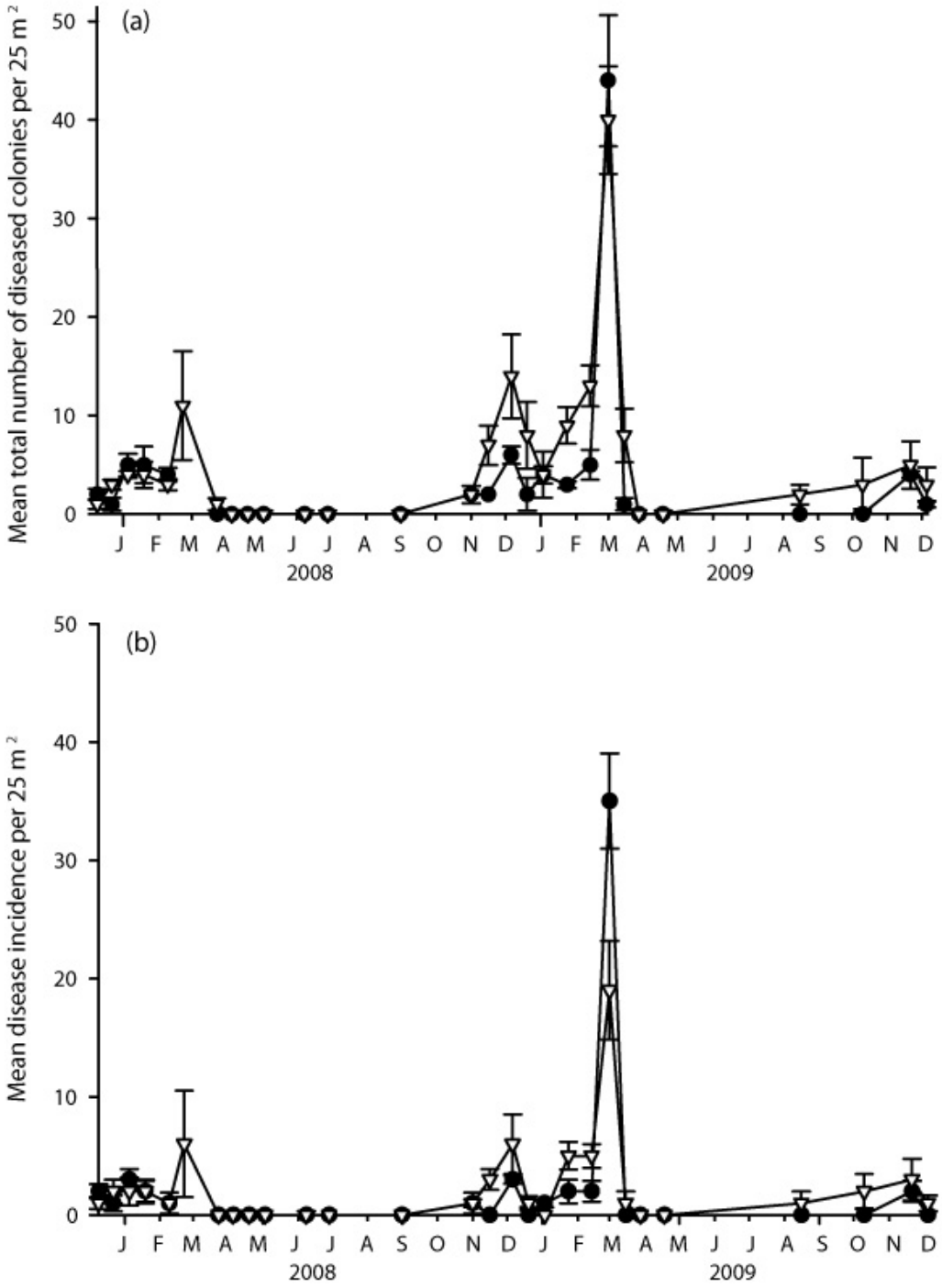


Figure 4-2. a) Mean number of corals demonstrating signs of atramentous necrosis (AtN) per 25 m^2 during the two-year study. The highest numbers were found in February 2009 in Geoffrey Bay (GB). b) Mean number of new infections (i.e. incidence) of AtN per 25 m^2 during the two-year survey. The highest numbers were found in February 2009 in GB. (GB=dark circles, NB=white triangles).

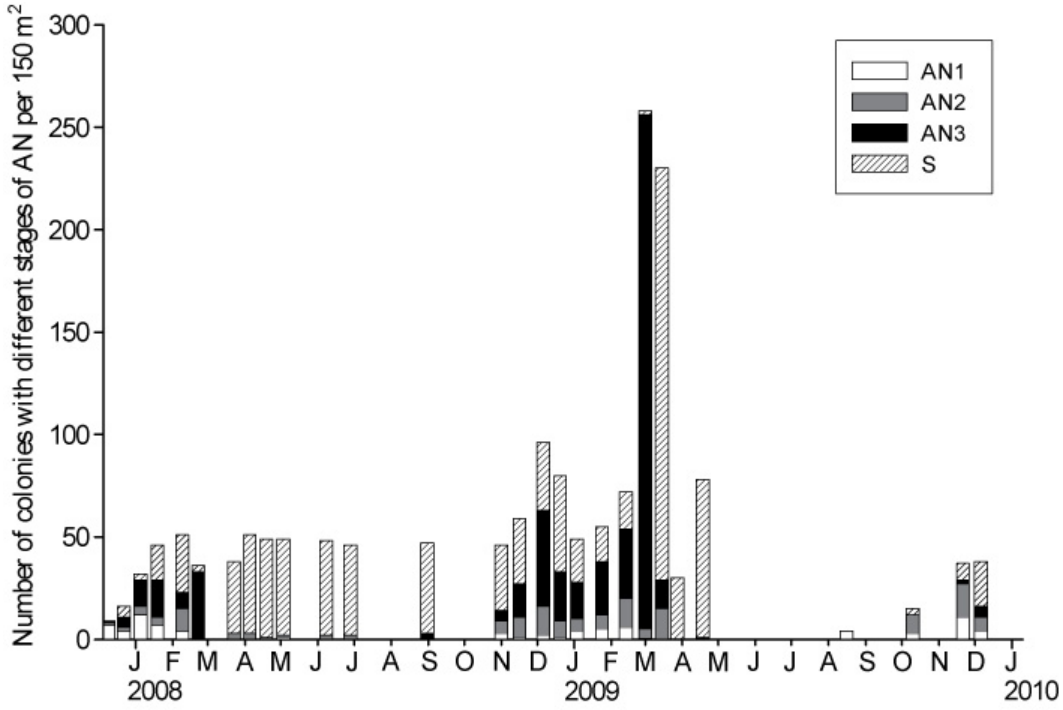


Figure 4-3. Disease stages in time. Disease stages in time per 150 m^2 (AN1=first stage of atramentous necrosis (AtN) characterised by a small (1-2 cm diameter) initial area of bleached but intact tissue; AN2= a lesion of white skeleton devoid of tissue; AN3= lesions covered with a white bacterial film and a black, sulphurous-smelling deposit, subsequently accumulating under the white film; S= disease progression stopped). The third stage was most common during the summer disease peak whereas the disease stopped in winter.

4.4.2 Environmental conditions at the study sites

The highest values for both mean abundance of diseased colonies and mean disease incidence corresponded with the highest values in all of the environmental parameters investigated except for salinity, for which the lowest values were recorded at the disease peak (Figure 4-2 and Figure 4-4). An increasing trend in environmental parameters (decreasing for salinity) was observed preceding the disease outbreaks in both years, with values tending to be higher in 2009 than in 2008. The summer of 2009 was the wettest in 10 years in the Townsville region, with a total rainfall of 1901.6 mm compared to

1187 mm in 2008. A dramatic (~40%) decrease in salinity over four weeks was observed in 2009 prior to the disease outbreak (from 31.7 to 19.0 in NB, and from 32.3 to 20.1 in GB). Salinity data prior to the 2008 outbreak are lacking because measurements for this study started in February 2008. Sedimentation was very seasonal, with higher values during summer rain events, especially in 2009. Mean water temperatures increased by only 0.3°C (to 30.5°C) in the month prior to the disease outbreak in 2008, whereas temperatures increased by 1.7°C in the month prior to the 2009 outbreak and reached 31.7°C. The highest value of particulate nitrogen (PN) was observed one month prior to the 2008 outbreak, with lower and more even distribution of recorded values in 2009. Particulate phosphorus (PP) showed 10-fold higher values two weeks prior to the 2009 outbreak and particulate organic carbon (POC) values were higher in 2009 than in 2008 (Figure 4-4 and Table 4.1).

The exploratory multivariate ordination of the nine environmental parameters in a principal coordinates analysis (PCA) showed that some of the environmental variables were highly correlated (

Figure 4-4. Temporal patterns in environmental variables. Temporal patterns in (a) salinity and rainfall, (b) daily mean sea water temperature combining temperatures from both bays, (c) ash-free dry weight of sediment (AFDW), (d) suspended solids (SS), (e) dissolved organic carbon (DOC), (f) chlorophyll *a* (chl-*a*), (g) particulate organic carbon (POC), (h) particulate nitrogen (PN), and (i) particulate phosphorus (PP) during the two-year study in Nelly and Geoffrey Bays. Values represent means of two samples at each study site. (NB=dark circles, GB=white circles).

). The first principal component, PC1, was associated with water column concentrations of PP (eigenvalue -0.402), chlorophyll-*a* (chl-*a*; -0.382), PN (-0.361) and salinity (0.341). PC2 was associated with sedimentation (0.498),

maximum temperature 14 days preceding and including the sampling date (Tmax 14d; 0.452), maximum temperature 7 days preceding and including the sampling date (Tmax 7d; 0.423) and POC (-0.417) (

Figure 4-4. Temporal patterns in environmental variables. Temporal patterns in (a) salinity and rainfall, (b) daily mean sea water temperature combining temperatures from both bays, (c) ash-free dry weight of sediment (AFDW), (d) suspended solids (SS), (e) dissolved organic carbon (DOC), (f) chlorophyll *a* (chl-*a*), (g) particulate organic carbon (POC), (h) particulate nitrogen (PN), and (i) particulate phosphorus (PP) during the two-year study in Nelly and Geoffrey Bays. Values represent means of two samples at each study site. (NB=dark circles, GB=white circles).

). Together, PC1 and PC2 explained 59.3% of the total variation in the data.

Table 4.1. Values for environmental parameters in 2008 and 2009 Values for the ten environmental parameters measured one month and two weeks prior to and during the disease outbreaks of 2008 and 2009. Values represent means of two samples at each study site (NB=Nelly Bay, GB=Geoffrey Bay).

Environmental parameter	**1 month prior to dis. peak 2008**		**2 wks prior to dis. peak 2008**		**Disease peak 2008**		**1 month prior to dis. peak 2009**		**2 wks prior to dis. peak 2009**		**Disease peak 2009**	
	NB	GB	NB	GB	NB	GB	NB	GB	NB	GB	NB	GB
Salinity	-	-	-	-	33.1	-	31.7	32.2	20.8	28.3	19.0	20.1
Temp (°C)	30.2		29.9		30.5		29.8		28.5		31.7	
Rainfall (mm) Dec-Apr				1187.0						1901.6		
Sedimentation (ash-free dry weight mg cm^{-2} day^{-1})	-	-	0.4	0.2	1.0	-	0.7	0.6	1.4	1.0	1.8	0.8
Suspended solids (mg L^{-1})	1.6	2.0	1.3	2.1	4.3	-	2.4	3.0	3.7	3.8	4.5	2.4
Dissolved Organic Carbon (µM C)	70.2	74.5	85.1	196.4	116.09	-	101.7	103.4	106.5	126.5	146.1	156.2
Chlorophyll-*a* (µg L^{-1})	0.4	0.4	0.6	0.4	1.6	-	0.3	0.7	3.4	1.1	1.1	1.3
Particulate Organic Carbon (µM C)	14.4	12.4	17.2	14.8	26.9	-	18.5	21.8	-	-	39.8	22.2
Particulate Nitrogen (µM N)	8.5	7.0	7.5	0.8	1.6	-	1.4	1.7	3.79	2.07	2.5	1.8
Particulate Phosphorus (µM P)	0.1	0.1	0.1	0.1	0.2	-	0.1	0.1	0.1	0.1	0.2	0.1

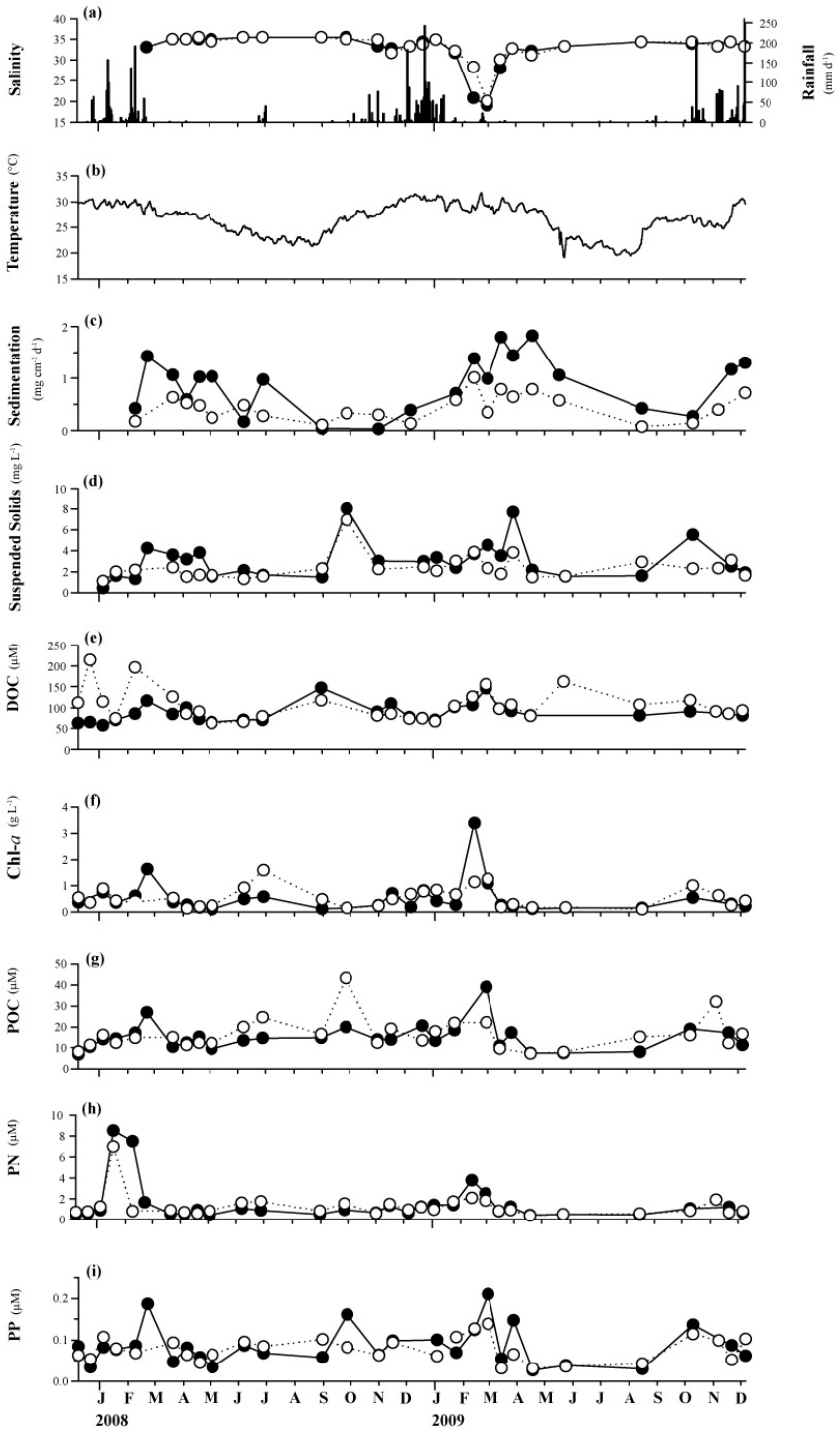


Figure 4-4. Temporal patterns in environmental variables. Temporal patterns in (a) salinity and rainfall, (b) daily mean sea water temperature combining temperatures from both bays, (c) ash-free dry weight of sediment (AFDW), (d) suspended solids (SS), (e) dissolved organic carbon (DOC), (f) chlorophyll *a* (chl-*a*), (g) particulate organic carbon (POC), (h) particulate nitrogen (PN), and (i) particulate phosphorus (PP) during the two-year study in Nelly and Geoffrey Bays. Values represent means of two samples at each study site. (NB=dark circles, GB=white circles).

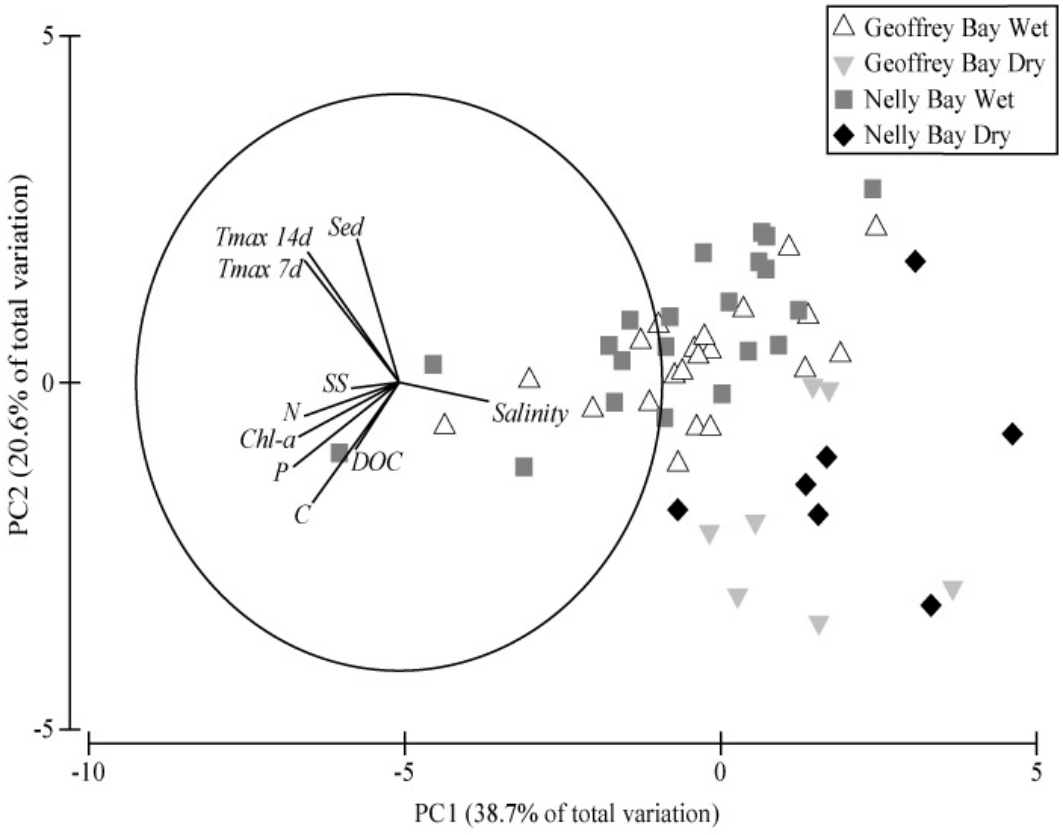


Figure 4-5. PCA of environmental variables. Principal coordinates analysis (PCA) of the nine measured environmental variables. Vector overlays represent multiple correlations between ordination axes and environmental parameters. PC1 is associated with particulate phosphorus (P) and nitrogen (N), chlorophyll-*a* (Chl-*a*) and salinity whereas PC2 is associated mainly with sedimentation (Sed), maximum temperature 14 days preceding and including the sampling date (Tmax 14d) maximum temperature 7 days preceding and including the sampling date (Tmax 7d) and particulate organic carbon (C). Together PCA1 and PCA2 axes capture 59.3% of the total variation of numbers of coral colonies with AtN.

4.4.3 PLS regression

In the initial Partial Least Squares (PLS) regression model containing all nine environmental variables, many of the variables had low regression coefficients (R) and hence were not good predictors of the variance in the number of AtN cases. These predictors were therefore removed from the model in a stepwise manner.

The variance associated with AtN abundance in the PLS model conducted using the combined datasets from both bays was significantly related to salinity, POC and Tmax 7d ($F_{3,167}$=111.88, p<0.0001). More than 95% of the variance in the model was found within its first component. The model explained 67% of the variance within the first three components (Global R^2=0.67). Following cross-validation, the model for both bays still explained 63% of the variation (predicted Global R^2=0.63). When the model was broken down to regression coefficients, AN abundance was negatively correlated with salinity (R=-0.6) and positively correlated with POC (R=0.32) and Tmax 7d (R= 0.08) (Table 4.2). The Durbin-Watson statistic was 1.39 indicating that there was no autocorrelation in the data set.

Table 4.2. Results of the PLS regression for the whole data set. Results of the final model for the combined data set of AtN versus the most important disease predictors: salinity, particulate organic carbon (POC) and maximum temperature 7 days preceding and including the sampling date (Tmax 7d). The PLS model was highly significant with its first three components explaining 74% of the variation. Following cross-validation the model still explained 36% of the variation.

	ANOVA				Model Selection and Validation		Disease Predictors		
All data	P-value	F	D.F.	Component	Global R2	Predicted Global R2	Salinity	POC	Tmax 7d
PLS ANOVA	<0.0001	111.88	167	1	0.65	0.62	-0.6		
PLS Model				2	0.67	0.63		0.32	
				3	0.67	0.63			0.08

4.5 DISCUSSION

As coastal human populations continue to increase, nutrients, terrigenous silt, pollutants and even pathogens themselves can be released to nearshore waters (Harvell et al., 2007). While the link between anthropogenic stress and disease susceptibility is currently poorly understood, it is thought that coral disease is facilitated by a decrease in water quality (Bruno et al., 2003). Evidence of this exists from the Caribbean (Bruno et al., 2003, Voss and Richardson, 2006b, Kaczmarsky et al., 2005) and the Philippines (Kaczmarsky, 2006) and suggests that anthropogenic stressors and coral disease are linked in complex ways (Harvell et al., 2007).

The present study documents a direct correlation between temporal coral disease dynamics and environmental parameters associated with water quality. The summer outbreaks of atramentous necrosis (AtN) corresponded to minima in seawater salinity but maxima in all other water quality parameters investigated. The disease was strongly and negatively correlated with salinity and positively correlated with seawater concentrations of particulate organic carbon (POC).

The more pronounced AtN outbreak in the summer of 2009 than in 2008 may be attributed to a greater terrestrial runoff caused by higher rainfall and higher values for environmental parameters (lower for salinity) preceding the outbreak in 2009. This may have lead to increased stress on corals that may have reduced their immune responses, and/or increased virulence of pathogen(s) causing the disease. Decreased resistance of the host coral caused by adverse environmental conditions may also increase opportunistic diseases (Harvell et al., 1999). Intense wet seasons may become more common in the future since strong

rainfall events are a likely scenario associated with climate change (Trenberth, 1998). Rainfall may be more variable from month to month, with longer dry spells and possibly with an increased frequency of disturbance events such as flooding rains and cyclones (Easterling et al., 2000, Walsh and Ryan, 2000, Milly et al., 2002, Palmer and Ralsanen, 2002) which may lead to drastic changes in inshore salinity levels.

While black band disease prevalence showed no relationship with salinity in the Caribbean (Kuta and Richardson, 2002), the results of the present study indicate that low salinity promoted AtN outbreaks. In 2009, salinity decreased in one month rapidly from above 30 to 20 in GB and to 19 in NB. Salinity measurements only commenced during the 2008 disease peak once this parameter was identified as a likely driver of AtN, therefore lower salinity values may have occurred in the preceding weeks. Low salinity adversely affects corals (Veron, 2008) by harming coral fertilization (Humphrey et al., 2008), by affecting the processes of photosystem II (Chartrand et al., 2009) and, in extreme cases, by causing a breakdown in coral-zooxanthellae symbiosis leading to coral bleaching (DeVantier et al., 1997).

The role of POC in coral infections has not been investigated previously; however, dissolved organic carbon (DOC) has been linked to coral disease (Kline et al., 2006, Smith et al., 2006). High levels of DOC increased the growth rates of microbes and DOC was more detrimental to coral health than nutrients (nitrate, phosphate, ammonia) (Kline et al., 2006). The authors suggested that a disruption in the balance between the coral and its associated microbes could have subsequently shifted the microbial consortia resulting in disease. Smith et al.

(2006) suggested that DOC compounds released by macroalgae may increase microbial activity. These findings suggest that increasing DOC levels associated with inputs of sewage and organic waste from coastal development could contribute to the high incidence of disease on highly polluted reefs (Kline et al., 2006). In the present study, the higher summer dissolved organic carbon (DOC) values could have facilitated AtN infections by increasing the growth rates of microbes. High values of DOC and POC at the study sites were likely associated with increased pelagic and benthic primary production in the water after increased nutrient inputs following heavy rainfall and runoff (Alongi and McKinnon, 2005, Furnas et al., 2005).

In the future, a combination of sea-level rise and an increase in rainfall due to climate change (Trenberth, 1998) could synergistically alter runoff and salinity in coastal ecosystems (Sokolow, 2009). The duration and intensity of the rainy season will be important factors in determining the stress caused to corals since a long duration could lead to chronic stress. Previous studies have concluded that chronic stressors may be more harmful to corals than acute stressors but their impact will depend on the period of exposure to those stressors (Kuntz et al., 2005). With the increasing probability of strong rainfall events leading to increased runoff in the future, both low salinity and high POC levels may lead to serious impacts on inshore reefs. It is likely that most inshore reefs of the GBR are heavily impacted by runoff during the wet season and that other reefs with high *Montipora* cover may experience similar outbreaks of AtN like the ones on Magnetic Island. To date, no studies of how runoff impacts other coral diseases

and other coral genera have been undertaken on the GBR and investigating this should be a priority in coral disease research.

Previous studies have identified clear seasonal patterns related particularly to warm temperatures for other coral diseases, including white syndrome (WS) (Bruno et al., 2007, Willis et al., 2004), black band disease (BBD) (Sato et al., 2009), and ulcerative white spots (Haapkyla et al., 2010) on the GBR, and aspergillosis (Harvell et al., 2001), white pox (Patterson et al., 2002) and BBD (Kuta and Richardson, 2002) in the Caribbean. Earlier studies on AtN (Jones et al., 2004b) documented an outbreak on reefs around Magnetic Island when the water temperature was higher than 31.5°C. In the present study, the water temperature was 31.7°C during the outbreak of 2009. In the month preceding the outbreaks (

Figure 4-4 and Table 4.1), temperature increased more in 2009 than in 2008, which may also have contributed to the larger number of recorded disease cases in 2009.

It is important to recognize that ecological responses to multiple interacting environmental variables are highly dynamic and rarely linear across both space and time with natural processes characterized by thresholds and limiting functions (Farnsworth, 1998, Koch et al., 2009). Temperature, although not showing a strong correlation in the PLS regression, is still likely to contribute to disease abundance but the response may not be linear. The PCA analysis revealed that maximum temperature 7 (Tmax 7d) and 14 days (Tmax 14d) preceding and including sampling dates explained some of the variability in disease abundance (

Figure 4-4). Temperature preceding the outbreak may be important in AtN dynamics as the PLS regression based on the whole dataset revealed that, although having a low regression coefficient, Tmax 7d was the third most significant environmental variable after salinity and POC (Table 4.2). The rate of temperature change is potentially important in AtN dynamics and merits further investigations (Lonergan, 2006). Further studies should also measure the duration of warm and cold periods that may impact AtN dynamics to better understand the role of temperature in AtN dynamics. Periods of hot and cold seawater, or hot and cold 'snaps', had an effect on patterns of white syndromes (WS) on the GBR, with most outbreaks occurring after mild winters and during hot summers (Heron et al., 2010).

In the present study, the highest water column nutrient concentrations were measured during the wet season. This agrees with previous studies that found that most water quality parameters other than salinity are higher during the wet season in the inshore GBR lagoon, when water quality conditions can change abruptly and nutrient concentrations increase dramatically for short periods following major disturbance events (cyclonic mixing, river flood plumes) (Schaffelke et al., 2007). Flood plumes are the main delivery mechanism for nutrients (in dissolved and particulate form) and suspended sediments to GBR coastal waters, with concentrations 10 to 400 times higher than in non-flood conditions (Devlin et al., 2001, Devlin and Brodie, 2005). The coastal zone of the Burdekin region, where Magnetic Island is located, had the highest values of PN, PP and SS and second highest for chl-*a* of the whole GBR (De'ath and Fabricius, 2008). The Burdekin River exports very large amounts of sediment and associated nutrients during

large floods (Furnas, 2003) and significantly affects the water quality of Magnetic Island (Wolanski and Vansenden, 1983, King et al., 2001, King et al., 2002), together with local runoff from the island itself and from smaller rivers in the vicinity.

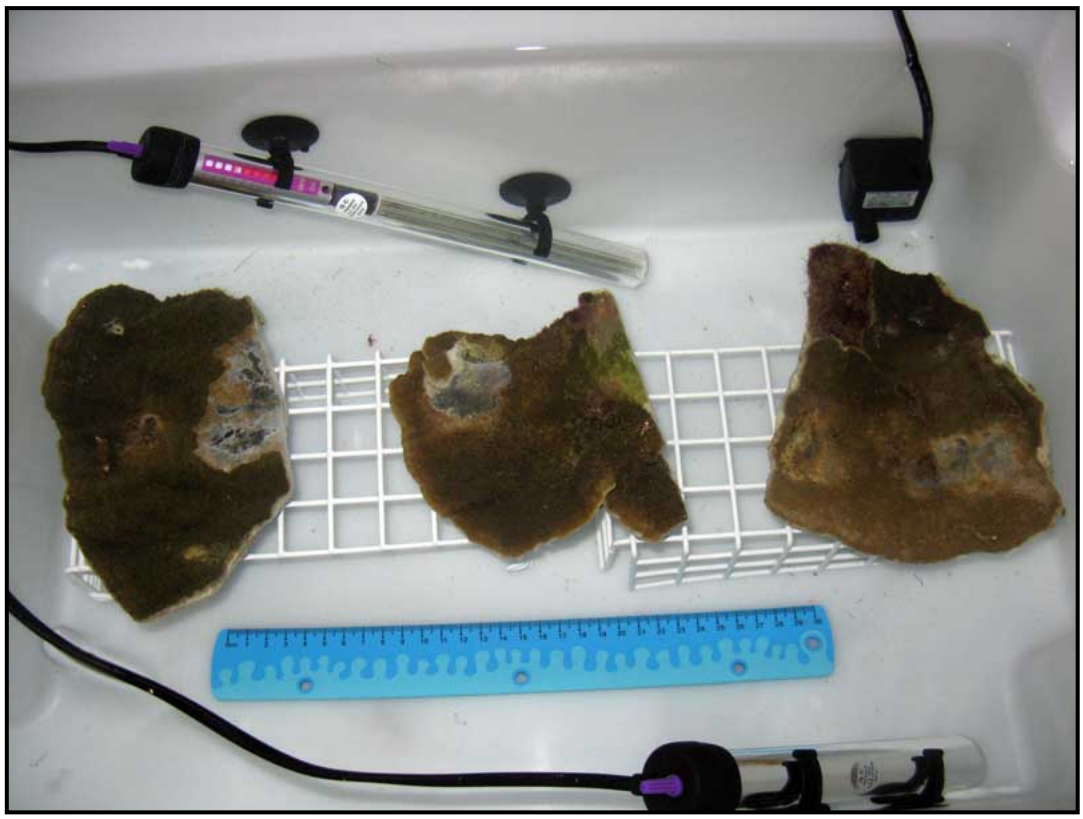
Sediments may not only be a cause of physical stress to corals but may also act as a pathogen reservoir (Voss and Richardson, 2006b). For example, significantly higher sedimentation rates were found on sites with black band disease than on sites with no signs of disease in the Caribbean (Voss and Richardson, 2006b). The highest sedimentation rates were found during the summer in the present study when AtN abundance was high. By stressing corals, sediments may make the corals more susceptible to infections by microbial pathogens and may also act as disease reservoirs (Voss and Richardson, 2006b). Fine sediment often settles on Magnetic Island reefs during periods of calm weather, and can result in smothering and tissue mortality of corals if the sediment is not re-suspended during rough weather or removed by the coral itself (Fabricius, 2005, Roy and Smith, 1971, Rogers, 1983). It is possible that the sediments act as pathogen reservoirs on Magnetic Island, however this was beyond the scope of the present study.

Coastal areas are globally under increasing pressure by human population growth, intensifying land use, urban and industrial development. However, previous studies on terrestrial influences on coral disease prevalence in the Indo-Pacific have not included direct measurements of water quality (Kaczmarsky, 2006, Page and Willis, 2006). Our study highlights a previously unrecognized adverse effect of land runoff on the health of key reef-building corals: the

promotion of coral disease. The findings of this study are of wide importance because improving water quality in areas affected by runoff is one of the few management options that will enhance reef resilience in the face of climate change (Veron et al., 2009, Bellwood et al., 2004).

Chapter 5

Experimental study to elucidate the effects of temperature and salinity on atramentous necrosis



5.1 ABSTRACT

Environmental drivers of coral diseases, particularly those relating to water quality, remain poorly understood. The results of an earlier field study (Haapkyla et al., 2011) highlighted the role of low salinity and high temperature as drivers of the disease atramentous necrosis (AtN), which primarily affects the coral *Montipora aequituberculata*. The impacts of three temperatures (28, 30 and 32°C) and three salinities (20, 26 and 34 ppt) on rates of mortality caused by AtN were investigated in separate aquarium studies and the interactions between them in a combined analysis. The results suggested that disease progression and resulting mortality of coral fragments may have been more rapid and extensive under salinity stress than under temperature stress. Mortality increased over time in all experiments but the difference in the pattern of change over time was not statistically detectable for either temperature or salinity. This suggests that there may be a 'point of no return' in the progression of AtN, beyond which the disease progresses no matter what the surrounding environmental conditions are. The results of this study indicate that low salinity, for example resulting from heavy rainfall during the monsoon season, may negatively affect inshore coral communities, particularly when seawater temperatures are high.

5.2 INTRODUCTION

Coral diseases have emerged as an important driver of ecological change on coral reefs in recent decades (Harvell et al., 2002), thus understanding environmental factors that trigger or promote these diseases is becoming increasingly urgent. Recent research supports a connection between high temperatures and coral disease (Harvell et al., 2001, Harvell et al., 2002, Bruno et al., 2007, Maynard et al., 2011), but links between disease and other environmental factors are less well understood (Bruckner, 2002). While disease outbreaks are likely to increase due to climate warming in many ecosystems, corals are thought to be among the most susceptible of organisms because of their narrow thermal tolerance (Harvell et al., 2002).

Field experiments demonstrate that moderate increases in dissolved inorganic nutrient concentrations can substantially increase the severity of coral diseases. In the Caribbean, for example, increased phosphorus, nitrate and ammonium levels enhanced aspergillosis and Caribbean yellow band disease (Bruno et al., 2003), increased ammonium, nitrate and nitrite levels enhanced the prevalence of aspergillosis (Kim and Harvell, 2004), and increased levels of nitrate correlated with enhanced progression rates of black band disease (Voss and Richardson, 2006b). No relationship was detected in a correlative study between the prevalence of black band disease on Great Barrier Reef corals and distance to the mainland, which was used as a proxy for terrestrial influences (Page and Willis, 2006), whereas the distribution of growth anomalies (GA) was strongly associated with coral host density and human population density in the Indo-Pacific (Aeby et al., 2011).

Atramentous necrosis (AtN) is one of the few coral diseases that is more common on coastal reefs than on offshore reefs (B. Willis and C. Page, pers. comm.). AtN is characterised by a spreading bleached patch that subsequently becomes covered with a white and black bacterial film (Anthony et al., 2008) likely the result of opportunistic secondary microbial community (Bourne, 2005). AtN was first recorded around Magnetic Island, a fringing reef in the central Great Barrier Reef (GBR), Australia, in 2001. AtN prevalence is linked to high temperatures, for instance, the disease was first recorded during a mass bleaching event in 2001 when water temperatures higher than 30 ºC were detected (Jones et al., 2004b). An aquarium study demonstrated that the progression rate of AtN was fastest at 29 °C (Lonergan, 2006). AtN maxima in two consecutive years on Magnetic Island coincided with water temperatures higher than 30 ºC, but also with low salinities and high concentrations of particulate organic carbon in the water column (Haapkyla et al., 2011). Since many factors vary in field studies, it is not possible to tease apart the underlying casual relationships between environmental drivers and coral disease.

To further explore whether salinity or temperature is the primary environmental factor driving prevalence and progression rates of AtN found in field studies, controlled experiments that manipulate single factors while maintaining others constant are required. The aim of the present study was to compare progression rates of AtN on the host coral *Montipora aequituberculata* among a number of salinity and temperature treatments in controlled aquarium experiments.

5.3 METHODS

5.3.1 Sample collection

Fragments of the foliose coral *Montipora aequituberculata,* ranging in size from 10x10 cm to 25x25 cm, were collected from 3-5 m depth in two adjacent bays (Nelly and Geoffrey Bay) on the south-eastern side of Magnetic Island (19°S, 147°E) on three occasions during 8th of January and 7th and 27th of February 2010. Fragments were selected based on the presence of signs of the initial tissue loss phase of atramentous necrosis (AtN) (see Anthony et al., 2008). Apparently healthy fragments were collected and used as controls. The fragments were cut underwater with a saw, immediately wrapped in bubble wrap for protection and transported immersed in seawater in insulated containers. Within 5-6 hours of collection, corals were transferred to holding tanks with flow-through seawater at the Aquarium Complex of the Marine and Aquaculture Research Facilities Unit (MARFU) at James Cook University (JCU).

5.3.2 Experimental set-up and conditions

Corals were acclimated for 48 hours at 28 ºC and ambient salinity (34) before each experiment. Experiments were conducted in plastic tanks holding 40 L of seawater. A portion of the seawater in each tank (10-15 L) was exchanged every 12 hours for the first 72 hours, and every 24 h thereafter to maintain water quality. Seawater was sand-filtered, protein-skimmed and then further filtered to 1µm. Air stones circulated the water in all experimental tanks and the temperature in all tanks was maintained at treatment temperatures using electrical heaters. White fluorescent lights (New Generation T8 Tri-phosphor Fluorescent Lamp

Sylvania SYL FL58W/840 5200 Lumens 1520 mm) provided a light intensity of 97 μM/m^2/s (Li-Cor Model LI-250 light meter).

All experiments included one control tank containing three apparently healthy coral fragments (tank controls) that were maintained at ambient temperature (28 °C) and salinity (34) throughout. Tank controls remained alive and showed no signs of AtN throughout any of the three experiments (described below), demonstrating that the experimental set-up had no or negligible effects on the health of the coral fragments.

Experiments were terminated once all coral fragments in the treatments had died, with the exception of the 28 ºC treatment in the temperature experiment, which was terminated after 15 days because disease progression had levelled out over the final four days of the experiment and no further response was expected. All experiments were completed between January 9th and March 10th 2010.

Disease progression rates were calculated by measuring changes in the percent area of coral fragments that were dead in all three experiments. Changes in dead areas were measured from photographs of each coral fragment that were taken daily from the same angle and included a ruler for scale. Images were analysed using Canvas^{TM}X (System version 10.5.5).

5.3.3 Experimental Design

1. Temperature experiment

Three temperature treatments were established, each in three replicate tanks, representing an ambient summer temperature treatment (control; mean ±SE: 28°C ±0.09) and two elevated temperature treatments (30 °C ± 0.14 and 32 °C 0.04 ºC).

Each tank contained three diseased coral fragments, resulting in 9 tanks and a total of 27 fragments of *M. aequituberculata* infected by AtN. Temperatures were measured twice a day using glass thermometers (GT-RL series, Omega, USA) assigned to each tank. Salinity was held constant at 34 ppt.

2. Salinity experiments

Salinity was manipulated in two experiments, one where temperature was fixed at ambient summer temperatures (28 °C) and one where temperature was elevated (32 °C) to simulate heat stress. In the salinity experiment at ambient summer temperatures, three salinity treatments: 20 ppt (± SE 0.13), 26 ppt (± 0.09) and 34 ppt (± 0.03) were established. As above, each treatment consisted of three replicate tanks, each containing three coral fragments infected by AtN, resulting in 9 tanks and a total of 27 fragments of *M. aequituberculata* with signs of AtN.

In the salinity experiment run in combination with heat stress, only the effects of lowered salinity at 20 ppt (± SE 0.85), and 26 ppt ± 0.22) were investigated. Due to difficulties in finding diseased colonies, it was not possible to obtain enough replicate fragments to test the effects of heat stress under an ambient (34 ppt) salinity regime, which had implications for the statistical analyses (see below). However, this was tested in the temperature experiment where a combination of high temperature (32 °C) and ambient (34 ppt) salinity were used. The salinity experiment under heat stress used a total of six experimental tanks, each containing three diseased coral fragments.

Salinities were measured twice a day with a hand-held refractometer (r^2 Mini, Reichert GmbH, Germany). Reverse osmosis (RO) water was used to adjust salinities, if required. The volumes and salinities were calculated using the following formula: $C_1 \times V_1 = C_2 \times V_2$ where C_1=existing concentration of seawater V_1=existing volume of seawater, C_2=desired concentration of seawater, V_2=desired volume of seawater.

5.3.4 Data analyses

To analyse changes in partial mortality of fragments through time, the percent area of fragments that were dead was compared among treatments using nonparametric repeated measures analyses in R (version 2.10.1). The 'nparLD' package with an 'F1-LD-F1' design (Noguchi et al., 2009, Brunner et al., 2002) was used in the analysis. Because the covariance matrix was singular in all cases (most likely due to the many instances of values of 100% mortality in the data set), p-values were based on tests using an ANOVA-type statistic (i.e. a non-parametric approximation to an F-distribution as recommended by Brunner et al. (2002).

The combined effects of salinity and temperature were investigated by combining the results of the salinity experiments conducted at 28 °C and 32 °C. While the combined design is equivalent to a 2-factor ANOVA (with two levels of temperature and two levels of salinity), the temperature effect must be interpreted as a combined 'temperature-experiment' effect, because the two levels of temperature (28 °C and 32 °C) were tested in two separate experiments. While every effort was made to maintain consistent conditions between experiments,

uncontrolled variables may have contributed to the differences in the combined 'temperature-experiment' treatment. Due to the short duration of the second salinity experiment (5 days), only data from days 1-5 were used from the first salinity experiment.

5.4 RESULTS

Mean percent partial mortality of *M. aequituberculata* fragments differed among the three temperature treatments. Coral fragments in the 32 °C temperature treatment showed the highest disease progression rates and reached 100% mortality within 7 days, whereas corals in the 30 °C treatment reached 100% mortality only after 15 days (Figure 5-1). Fragments in the 28 °C treatment reached 70% partial mortality after 7 days and mortality remained constant until day 15 (Figure 5-1), after which time the experiment was terminated.

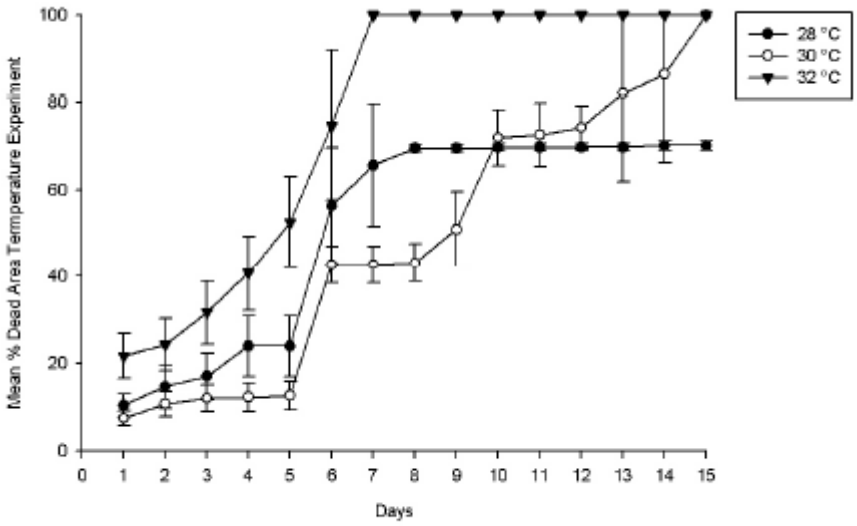


Figure 5-1. Comparative patterns of partial mortality (mean ± SE) on *Montipora aequituberculata* fragments among three temperature treatments over a 15 day experimental study. Day 0 was part of the acclimation period; heating treatments commenced on Day 1.

In the absence of heat stress, the highest rates of mortality were observed in the low (20 ppt) salinity treatment, and the least in the ambient salinity treatment (Figure 5-2). Overall, the most rapid mortality was observed when high temperature (32 °C) and low salinity (20 ppt) were combined in the salinity

experiment conducted under heat stress, in which 100% mortality was reached in 4 days on all fragments (Figure 5-3). In the high temperature (32 °C) and medium salinity (26 ppt) treatment, 100% mortality was detected in 5 days, whereas mortality was slowest in the low temperature (28 °C) and ambient salinity (34 ppt) treatment (Figure 5-2 and Figure 5-3).

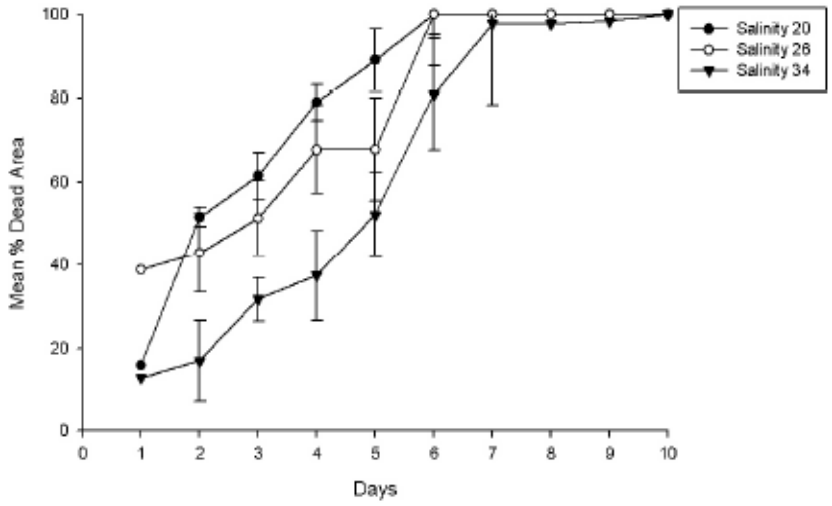


Figure 5-2. Comparative patterns of partial mortality (mean ± SE) on *M. aequituberculata* fragments among 3 salinity treatments when ambient (28 °C) temperatures were maintained.

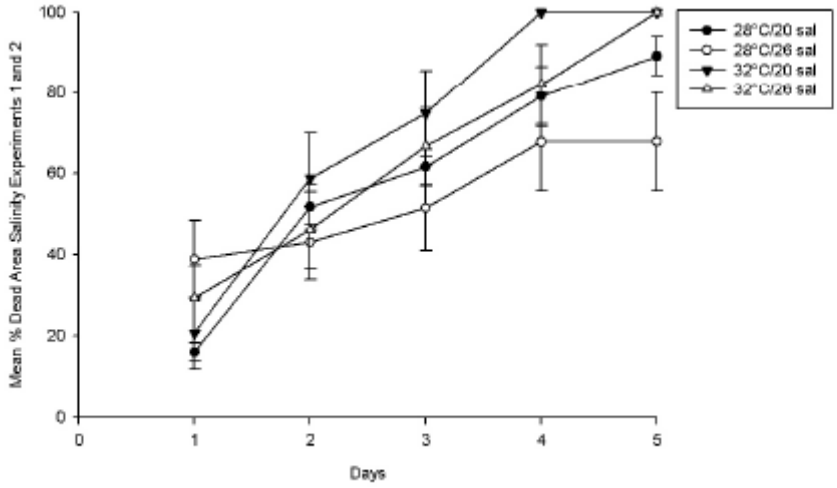


Figure 5-3. Mean (± SE) percentage cover of dead area on fragments of *M. aequituberculata* per treatment per day when salinity experiments conducted at ambient and high temperatures were combined.

Differences in partial mortality through time were highly significant in all experiments ($p<0.0001$; Table 5.1) but the pattern of change over time did not differ detectably when salinity stress experiments were run at ambient versus elevated temperatures. A post-hoc test detected a statistically significant difference in patterns of partial mortality among salinity treatments through time (salinity * time treatment, $p=0.02$), in the combined data set (salinity experiments 1 and 2) (Table 5.1), suggesting that lowering salinity affected the mean percentage of dead area between the two temperature treatments in the combined data set. However, this was not a consistent effect since salinity was not found to have a significant effect in the salinity experiment 1 (Table 5.1)

Table 5.1. Summary of results from the nonparametric repeated measures analysis with an ANOVA-like statistic. Shaded cells: significant effects at $\alpha = 0.05$.

	Effect	Anova-type statistic	df_1	df_0	*p*-value
Temp experiment	Temp	0.911	1.235	2.681	0.4442
	Time	23.11	1.953	∞	<0.0001
	Temp*Time	0.715	3.008	∞	0.0543
Salinity experiment 1	Sal	2.016	1.684	4.364	0.2368
	Time	54.24	1.781	∞	<0.0001
	Sal*Time	0.861	2.942	∞	0.4589
Salinity experiment 2	Sal	0.967	1	2.833	0.4018
	Time	39.21	2.155	∞	<0.0001
	Sal*Time	0.899	2.155	∞	0.4136
Combined data (Sal experiments 1 and 2 – 5 days)	Temp-exp	1.247	1	5.977	0.3070
	Sal	0.360	1	5.977	0.5706
	Time	52.84	2.905	∞	<0.0001
	Temp-exp*Sal	0.054	1	5.977	0.8245
	Temp-exp*Time	1.668	2.905	∞	0.1731
	Sal*Time	3.115	2.905	∞	0.0264
	Temp-exp*Sal*Time	0.819	2.905	∞	0.4797

DISCUSSION

Despite increasing research efforts focussed on Indo-Pacific coral diseases, significant knowledge gaps remain regarding environmental controls, triggers or drivers of coral disease in this region. Temperature and salinity were selected as environmental variables to be tested in the present manipulative study based on the results from a field study, in which outbreaks of atramentous necrosis (AtN) co-occurred with a salinity of 20 ppt and temperatures above 30 °C during two consecutive wet seasons on an inshore reef of the GBR (Haapkyla et al., 2011). My results suggested that disease progression leading to total mortality may have been most rapid when experimental fragments were exposed to a combination of low salinity (20 ppt) and high temperature (32 °C). When each stressor was tested independently, rates of mortality were greater when fragments were exposed to salinity stress compared to temperature stress alone (Figure 5-1 and Figure 5-3).

There have been few studies of the effects of low salinity on reef corals, and these have primarily focused on cyclone impacts on corals (Brodie, 1996, Crossland, 1928, Goreau, 1964, Byron and O'Neill, 1992, VanWoesik et al., 1995). Salinity may affect corals adversely in a number of ways. For example, low salinity may impair fertilization (Humphrey et al., 2008), affect the functioning of photosystem II (Chartrand et al., 2009), and lead to coral bleaching (DeVantier et al., 1997). A recent review of salinity tolerances of GBR corals found that the most sensitive species tolerated between 26 and 30 ppt for many months, whereas more tolerant species of coral could survive salinities as low as 23 ppt for an equal amount of time (Berkelmans et al., 2011). More detailed aquarium studies have shown that colonies of the sensitive genus *Acropora* from

the Keppel Islands tolerate salinities ranging from 20 to 27 PSU (Practical Salinity Units, used when data is derived from data loggers) for an exposure time of 2-14 days at the lowest and highest salinities, respectively (Berkelmans et al., 2011). The only study, prior to the present, that investigated potential links between low salinity and coral disease, found no correlation between black band disease prevalence and salinity (Kuta and Richardson, 2002). There is need for more studies of the impacts of salinity stress on corals, especially given predictions of increasing storm activity with climate change.

The faster progression rates of AtN observed in the salinity stress compared to the temperature stress experiments could be attributable to the later collection date of coral fragments for the salinity experiments. Corals would have been exposed to a longer period of stressful field conditions and a longer period of disease prior to collection for the salinity stress experiment. Ambient salinities at the time of collection of coral fragments for all experiments were lower than normal (between 23 and 27 ppt compared to normal values of 33-35 ppt) due to heavy seasonal rainfall. In the field, corals get progressively more infected during the course of the summer period and the number of diseased corals decrease drastically after the disease peak (Haapkyla et al., 2011). Differences in the extent of partial mortality on the collected fragments could not have been avoided because of difficulties in finding sufficient numbers of diseased corals. It could be that after a certain period of time, the progression of AtN reaches a 'point of no return' i.e. the disease progresses regardless of the prevailing environmental conditions. This could be a characteristic of AtN, and possibly also apply to other coral diseases. What makes corals reach this 'point of no return', and whether or

not a change in the environmental conditions could reverse this, warrant further investigations.

The present study is the first to test the effect of salinity on a coral disease under experimental conditions and it should therefore be considered as a pilot study. The fact that no statistically significant difference between the salinity and temperature treatments was detected may be due to a low number of replicates and high variability in the data set that led to a reduced power of the analysis. The results of a field study that preceded the experimental study revealed that the peak in AtN corresponded to low salinity values and high particulate organic carbon content, whereas temperature was the third most significant environmental driver of AtN (Haapkyla et al., 2011). The role of temperature in outbreaks of AtN is important since AtN outbreaks only occur at water temperatures above 30°C (Jones et al., 2004b, Haapkyla et al., 2011). A previous experiment testing the effect of temperature on the progression rate of AtN found that the rate of change in temperature was important in the onset of this disease (Lonergan, 2006).

In the future, rising CO_2 levels in the atmosphere are likely to lead to stronger and more variable rainfall events (Trenberth, 1998). Moreover, it is likely that there will be longer dry spells and possibly an increased frequency of flooding and cyclones (Easterling et al., 2000, Walsh and Ryan, 2000, Milly et al., 2002, Palmer and Ralsanen, 2002). Since the last century, average rainfall and extreme weather events have significantly increased in northeast Queensland (Lough, 2011), which is likely to lead to decreased salinity and increased stress levels that may seriously challenge the resilience of inshore coral reefs.

Chapter 6

General Discussion

6.1 COMPARATIVE SPATIO-TEMPORAL DYNAMICS OF CORAL DISEASE ON A HIGH AND LOW LATITUDE INDO-PACIFIC REEF

To determine the range of diseases that affect Indo-Pacific corals and gain insights into their etiology, this thesis investigated spatio-temporal disease dynamics at two latitudes 20 degrees apart in the western Pacific: a low latitude coral reef in the Wakatobi Marine National Park (WMNP), South-East Sulawesi, Indonesia, and the high latitude reef, Heron Island, in the southern Capricorn-Bunker sector of the Great Barrier Reef (GBR) (Chapters 2 and 3). Overall, levels of disease prevalence were highest on the southern Heron Island reef, reaching a maximum of 4.2 ± 1.72%. Indeed, the lowest overall mean disease prevalence at Heron Island (1.9 ± 0.75%; mean ± SE) was almost twice as high as the highest overall disease prevalence encountered in the WMNP (1.1 ± 0.42% in 2010) (Chapters 2 and 3). Overall levels of disease prevalence were lower at both locations than values reported from the Philippines (8.3 ± 1.2%, Raymundo et al., 2005) and the GBR in the summer of 2003 (8.9 ± 0.79%, Willis et al., 2004), and generally lower than mean levels reported for the Caribbean (4.2%, Croquer and Weil, 2009). The low (less than 2%) overall levels of disease prevalence in the WMNP are similar to levels observed on pristine reefs in the central Pacific (Vargas-Angel, 2009), however, temporal studies are important for interpreting the impact that even low levels of disease prevalence may have on coral populations. Even with a low overall disease level, the impact of diseases on the coral community may be significant since diseases may affect important reef-building genera and may have fast progression rates.

Trends in both the number of diseases observed and levels of disease prevalence between 2005 and 2010 suggest that coral health has been deteriorating in the WMNP over the past 5 years. In 2005, only two diseases, white syndromes (WS) and growth anomalies (GA) were detected, whereas four diseases were detected in 2007: WS, GA, black band disease (BBD) and *Porites* ulcerative white spots (PUWS). By 2010, six diseases were detected: WS, GA, skeletal eroding band (SEB), PUWS, ulcerative white spots (UWS) and BBD (Chapter 2). A total of six syndromes were also encountered at Heron, but in contrast to WMNP, they included BrB rather than PUWS (Chapter 3). The diseases encountered in surveys at both sites have been detected in disease surveys conducted elsewhere in the Indo-Pacific, including the Philippines (Raymundo et al., 2003, Raymundo et al., 2005) and Australia (Willis et al., 2004, Page, 2009), thus no new coral diseases were found at the two sites.

My study at Heron Island revealed that the most common disease was brown band syndrome and that its prevalence had increased three-fold since the previous survey, i.e. from <1% (Willis et al., 2004) to 3.3% ± 0.58 (Chapter 3). BrB is the most rapidly progressing coral disease known, both on the GBR (2.1 cm d^{-1}; (Boyett, 2006) and in the WMNP (1.2 cm d^{-1}; Chapter 2). The combined effects of increasing prevalence and rapid progression rates suggest that this disease may have significant impacts on Heron Island coral assemblages (Chapter 3). Also, this study is the first to record a higher prevalence of BrB in cooler winter months (Chapter 3), suggesting that it is one of the few coral diseases whose virulence does not increase with temperature. It is possible that declining coral health on this southern latitude reef, potentially because of localised cold-

water bleaching events that have been known to occur at Heron Island (Hoegh-Guldberg et al., 2005), could have facilitated the increasing prevalence of BrB infections in winter. Alternatively, mortality of susceptible corals over time may contribute to reasons underlying the lower prevalence of BrB found in the summer survey conducted in 2009. Epidemiological studies of disease dynamics have shown that host resistance to disease increases as susceptible individuals are lost from populations, leading to a waning in the number of disease cases (Sokolow, 2009). This phenomenon has been documented for gorgonians affected by aspergillosis in the Florida Keys (Kim and Harvell, 2004).

Declining coral cover in both the WMNP and at Heron Island throughout the period of my study highlights the possibility that coral diseases have had an impact on these populations. A highly significant drop in coral cover was detected between 2005 and 2007 in the WMNP, and cover continued to decline between 2007 and 2010, although declines were smaller than in the first two years (Chapter 2). Only a small decrease in coral cover was observed at three study sites around Heron Island over the three years of my study. Coral cover remained higher than 20% at all sites, which was higher than coral cover at several sites in the WMNP (Chapters 2 and 3). A correlation between coral cover and disease prevalence was detected on Heron, but the highest taxon-specific disease prevalences were found at sites with intermediate numbers of host corals, which is consistent with density-independent effects (Chapter 3). A correlation between coral cover and disease was previously documented on the GBR by Bruno et al. (2007) who found a higher prevalence of WS at reefs with greater than 50% coral cover. In the WMNP, decreasing coral cover could be due to human population

growth in the area, which has led to over-use of resources, e.g. by overfishing (McMellor, 2007). The link between fish diversity and coral diseases was investigated in the Philippines, where disease prevalence was negatively correlated with fish taxonomic diversity and positively correlated with the density of fish in the family Chaetodontidae (Raymundo et al., 2009). The authors suggest that over-fishing releases non-targeted fishes such as corallivorous Chaetodontidae, which then possibly act as vectors of coral disease. The possible link between coral disease and high fishing pressures in the WMNP should be investigated further to gain insights into the importance of intact functioning reef communities for coral health.

The dramatic decline in coral cover (from 74.5% ± 4.4 in 2007 to 18.3% ± 7.7 in 2010) observed at the WMNP site with the highest rates of sedimentation was most likely a consequence of the combined impacts of four coral diseases (BBD, PUWS, UWS and WS), which were first documented at this site in 2006 (Haapkylä, unpublished data). Although the co-occurrence of declining coral cover and increasing disease at this site suggests that poor water quality enhances the virulence of these diseases, further research is required to better evaluate the links between sedimentation and disease and to identify which aspects of water quality are important as environmental drivers of disease. The decline in coral cover combined with rates of disease progression, which are similar to rates recorded in the Caribbean and the GBR (Chapter 2), suggest that disease may play an important role in shaping coral communities in the WMNP, potentially threatening the reef framework at some sites, as has been documented in the Caribbean (Nugues, 2002).

Changes in coral community structure on Heron Island reefs, from tabular species of *Acropora* in 2007 to dominance by *Goniastrea*, bushy *Acropora*, *Coscinarea* and *Stylophora* species in 2009 (Chapter 3), are likely to lead to a structurally less diverse reef community, given that plate acroporid corals provide important habitats and food sources for many invertebrates and reef fish (Jones et al., 2004a). Tabular acroporids have been the primary target of white syndromes (WSs) recorded on Heron Island reefs in previous studies (Willis et al., 2004, Roff et al., 2006, Ainsworth et al., 2006, Roff et al., 2011), suggesting that this group of diseases may have played a role in the shift in coral community structure detected. Given the rapid tissue loss caused by WSs (up to 124.6 cm^2 per day) (Roff et al., 2006), this group of diseases certainly has the potential to significantly alter coral community structure. WS abundance has been linked to high seawater temperature anomalies (Bruno et al., 2007, Maynard et al., 2011), however, no temperature anomalies were detected at Heron Island during the survey period (Chapter 3). However, cold water bleaching did occur earlier at Heron Island (Hoegh-Guldberg et al., 2005) and may similarly deplete nutritional resources and increase the susceptibility of corals to WSs. These results highlight the need for more frequent disease monitoring in order to understand the role that coral diseases have had on Heron Island reefs and their environmental drivers.

Differences in disease susceptibility among coral taxa and coral community composition are also likely to have contributed to the community shift at Heron Island, as diseases were more common at sites dominated by the most susceptible taxa: staghorn *Acropora* and *Montipora*. Studies of GBR corals have found that staghorn *Acropora* are host to several coral diseases, including the virulent

diseases BBD (Page and Willis, 2006), BrB (Willis et al., 2004) and SEB (Page and Willis, 2008). In the Caribbean, acroporids have been subject to outbreaks of both white pox (Patterson et al., 2002) and white band (Aronson and Precht, 2001) diseases, making two acroporid species critically endangered. It has been hypothesised that fast-growing acroporids are more vulnerable to disease than other genera because they direct resources to growth (Jackson, 1979) rather than disease resistance (Willis et al., 2004), whereas corals with slow growth rates such as *Porites* may direct more resources towards disease resistance (Willis et al., 2004, Palmer et al., 2010). However, in contrast to my finding that acroporid corals were primarily affected by diseases on the high latitude Heron Island reef, the genus *Porites* were most affected by disease in the 2005 survey in WMNP. *Porites* was also identified as the main host of diseases in the Philippines (Raymundo et al., 2005). However, in the later 2007 and 2010 surveys in WMNP, *Acropora* species became the primary disease targets (Chapter 2), highlighting the susceptibility of this genus across its distributional range. The fast progression rates of BrB and WS, the principal diseases affecting acroporids, may lead to serious impacts on the coral community structure. Overall, the number of diseased coral taxa was similar in both the WMNP and on Heron Island reef, with 13 and 14 diseased taxa, respectively (Chapters 2 and 3).

My prevalence studies in the WMNP and around Heron Island highlight the crucial role of long-term monitoring for detecting changes in reef ecosystems and the potentially negative impact coral diseases may have on the reef framework at both latitudes.

6.2 ENVIRONMENTAL DRIVERS OF INDO-PACIFIC CORAL DISEASE

Changes in land use over the past 200 years of European settlement are now potentially affecting the health of coastal and inshore ecosystems on the GBR by bringing elevated concentrations of nutrients, suspended sediments and pesticides into the marine environment (Furnas, 2003, Brodie and Mitchell, 2005, Fabricius, 2005, Schaffelke et al., 2005). Despite the increase in coral disease studies in the last 15 years, the link between anthropogenic stress and disease susceptibility remains poorly understood. Caribbean field studies suggest that the incidence of some coral diseases may be facilitated by decreases in water quality (Bruno et al., 2003). Evidence of higher disease prevalence in close proximity to a sewage outfall in the Caribbean (Bruno et al., 2003, Voss and Richardson, 2006b, Kaczmarsky et al., 2005) and the Philippines (Kaczmarsky, 2006) supports a link between poor water quality and disease, but there are no previous studies on possible links between coral disease prevalence and poor water quality on the GBR.

My study of atramentous necrosis (AtN), a coral disease occurring predominantly on inshore reefs of the GBR, revealed a link between poor water quality and coral disease on the GBR (Chapter 4). This study also revealed, for the first time, the important role of low salinity resulting from monsoonal rain in driving outbreaks of AtN. Results of the experimental study on drivers of AtN were concordant with the findings of the field study, with the fastest mortality rate being observed in the low salinity (20) and high temperature (32°C) treatment, although the differences were not statistically significant, possibly due insufficient

replication, unknown intrinsic disease processes or both (Chapter 5). The progression of AtN may include a 'point of no return' after which the disease progresses regardless of the surrounding environmental conditions (Chapter 5). However, both field and experimental studies on AtN highlight the role of low salinity and high temperature as being important drivers of this disease (Chapters 4 and 5).

Strong rainfall events are likely to become more frequent in the future as climate changes (Trenberth, 1998), with the consequence that the frequency of significant flood events is predicted to increase substantially over the next few decades (Milly et al., 2002). Associated decreases in water quality are likely to lead to decreased resistance of corals and an increase in diseases (Harvell et al., 1999). My findings that low salinity and increased particulate organic carbon are linked to the abundance of AtN are of wide importance, since they suggest that drastic changes in inshore salinity levels and an increase in runoff from land, e.g. by more extreme floods, could potentially lead to serious impacts on inshore coral communities on the GBR and elsewhere in the Indo-Pacific. Studies of the links between water quality and other coral diseases are important areas for future research. Apart from increasing coral disease prevalence, low salinity can have other disastrous effects on coral populations. For example, fertilization of coral eggs did not occur at salinities of 28 ppt and low salinity associated with heavy rainfall destroyed the whole reproductive output of a coral reef flat on the GBR (Harrison et al., 1984, Humphrey et al., 2008).

The co-occurrence of AtN outbreaks with large rainfall events that resulted in high sedimentation rates at Magnetic Island (Chapter 4) provides strong

evidence that water quality is an important driver of coral disease. Sediment loads to the GBR have increased four to five-fold in the last 200 years (Maughan et al., 2008), and five to ten-fold in some catchments (McCulloch et al., 2003). Sedimentation has many harmful effects on coral reefs, including reducing coral recruitment rates and coral biodiversity (Fabricius, 2011). Sediment-related stress may make corals more susceptible to infections by microbial pathogens, and sediments may also act as disease reservoirs (Voss and Richardson, 2006b). Therefore, predicted extreme wet seasons and major flood events in the larger GBR drainage basins will have widespread and long-lasting influences on water quality and shelf ecosystems (King et al., 2002, Wolanski and Vansenden, 1983). High sedimentation rates were linked to an outbreak of BBD in the WMNP (Chapter 2). Previous studies have linked BBD with high sedimentation rates in the Caribbean (Bruckner and Bruckner, 1997a, Voss and Richardson, 2006b), the Indo-Pacific (Antonius, 1985, Littler and Littler, 1996) and the Red Sea (Al-Moghrabi, 2001) (Chapter 2). Harmful effects of sedimentation increase with increasing organic content and bacterial activity, and with decreasing grain size of sediments (Hodgson, 1990, Weber et al., 2006). At Magnetic Island, fine sediments are re-suspended under conditions of river flooding, strong wind events and cyclones, and currents transport the sediment northward from Cleveland Bay towards Magnetic Island reefs and other more northerly reefs (Lambrechts et al., 2010). Evidence that Cleveland Bay is slowly filling with sediment under present land-use conditions (Lambrechts et al., 2010) indicates that coral reefs around Magnetic Island are highly threatened.

Numerous studies have detected a link between warm seawater temperatures and coral diseases, including WSs (Bruno et al., 2007, Willis et al., 2004) and BBD (Sato et al., 2009) on the GBR, and aspergillosis (Harvell et al., 2001), white pox (Patterson et al., 2002) and BBD (Kuta and Richardson, 2002) in the Caribbean. Although the correlation found between warm seawater temperatures and AtN was weak in my study (Chapter 4), the fact that outbreaks of AtN occurred when seawater temperatures were higher than 31°C in both my study (Chapter 4) and the one by Jones et al. (2004b) suggests that the role of temperature is important in AtN dynamics. Results of an experimental study demonstrating that the rate of temperature change may affect AtN dynamics (Lonergan, 2006) provides further support for the importance of temperature as an environmental driver of AtN. Accordingly, patterns in the duration of warm anomalies preceding AtN outbreaks merit further investigation, and these should be combined with patterns in winter seawater temperatures, given that most outbreaks of WSs on the GBR occurred after mild winters and during hot summers (Heron et al., 2010).

Studies of a potential link between temperature and coral disease at Heron Island were inconclusive. Despite the clear seasonal trends found for UWS and BrB, a direct link between coral disease prevalence and temperature could not be demonstrated, in large measure because temperature could not be isolated from other environmental parameters that vary concurrently in field studies. Notably, rainfall, light levels, water clarity, run-off, ocean circulation, and nutrients also vary seasonally (Delcroix and Henin, 1991, Lima et al., 1996, Poulos et al., 1997). On Heron Island, no clear correlations between growth anomalies (GAs) and

temperature were detected, a finding that was in contrast to results from other studies that have detected a correlation between warm temperatures and GAs (McClanahan et al., 2009), and coral bleaching and GAs (Maina et al., 2008).

FUTURE RESEARCH DIRECTIONS

Continuing long-term disease monitoring programmes, both in the Indo-Pacific and the Caribbean, are important for understanding the impacts diseases have on the reef environment, particularly now because reefs worldwide are increasingly subject to multiple stressors. In order to manage and preserve the health of the world's coral reefs, it is critical to understand what causes disease outbreaks in the ocean, thus knowledge gaps in the understanding of drivers of coral disease should be addressed with urgency. So far, most studies on disease drivers have concentrated on proving the role of warm water temperature in increasing the severity of coral diseases. However, as the results of this thesis reveal, environmental factors other than elevated temperature, such as poor water quality, sedimentation and low salinity levels, may lead to disease outbreaks that have serious impacts on the reef ecosystem (Chapters 2, 4 and 5). Therefore, increasing water quality and preventing nutrient enrichment in coastal areas should be a priority around reefs worldwide. In the future, reef management decisions will hopefully be enhanced by a better understanding of long-term coral disease dynamics and disease drivers, which will enable insights into how disease outbreaks could be prevented and how impacts of disease outbreaks could be mitigated.

In order to better understand possible future scenarios on reefs, another priority area for future research is pathogen ecology, specifically how pathogens will be affected by environmental changes caused by climate change and ocean acidification and which environmental factors favour pathogen growth. Long-term monitoring of reef health coupled with cellular and molecular level studies of innate immunity, disease resistance and pathogen virulence are research areas requiring further study to understand how corals might build resilience to stressors related to climate change and minimise increasing impacts of coral disease in the future.

6.3 RELEVANCE OF THE RESULTS OF THIS THESIS FOR REEF MANAGEMENT

Currently, the highest management priorities set by the Great Barrier Reef Marine Park Authority (GBRMPA) are to reduce the impacts of climate change and land runoff on the GBR (GBRMPA, 2009, Brodie et al., 2011). Almost all work conducted on water quality is now directed at building a modelling framework that will enable predictions of the effect of management interventions on reef health (Brodie et al., 2011). Several programs such as Reef Water Quality Action Plan (Plan, 2003), Reef Rescue and the Great Barrier Reef Protection Act 2009 are in place to better manage the water quality of the GBR (Brodie et al., 2011)

The results of this thesis highlight the role of rainfall and land runoff as drivers of coral disease for the first time on an Indo-Pacific reef. Therefore, the current management priorities of the GBRMPA, to improve the health and

resilience of the world's largest coral reef by reducing the impacts of climate change and by increasing the water quality on the GBR, are well justified. However, even if target values for water quality are met in the near future, it may take years before a positive change can be seen in the reef ecosystem. The combined effect of increasing ocean temperature, acidification and extreme weather events, mean that critical times are on hand for corals.

6.4 SUMMARY AND CONCLUSIONS

1. Generally, higher disease prevalence was detected around the high latitude Heron Island reef than in the low latitude Wakatobi Marine National Park (WMNP), with some values similar to mean levels of disease prevalence reported for the Caribbean.
2. Increases in the number of diseases observed and their prevalence in the WMNP are signs of deteriorating coral health and reef condition.
3. Disease progression rates in the WMNP were similar to rates observed in the Caribbean and the Great Barrier Reef (GBR).
4. The highest disease prevalence in the WMNP was observed at the site with the highest sedimentation rate, suggesting that high sedimentation may be an important disease driver.
5. A total of six syndromes were found, both in the WMNP in 2010 and at Heron in 2009. The same diseases were detected at both locations, except that *Porites* ulcerative white spot syndrome (PUWS) was present in the WMNP, whereas brown band syndrome (BrB) was present at Heron Island.
6. Diseases affected a total of 13 coral taxa in the WMNP and 14 at Heron, with staghorn *Acropora* being the most susceptible taxon at both locations.

7. Ulcerative white spots (UWS) was the most common disease on Heron in the austral summer and BrB in the winter.
8. A shift in the coral community structure was observed at Heron Island, from tabular *Acropora* species to a dominance of *Goniastrea*, bushy *Acropora*, *Coscinarea* and *Stylophora* species, suggesting that disease may be having a significant impact on coral assemblages at Heron Is.
9. The results from the WMNP and Heron Island highlight the crucial role of long-term monitoring in detecting changes in reef ecosystems.
10. The mean number of corals with atramentous necrosis (AtN), an inshore coral disease affecting mainly *Montipora aequituberculata,* was clearly higher in the wet season than in the dry season.
11. The three most important environmental drivers of AtN were low salinity, high particulate organic carbon (POC) and temperature, when considered 7 days prior to and including the sampling date.
12. The results of the experimental study on drivers of AtN suggested that the corals died more rapidly in lowered salinity than in high temperature experiments, with the highest mortality observed in the high temperature (32ºC) and low salinity (20) treatment.
13. Extreme weather events in the future may threaten the health and resilience of inshore coral reefs.
14. The findings of this thesis support the GBRMPA management priorities of reducing impacts of climate change and runoff from land on the GBR.

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