VECTORS AND ENVIRONMENTAL DRIVERS OF CORAL DISEASE DYNAMICS ON THE GREAT BARRIER REEF

Thesis submitted by

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ABSTRACT

Coral diseases are causing significant levels of coral mortality at a global scale, based on the frequency of reports of disease and the rates of disease-related coral tissue loss worldwide. Of further concern is that the recent increase in the prevalence of coral diseases has been linked to environmental changes that can alter the outcome of host/pathogen interactions. Thus, coral diseases are expected to have increasing impacts on the structure and dynamics of coral populations and communities as the environment continues to change in the future. Despite the growing body of literature on the various diseases and pathogens that affect corals, the aetiology (i.e., causes of disease) and ecology (i.e., how the environment affects interactions between hosts and pathogens) and transmission of most coral diseases remain poorly understood. Understanding disease transmission mechanisms is critical to evaluate the potential impact of diseases on their host population. Diseases where pathogens cannot survive outside their hosts are unlikely to drive their host population to extinction, as the population would reach a threshold below which the pathogen cannot persist. In contrast, if the pathogen can infect multiple hosts, has reservoirs in the natural environment, and/or is transmitted by vectors, the disease prevalence can continue to increase even when host density is low, leading to disease-mediated population declines and extinctions. Ultimately, a comprehensive understanding of the ecology of pathogens, their vectors and environmental drivers, is required before outbreaks of coral disease can be understood and managed.

The overarching objective of this thesis was to understand the aetiology of coral diseases and gain insights into the effects of diseases on coral populations. More specifically, I investigated the links between environmental factors and disease prevalence and progression rate on coral reefs at Lizard Island, for 1.5 years, and evaluated the role that vectors play in transmission of coral diseases. I also examined interactions among potential vectors, coral disease dynamics and the relative importance of multiple environmental stressors in order to determine if the outcomes of coral-vector-pathogen-environment interactions are positive or negative for corals. The specific aims of my PhD research were to; i) Evaluate the role of corallivorous fish in

promoting or reducing progression rates of coral disease (Chapter 2); ii) Determine whether corallivores act as coral disease vectors, and understand the biological mechanism through which transmission occurs (Chapter 3); iii) Resolve coral disease contagiousness and transmission mechanisms by analysing spatio-temporal distribution patterns of naturally occurring coral diseases (Chapter 4); and iv) Understand the environmental drivers of disease dynamics, by quantifying how disease abundance and progression rates vary in response to environmental conditions (Chapter 5).

Coral-feeding fishes (such as butterflyfishes) are known to feed on disease lesions, potentially affecting the rate of coral tissue loss (disease progression rate), and/or transmitting diseases among coral colonies. Although selective predation on lesions by corallivorous fish was observed, I found no evidence that removal of pathogens by fish reduced progression rates of black band disease (BBD) either in a controlled laboratory setting or in the natural environment. Variability in disease progression rates in the field was explained by inherent variation among coral colonies (24%) and among sampling days (38%) rather than by predation treatment (<0.1%). Furthermore, selective feeding on diseased tissue and subsequent predation on healthy colonies by corallivorous fish did not transmit either BBD or brown band disease (BrB) in the laboratory or in the field. In contrast, *Drupella* transmitted BrB to healthy corals in 40% of cases immediately following feeding on infected corals, and even in 12% of cases 12 and 24 hours following feeding. These results indicate that polyp-feeding fishes are unlikely to be vectors of coral diseases, possibly because their feeding creates small lesions that are too shallow for pathogens to invade coral tissues.

Spatial and temporal distributions of disease prevalence and incidence provide insights into the cause, origin, and transmission mechanisms of diseases. For BBD and skeletal eroding band (SEB), the spatial patterns in disease incidence were often aggregated (i.e., in 78% and 66% of cases, respectively), suggesting that these two diseases are contagious. In contrast, incidences of white syndromes (WS) were randomly distributed; suggesting that this group of diseases is not

contagious. The lack of a clear pattern in the distribution of BrB in analyses of quadrats over the 1.5 years suggests that multiple interacting factors culminate in BrB disease signs. Furthermore, the spatial distribution of most diseases (WS, SEB and BrB) was independent of the distribution of feeding scars created by *Drupella* snails and crown-of-thorns starfish. BBD, however aggregated around feeding scars in 43% of cases, suggesting that physical injury of the coral host might play a role in the transmission of BBD.

My research demonstrates that progression rates of five common coral diseases (BBD, BrB, SEB, WS and atramentous necrosis) vary significantly with seasonal changes in environmental variables. Total dissolved nutrients (TDN) and seawater temperature were the most important factors affecting progression of coral diseases, with a general enhancement of progression rate at high temperature (>29°C) and high TDN (>6 μmol L^{-1}). Different environmental variables, however influenced the dynamics of the different diseases and non-linear, threshold relationships were observed. In contrast, there were no strong effects of environmental factors on the overall abundance of any of the five different coral diseases. Nevertheless, the increased rates of disease progression at increased seawater temperature and TDN suggest that declining water quality and ocean warming have the potential to exacerbate disease-related coral tissue loss.

Overall, the results of my research demonstrate that both biotic (corallivorous snails and invertebrates) and abiotic (seawater temperature and water quality) factors influence the progression and transmission rate of coral diseases. Host condition and natural resistance of corals were also of great importance and played a greater role in disease dynamics than selective feeding by corallivorous fishes (Chapter 2). Corallivorous invertebrates, however create deeper feeding scars, and either directly transmitted disease to new hosts (Chapter 3) or indirectly increased the transmission rate of diseases by disrupting the coral's protective barrier (Chapter 4). Lastly, seawater temperature and total dissolved nutrients were the most important environmental factors that affect the progression rate of all coral diseases on the reef (Chapter

5). Reducing carbon dioxide emissions responsible for global warming and the increase in seawater temperature should remain the first priority of any management response. However, reducing land-based pollution, terrestrial runoff and seafloor dredging would moderate the impact of environmental stressors on coral diseases and may therefore be a powerful tool for lessening indirect human impacts (i.e. global warming) on coral reefs.

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To standardize for sampling intensity in each field trip, total number of infected branches observed in each quadrat in each trip was divided by the number of observation days for that trip. Means presented here are thus mean abundance per quadrat (100 m^2) and not per m^2. Note the different y-axis scales for each disease: atramentous necrosis (AtN), black band disease (BBD), brown band disease (BrB), skeletal eroding band (SEB) and white syndrome (WS). Thick lines inside boxes represent the median (or second quartile), while the lower and upper lines of boxes represent quartiles 1 and 3. The box itself is the inter-quartile range. The whiskers show 1.5 times the inter-quartile range and individual points on the graph show data outside the range of the whiskers.

CHAPTER 1 – GENERAL INTRODUCTION

1.1 Increasing prevalence of coral diseases

Coral reefs are increasingly subject to rapid changes in environmental conditions, which is contributing to significant degradation of reef ecosystems worldwide (Pandolfi et al. 2003; Hughes et al. 2003; Bellwood et al. 2004; Knowlton and Jackson 2008). Sustained and ongoing increases in seawater temperature and ocean acidification, which are directly attributable to anthropogenic climate change, are now recognised as the greatest threats to the persistence of coral-dominated habitats into the future (Hughes et al. 2003; Harvell et al. 2007). Thus far, widespread mass-bleaching of scleractinian corals, which is unequivocally linked to anomalously high water temperatures, has been the most conspicuous effect of climate change on coral reefs (Hoegh-Guldberg 1999; Berkelmans et al. 2004; Eakin et al. 2010). However, other chronic and acute disturbances, such as sedimentation through coastal development and pollution through the transport of herbicides and pesticides from land to sea, have also contributed to the declining health and abundance of corals on reefs (Koop et al. 2001). Moreover, these various impacts of anthropogenic activities on coral reefs (indirect global warming and direct land-based pollutions) may act in synergy to cause further reef degradation (e.g., chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching, Vega Thurber et al. 2014). Overall, the literature demonstrates that coral reef degradation over the past several decades has occurred directly in response to environmental changes, but also indirectly as the changing environment alters the outcomes of species interactions (e.g. the 'winner' of competition between corals depends on seawater pH, Horwitz et al. 2017), and can lead to increased prevalence of diseases that cause coral mortality (Harvell et al. 2007).

Coral diseases are expected to have increasing impacts on the structure and dynamics of coral populations and communities as the environment continues to change. Both indirect (e.g.

warming of seawater) and direct (e.g. increased nutrients) human impacts can contribute to increases in coral disease prevalence, either by weakening the immunity of the coral or by increasing pathogen virulence. The first account of a coral disease was reported by Squires in 1965 (Sutherland et al. 2004). Since then, the prevalence of coral diseases has increased, with a proliferation of reports of newly discovered diseases, as well as an increase in the reports of disease-related coral mortality (Harvell et al. 2002; Sutherland et al. 2004; Precht et al. 2016). Coral diseases are already causing significant levels of coral mortality at a global scale, based on the frequency of reports of disease and the rates of disease-related coral tissue loss, both in the Caribbean (Porter et al. 2001; Weil et al. 2002; Weil 2004; Weil et al. 2006; Yee et al. 2011; Precht et al. 2016) and the Indo-Pacific (McClanahan et al. 2004; Raymundo et al. 2004; Willis et al. 2004; Onton et al. 2011). Of further concern is that recent increases in the prevalence of coral diseases (especially since 1998) have been attributed to climate change (Harvell et al. 2002; Maynard et al. 2011, 2016). More specifically, there are apparent links between the prevalence of numerous diseases, in both the Caribbean and the Indo-Pacific, and increasing temperature (Antonius 1985; Bruckner and Bruckner 1997; Harvell et al. 2001; Patterson et al. 2002; Remily 2004; Willis et al. 2004; Bruno et al. 2007; Maynard et al. 2011).

The dynamics of diseases in the natural environment depends on whether the disease is 'infectious' and/or 'contagious'. As a general definition, 'disease' is the occurrence of dysfunction in an organism that produces specific signs or symptoms. Here I focus on diseases caused by the presence of an agent, which can be – but is not limited to – a pathogenic microorganism (e.g. bacteria, fungi, Protozoa and viruses). An 'infection' is the presence of an agent (i.e., a 'pathogen') that has the ability to produce a disease (Wobeser 2006), but not all infected organisms are diseased. For example, bacteria causing meningitis in humans are typically common components of the human microbiome but only become 'infectious' (i.e., cause dysfunction) when triggered by some environmental factor or trauma. Diseases can also be 'contagious', meaning they are capable of being transmitted from one individual to the next (Dorland 2006), but not all diseases are contagious. For example, tetanus is caused by

microorganisms that reside in the soil, but the pathogens do not spread from one host to the next. In general, natural and human-induced environmental stressors are assumed to influence biotic diseases (i.e., caused by a living agent) by weakening host resistance, promoting the virulence of pathogens, triggering the pathogenic process and/or increasing the transmission rates of disease (Sutherland et al. 2004). For coral diseases, however most of these assumptions lack concrete evidence.

Despite the growing body of literature on the various diseases and pathogens that affect corals, the aetiology (i.e., causes of disease) and ecology (i.e., how the environment affects interactions between hosts and pathogens) of most coral diseases remain poorly understood (Pollock et al. 2011). For corals, diseases mainly result in tissue loss (partial colony mortality), reduced reproduction and growth, or total mortality of infected colonies (Weil et al. 2009; Borger and Colley 2010; Burns and Takabayashi 2011; Ruiz-Diaz et al. 2016). Moreover, a large number of factors (abiotic and biotic) may affect the prevalence and dynamics of individual coral diseases differently, likely due to specific traits of different coral hosts and pathogens. For example, while warmer water temperatures are known to increase black band disease severity and prevalence (Sato et al. 2009, 2011), brown band disease exhibits its highest prevalence either in summer (Nash 2003; Boyett 2006), or in winter (Haapkylä et al. 2010), and direct temperature treatments had no impact on disease progression rate (Boyett 2006). These examples demonstrate the complexity of interactions between disease pathogens, coral hosts and environmental factors. Further research is needed to resolve the drivers of coral disease dynamics.

In addition to lack of knowledge about the aetiology and ecology of coral diseases, there are clear gaps in current knowledge about the transmission of coral diseases (Chong-Seng et al. 2011). Transmission of a coral disease can be either direct (transmitted through contact between infected and healthy individuals) or indirect, whereby pathogenic organisms are transmitted by water movement, or transported between colonies by mobile organisms (often termed vectors).

Directly monitoring the transfer of (usually microscopic) pathogens between individuals under natural conditions is extremely difficult. Consequently, quantification of spatial and temporal patterns of disease prevalence, particularly when and where new cases of disease arise, is often used to help establish the cause, origin and transmission mechanism of diseases (Mayer 1983). Spatiotemporal information, for example, has been widely used to calculate the speed of pathogen spread through a population and to deduce whether spread is facilitated by specific vectors, through air borne or water borne transmission, or via direct contact (McCallum et al. 2003).

Knowledge of disease transmission mechanisms is critical to evaluate the potential impact of diseases on the host population (McCallum et al. 2003). Indeed, the transmission mechanisms of diseases influence whether or not the pathogen can survive outside its host population, and this shapes the dynamics of the disease. 'Density-dependant' diseases, where the pathogen cannot survive outside of its host, do not generally result in extinction of the host population because the pathogens would drive their hosts below threshold densities required for pathogens to persist in the population (Anderson and May 1992). However, many pathogens do not have density-dependent transmission; instead, the pathogens of some diseases can survive outside of the host and transmission is a function of the frequency of infected individuals rather than a function of host density (Smith et al. 2009). In particular, vector-borne pathogens are commonly 'frequency-dependent', and their prevalence can continue to increase even when host density is low, leading to disease-mediated population declines and extinctions (Thrall et al. 1993; Boots and Sasaki 2003). The same is true when pathogens have reservoirs where they remain viable outside of their hosts, or when pathogens are able to infect multiple hosts, which releases them from the dynamics of a specific one host-one pathogen system (van Riper et al. 1986; Fenton and Pedersen 2005; Pedersen et al. 2007).

A comprehensive understanding of the ecology of pathogens, their vectors and environmental drivers, is required before outbreaks of coral disease can be understood and managed. Most

coral diseases affect multiple coral species. Black band disease, for example, affects at least 40 coral species on the Great Barrier Reef (Willis et al. 2004; Page and Willis 2006), enabling the disease to circumvent density-dependent host-pathogen dynamics. Moreover, some coral pathogens have reservoirs and vectors that maintain pathogen loads, even when host population densities are low. The coral disease white pox, for example, is caused by the pathogen *Serratia marcescens*, which survives and remains virulent within the corallivorous snail *Coralliophila abbreviata* (Sutherland et al. 2011), enabling the snail to infect new coral colonies. Additionally, the relationship between corallivores and coral disease dynamics is rendered more complex if corallivores preferentially feed on coral disease lesions, thereby suppressing disease and raising the possibility that organisms can simultaneously be vectors of diseases and disease control agents (Cole et al. 2009; Chong-Seng et al. 2011). However, currently there is a paucity of knowledge about coral disease dynamics, the extent to which they depend on their host population density, and the role of vectors in disease transmission.

1.2 Diseases known to affect scleractinian corals in the Indo-Pacific

1.2.1 Coral diseases and their epidemiology

In the four decades since the first report of coral disease by Squires (1965), intensive research has been conducted in the Caribbean, long thought to be a coral disease "hot spot". It was not until 1999, when Antonius (1999) described a syndrome called skeletal eroding band (SEB) on reefs of Mauritius (Indian Ocean) and Lizard Island (GBR), that intensive research on coral diseases was initiated in the Indo-Pacific. Up until 2003, five distinct coral diseases had been described from the Indo-Pacific (Table 1.1). Thereafter, Willis et al. (2004) and Jones et al. (2004) described four new syndromes, increasing the total number of diseases in this region to nine. Identifying the agents of coral diseases is extremely challenging due to the complexity of the host. A coral is a holobiont consisting of the animal, its associated suite of internal and external microbiota, and its associated symbiotic algae (Rohwer et al. 2002; Rosenberg et al. 2007). One standardised method used to identify the primary agent(s) of coral diseases is Koch's postulates, which require that: (1) the agent must be found in abundance in all infected

hosts, but should not be found in healthy organisms; (2) the agent must be isolated from the diseased host and grown in pure culture; (3) re-infection of healthy hosts with the pure culture should produce characteristic signs; and (4) the causative agent should be re-isolated from the inoculated diseased experimental host and identified as being identical to the original specific causative agent (revised by Evans 1976). Of the 17 diseases currently recognized from the Indo-Pacific (if various different white syndromes are considered as separate diseases, Table 1.2), the epidemiology of seven diseases has been successfully demonstrated using Koch's Postulates (Table 1.3). Although many marine microbial organisms cannot be grown in pure culture, the fulfilment of Koch's postulates for some diseases provides insights into disease causes and origins.

Table 1.1: Coral diseases and their geographical distribution, date of first report in the Caribbean 'C', Indo-Pacific 'IP', Mediterranean Sea and Red Sea 'MR', and principal reference sources. Only diseases known to affect Indo-Pacific corals are reported here. The abbreviations in parentheses are used throughout the thesis to refer to each of these diseases.

Disease	C	IP	MR	Sources
Atramentous necrosis (AtN) Black lesion covered in a thin light coloured film, giving it a grey appearance.		2004		Jones et al. 2004; Bourne 2005 ; Anthony et al. 2008; Haapkyla et al. 2011, 2013 ; Miller et al. 2015
Black band disease (BBD) Bacterial mat forming a black band that migrates across apparently healthy coral, killing tissue and exposing skeleton	1973	1985	1985	Antonius 1973, 1981, 1985, 1988; Garrett and Ducklow 1975; Ducklow and Mitchell 1979b; Antonius and Weiner 1982; Ramos-Flores 1983; Rutzler et al. 1983; Rützler and Santavy 1983; Taylor 1983; Guzman and Cortes 1984, Peters 1984, Rogers 1985; Schnell et al. 1996; Dustan 1987, 1993; Williams and Bunkley-Williams 1990; Edmunds 1991, Liddell and Ohlhorst 1992, Richardson and Carlton 1993; Bythell et al. 1993; Garzon-Ferreira and Kielman 1993, Dinsdale 1994, Kuta and Richardson 1994, 1996, 1997, 2002; Santavy et al. 1994; Carlton and Richardson 1995; Littler and Littler 1996; Miller 1996; Richardson 1996, 1997, 2004; Richardson et al. 1997, 2007, 2009; Briggs et al. 1999; Bruckner and Bruckner 1997a, b, c, 1998; Grosholz and Ruiz 1997; Feingold 1988; Franklin 1998; Korrubel and Riegl 1998; Monserrate et al. 2001; Cooney et al. 2002; Frias-Lopez et al. 2002, 2003, 2004a,b; Richardson and Kuta 2003; Aeby and Santavy 2006; Page and Willis 2006; Sekar et al. 2006; Viehman et al. 2006; Voss and Richardson 2006; Barneah et al. 2007; Boyett et al. 2007; Myers et al. 2007; Voss et al. 2007; Richardson and Ragoonath 2008; Rodriguez and Croquer 2008; Sekar 2008; Arotsker et al. 2009; Cole et al. 2009; Gantar et al. 2009; Myers and Richardson 2009; Rasoulouniriana et al. 2009; Sato et al. 2009, 2010, 2011, 2013, 2016; Zvuloni et al. 2009; Bourne et al. 2011; Chong-Seng et al. 2011;Gantar et al. 2011; Klaus et al. 2011; Kuehl et al. 2011; Lamb and Willis 2011; Miller et al. 2011, 2012, 2015; Stanic et al. 2011; Thinesh et al. 2011, 2013, 2014 ; Glas et al. 2012; Jones et al. 2012; Miller and Richardson 2012; Montano et al. 2012, 2013; Weil et al. 2012 ; Bourne et al. 2013 ; Casey et al. 2014; Kramarsky-Winter et al. 2014; Lamb et al. 2014 ; Yang et al. 2014; Aeby et al. 2015; Arotsker et al. 2015; Montano et al. 2015, 2016a; Riegl and Purkis 2015 ; Séré et al. 2015 ; Arotsker et al. 2016; Buerger et al. 2016 a,b ; Den Uyl et al. 2016 ; Meyer et al. 2016; Séré et al. 2016
Brown band disease (BrB) Brown zone flanked by healthy tissue at the advancing front and exposed white skeleton at the		2004		Nash 2003 ; Willis et al. 2004 ; Boyett 2006 ; Boyett et al. 2007 ; Ulstrup et al. 2007; Yarden et al. 2007; Bourne et al. 2008; Nugues and Bak 2009, Page et al. 2009; Haapkyla et al. 2010, Qiu et al. 2010; Chong-Seng et al.2011 ; Lamb and Willis 2011 ; Lobban et al. 2011 ; Sweet and Bythell 2012 ; Weil et al. 2012 ; Nicolet et al. 2013 ; Katz et al. 2014; Pollock et al. 2014 ; Miller et al. 2015 ; Randall et al. 2015; Seveso et

trailing edge. Dense populations of ciliates, packed with zooxanthellae from coral tissue, cause the brown coloration of the band.			al. 2015; Montano et al. 2016a,b ; Sweet and Séré 2016
Growth anomaly (GA) Raised roughly spherical masses projecting above the surface of the colony. Two types: hyperplasia and neoplasia.	1983	1965	Squires 1965; Cheng and Wong 1974; Cheney 1975; Morse et al. 1977, 1981; Goldberg and Makemson 1981; Laydoo 1983; Bak 1983; Loya et al. 1984; Peters 1984; Goldberg et al. 1984; Peters et al. 1986; Glynn et al. 1989; Liddell and Ohlhorst 1992; Dinsdale 1994; Le Champion-Alsumard et al. 1995; Coles and Seapy 1998; Grygier and Cairns 1996; Aeby 1998; Smith et al. 1998; Ravindran et al. 2001; Yamashiro et al. 2001; Dube et al. 2002; Breitbart et al. 2005; Domart-Coulon et al. 2006; Kaczmarsky and Richardson 2007, 2011; McClanahan et al. 2009; Page et al. 2009; Vargas-Angel 2009; Haapkyla et al. 2010; Williams et al. 2010; Aeby et al. 2011, 2016; Burns et al. 2011, 2013; Irikawa et al. 2011; Lamb and Willis 2011; Williams et al. 2011 a, b; Chiu et al. 2012; Yasuda and Michio 2012; Yasuda et al. 2012; Becker et al. 2013; Spies and Takabayashi 2013; Williams 2013; Couch et al. 2014; Lamb et al. 2014; Work et al. 2014; Ng et al. 2015; Séré et al. 2015; Caldwell et al. 2016; Claar and Takabayashi 2016; Hussain et al. 2016; Ponti et al. 2016; Work et al. 2016; Yoshioka et al. 2016
Indo Pacific necrotic patch (INP) Brown to black zone formed by the presence of a fungus.	1991		Raghukumar and Raghukumar 1991; Ravindran et al. 1999; Cerrano et al. 2000 ; McClanahan et al 2004;
Skeleton eroding band (SEB) Caused by a ciliate that erodes the tissue and skeleton of corals as it produces a black lorica. Clusters of ciliates along the tissue-skeleton interface produce a black band and empty black	1999	2004	Antonius 1999; Antonius and Lipscomb 2001; Riegl and Antonius 2003; Winkler et al. 2004; Haapkyla et al. 2007, 2009, 2010 ; Yarden et al. 2007; Page and Willis 2008; Page et al. 2009 ; Lamb and Willis 2011 ; Onton et al. 2011; Montano et al. 2012, 2016a,b; Lamb et al. 2014; Séré et al. 2015 ; Ponti et al. 2016

loricae are visible on the exposed skeleton.			
***Vibrio coralliilyticus* induced bleaching (VCB)**	2011	2002	Ben-Haim and Rosenberg 2002; Ben-Haim et al. 2003; Vidal-Dupiol et al. 2011; Mills et al. 2013
White syndromes (WS) Collective term to describe conditions resulting in tissue loss exposing white bands of skeleton.	2004		Willis et al. 2004; Aeby 2005 Fine et al. 2006; Roff et al. 2006, 2008; Ainsworth et al. 2007; Bruno et al. 2007; Andersen et al. 2010; Sussman et al. 2008; Aeby et al. 2010; Dalton et al. 2010; Haapkyla et al. 2010 ; Heron et al. 2010; Hobbs and Frisch 2010; Luna et al. 2010; Williams et al. 2010; Lamb and Willis 2011 ; Maynard et al. 2011; Palmer et al. 2011; Roff et al. 2011 ; Work and Aeby 2011; Work et al. 2011, 2012 ; Montano et al. 2012; Sweet and Bythell 2012 ; Weil et al. 2012 ; Wilson et al. 2012 ; Ban et al. 2013 ; Pollock et al. 2013, 2014a,b, 2015 ; Redding et al. 2013 ; Sweet et al. 2013 ; Zhenyu et al. 2013 ; Lamb et al. 2014; Sheridan et al. 2014 ; Ushijima et al. 2014; Ainsworth et al. 2015 ; Bourne et al. 2015 ; Heintz et al. 2015 ; Hobbs et al. 2015 ; Lozada-Misa et al. 2015 ; Miller et al. 2015 ; Riegl and Purkis 2015 ; Séré et al. 2015a,b ; Smith et al. 2015; Sweet and Bythell 2015 ; van de Water et al. 2015 ; Weynberg et al. 2015; Wright et al. 2015 ; Montano et al. 2016a ; Raymundo et al. 2016 ; Rouze et al. 2016 ; Sweet and Séré 2016
Yellow blotch/band (YBD) Begins as a small pale round blotch that expands in diameter as a ring of pale tissue encircling an increasingly large area of dead coral. In advanced stages, the ring becomes less defined, but continues to increase in diameter.	1997	1998	Santavy and Peters 1997; Hayes and Goreau 1998; Korrubel and Riegl 1998; Cervino et al. 2001, 2005, 2004a,b, 2008; Garcia et al. 2003, 2004; Gil-Agudelo et al. 2004; Nowak 2004; Foley et al. 2005; Bruckner and Bruckner 2006; Bruckner and Hill 2009; Mydlarz et al. 2009; Vu et al 2009; Weil et al. 2009; Bruckner and Borneman 2010; Jordan-Garza and Jordan-Dahlgren 2011; Yee et al. 2011; Muller and van Woesik 2012; Apprill et al. 2013; Anderson et al. 2013; Kimes et al. 2013; Sweet et al. 2013; Closek et al. 2014; Hauff et al. 2014; Guerra et al. 2014; Soto-Santiago and Weil 2014; Zaragoza et al. 2014; Morgan et al. 2015; Munn 2015; Anderson et al. 2016; Montilla et al. 2016;

Note: The term Growth Anomaly (GA) includes a number of different categories, including tumors, hyperplasias and neoplasias, along with *Porites* growth anomaly.

Table 1.2: Known types of white syndromes, their geographical distribution and principal references. The term white syndromes (WS) was coined by Willis and colleagues in 2004, and was used as a collective term for diseases producing tissue loss exposing white skeleton on Indo-Pacific corals. Subsequently, different 'types' of WS have been described and are likely to have varying aetiologies and pathogenesis. Table is based on information in Bourne et al. 2015.

Type of White Syndrome	**Geographical Region**	**Sources**
White syndromes (WSs)	Australia, USA, Pacific remote Island area, Guam	Willis et al. 2004; Vargas-Angel 2009; Myers and Raymundo 2009
Acropora white syndrome (AWS)	Hawaii, Marshall Islands, Indonesia, American Samoa, Australia, Palmyra Atoll, Japan	Aeby 2005 ; Jacobson et al. 2006 ; Haapkyla et al. 2007; Aeby et al. 2008; Hobbs and Frisch 2010; Roff et al. 2011; Williams et al. 2011; Weil et al. 2012; Wilson et al. 2012 ; Ushijima et al. 2016
Australian subtropical white syndrome (ASWS)	Solitary Islands of Australia	Dalton and Smith 2006; Dalton et al. 2010; Godwin et al. 2012
Montipora white syndrome (MWS)	Hawaii	Aeby et al. 2010, 2016; Williams et al. 2010; Ushijima et al. 2012, 2014 ; Caldwell et al. 2016
Porites white patch syndrome (PWPS)	Western Indian Ocean	Séré et al. 2012, 2013, 2015; Lawrence et al. 2015
Porites tissue loss (PorTL)	Hawaii	Williams et al. 2010; Aeby et al. 2011; Caldwell et al. 2016
Porites bleaching with tissue loss (PBTL)	Hawaii	Sudek et al. 2012, 2013, 2015
Porites ulcerative white spot (PUWS)	Philippines	Raymundo et al. 2003, 2005; Kaczmarsky 2006; Haapkyla et al. 2010; Kaczmarsky and Richardson 2011; Weil et al. 2012
Ulcerative white spot disease (UWS)	Philippines	Raymundo et al. 2008; Kaczmarsky and Richardson 2011; Montano et al. 2016a

Table 1.3: Pathogens associated with coral diseases and their present status in terms of fulfilling Koch's Postulates.

Disease	Pathogen(s)	Koch's Postul.	Sources
AtN	*Alphaproteobacteria* (bacteria) *Firmicutes* (bacteria) *Bacteroidetes* (bacteria)	No	Jones et al. 2004; but proved to be a secondary community by Bourne 2005
ASWS	*Beggiatoa* (bacteria)	No	Dalton et al. 2010
AWS	*Vibrio coralliilyticus*	Yes	Ushijima et al. 2016
BBD	*Phormidium corallyticum* (cyanobacterium) *Trichodesmium spp.* (cyanobacteria) *Cyanobacterium* *Desulfovibrio spp.* (bacteria) *Beggiatoa spp*. (bacteria) Heterotrophic bacteria Marine fungus *Alpha-proteobacteria* (bacteria) *Vibrio spp*. (bacteria) *Pseudoscillatoria coralii* (cyanobacteria)	No	Rützler and Santavy 1983 ; Frias-Lopez et al. 2002, 2003; Cooney et al. 2002; Frias-Lopez et al. 2003; Garrett and Ducklow 1975; Schnell et al. 1996; Cooney et al. 2002; Ducklow and Mitchell 1979b; Garrett and Ducklow 1975; Cooney et al. 2002; Frias-Lopez et al. 2002; Ramos-Flores 1983; Sekar et al. 2006; Arotsker et al. 2009; Rasoulouniriana et al. 2009; Glas et al. 2010; Sato et al. 2010, 2013, 2016
BrB	*Scuticociliatia* (protozoan) *Philaster guamensis* (protozoan) *Philaster digitiformis* (protozoan)	No	Bourne et al. 2008 Lobban et al. 2011 Sweet and Bythell 2012
FPS	*Trichoderma spp.* (fungi) *Clodosporium spp.* (fungi) *Penicillum spp.* (fungi) *Humicola spp.* (fungi) Ciliate (protozoan)	No	Cerrano et al. 2000
GA	*Petrarca madreporae* (crustacean) *Podocotyloides stenometra* (trematode) Endolithic fungi *Aspergillus sydowii* (fungus) Order Siphonales (algae) *Entocladia endozoica* (algae) *Vibrio spp.* (bacteria)	No	Grygier and Cairns 1996; Cheng and Wong 1974; Aeby 1998; Le Champion-Alsumard et al. 1995; Ravindran et al. 2001; Smith et al. 1998; Dube et al. 2002; Morse et al. 1977, 1981; Goldberg et al. 1984; Breitbart et al. 2005
INP	*Scolecobasidium spp.* (fungi)	No	Raghukumar and Raghukumar 1991
MWS	*Vibrio coralliilyticus* (bacteria) *Vibrio owensii* (bacteria)	Yes	Ushijima et al. 2014 Ushijima et al. 2012

PUWS	*Vibrio spp*. (bacteria)	Yes	Arboleda and Reichardt 2010; Weil et al. 2012
PWPS	Vibrionacea, Rhodobacteraceae	Yes	Séré et al. 2013
	Shimia marina (bacteria)		
	Vibrio hepatarius (bacteria)		
	Virus-like particles	No	Lawrence et al. 2015
SEB	*Halofolliculina corallasia* (protozoan)	No	Antonius and Lipscomb 2001
VCB	*Vibrio coralliilyticus* (bacterium)	Yes	Ben-Haim and Rosenberg 2002; Ben-Haim et al. 2003a,b
YBD	*Vibrio* consortium	Yes	Cervino et al. 2004a,b, 2008
	Phycodna-like viruses	No	Cervino et al. 2004a
WS	*Vibrio coralliilyticus* strains (bacteria)	Yes	Sussman et al. 2008
			Weynberget al. 2015
	Vibrio spp. (bacteria)		Ainsworth et al. 2007
	Vibrio harveyi (bacteria)		Luna et al. 2010
	Virus-like particules		Pollock et al. 2014

1.3 Biotic and abiotic factors affecting coral disease dynamics

The severity of the impacts of coral diseases on coral populations and communities can be assessed by quantifying 1) the prevalence and spatial distribution of diseases, which may also inform how diseases are transmitted between colonies, and 2) the rate of coral tissue loss due to disease. As both disease prevalence and tissue loss are likely to depend on environmental conditions, quantifying the environmental drivers of disease dynamics is also important. Of the 17 recognized Indo-Pacific diseases (including all types of WSs as separate diseases), research required to establish factors affecting their dynamics has been conducted for 16 diseases, with only 13 articles investigating the influence of these factors on progression rates of 5 different coral diseases (Table 1.4). This literature suggests that the dynamics of different diseases are likely to be influenced by different factors (abiotic and biotic), and/or to react to the same factors in different ways. For example, warmer seawater temperature has been correlated with increased prevalence of black band disease (BBD) in many coral reef regions (Antonius 1981; Kuta and Richardson 1996; Bruckner et al. 1997; Borger 2005; Rodriguez and Croquer 2008; Zvuloni et al. 2009); however, brown band disease (BrB) was shown to exhibit higher prevalence in summer in warmer regions (Nash 2003; Boyett 2006) but higher prevalence in

winter in cooler regions (Haapkyla et al. 2010). Furthermore, while a number of diseases appear to be present at increased prevalence in warmer seasons (Harvell et al. 2002; Willis et al. 2004; Bruno et al. 2007; Sato et al. 2009; Haapkyla et al. 2010; Heron et al. 2010), many factors, such as rainfall, light levels, water clarity, run-off, ocean circulation and nutrients, also vary seasonally. Seasonal covariation of many environmental variables confounds identification of the primary drivers of disease dynamics. For example, atramentous necrosis (AtN) was first described to cause extensive mortality to corals around Magnetic Island, and to co-occur with a bleaching event driven by high seawater temperatures (Jones et al. 2004). However, seawater temperature was only weakly correlated with AtN prevalence and highly correlated with low salinity in more extensive studies, which suggests that heavy rainfall characteristic of tropical summers, and not increased temperature, are driving AtN dynamics (Haapkyla et al. 2011, 2013).

To identify the main environmental drivers of the dynamics of each disease type, the association between both disease prevalence (i.e. number of disease cases present at a certain time) and disease progression rate (i.e. the rate of disease-related coral tissue loss) and multiple environmental factors must be elucidated. To date, however most studies assessing multiple diseases have been conducted using annual sampling of permanent transects at specific months of the year (not allowing for the investigation of seasonality or spatial variation in diseases), or at different locations across a specific environmental gradient (not allowing for the assessment of disease progression rate or temporal variation). Moreover, holistic studies evaluating both progression and transmission rates of disease over time and space have, to date, been conducted for single diseases in isolation. As a result, the literature currently available limits inferences about environmental drivers of coral disease dynamics on reefs.

Table 1.4: Coral diseases and known abiotic and biotic stressors influencing either their prevalence or progression rate.

Disease	Factors	Source	
		Prevalence	**Progression**
ASWS	Elevated temperature		Dalton et al. 2010
AtN	Elevated temperature	Jones et al. 2004	
	Organic carbon	Haapkyla et al. 2011	
	Salinity	Haapkyla et al. 2011	
AWS	Elevated temperature	Ushijima et al. 2016	
	Host cover	Hobbs and Frisch 2010; Roff et al. 2011	
BBD	Elevated temperature	Antonius 1985 ; Kuta and Richardson 2002 ; Boyett et al. 2007; Sato et al. 2009; Zvuloni et al. 2009 ; Kuehl et al. 2011	Boyett et al. 2007; Muller and van Woesik 2011; Sato et al. 2011
	Light level	Antonius 1985; Boyett et al. 2007; Sato et al. 2009; Kuehl et al. 2011	Antonius 1985; Boyett et al. 2007; Muller and van Woesik 2011; Sato et al. 2011
	Nutrient enrichment	Antonius 1981 ; Kuta and Richardson 2002	Voss and Richardson 2006
	Sewage contamination	Kaczmarsky et al. 2005	
	Sedimentation	Bruckner et al. 1997	
	Injury	Aeby and Santavy 2006	
BrB	Elevated temperature	Boyett et al. 2006; Page et al. 2009	
	Dredging plum	Pollock et al. 2014	
	Injury	Lamb and Willis 2011; Nicolet et al. 2013; Katz et al. 2014 ; Lamb et al. 2015, 2016	
GA	Elevated temperature	McClanahan et al. 2009	
	Winter conditions	Caldwell et al. 2016	
	Nutrient enrichment	McClanahan et al. 2009; Kaczmarsky and Richardson 2011	
	Host cover	Aeby et al. 2011a,b	
	Turbidity	Williams et al. 2010; Burns et al. 2011; Heintz et al. 2015	
	Organic carbon	Kaczmarsky and Richardson 2011	
	Chlorophyll *a*	Becker et al. 2013	
	Human population	Aeby et al. 2011a,b; Becker et al. 2013	
MWS	Elevated temperature	Caldwell et al. 2006; Williams et al. 2010	
	Winter conditions	Caldwell et al. 2006	
	Host cover	Aeby et al. 2010	
	Butterflyfish abundance	Williams et al. 2010	
PBTL	Elevated temperature	Sudek et al. 2015	

	Water motion	Sudek et al. 2015	
	Parrotfish density	Sudek et al. 2015	
PorTL	Elevated temperature	Williams et al. 2010	
	Winter conditions	Caldwell et al. 2006	
	Coral cover	Caldwell et al. 2006	
	Butterflyfish abundance	Williams et al. 2010	
PUWS	Nutrient enrichment	Kaczmarsky and Richardson 2011	
	Organic carbon	Kaczmarsky and Richardson 2011	
	Human population	Kaczmarsky 2006	
PWPS	Elevated temperature		Séré et al.2015
SEB	Injury	Page and Willis 2008 ; Lamb et al. 2014	
	Human population	Ponti et al. 2016	
	Chemical pollution	Ponti et al. 2016	
	Dead coral cover	Montano et al. 2016	
	Drupella scars	Onton et al. 2011	
	Tourism-related impact	Winkler et al. 2004; Lamb and Willis 2011	
UWS	Nutrient enrichment	Kaczmarsky and Richardson 2011	
	Organic carbon	Kaczmarsky and Richardson 2011	
VCB	Elevated temperature	Ben-Haim and Rosenberg 2002 ; Ben-Haim et al. 2003a,b; Vidal-Dupiol et al. 2011	
WS	Elevated temperature	Willis et al. 2004; Bruno et al. 2007 ; Heron et al. 2010 ; Maynard et al. 2011; Ban et al. 2013	Dalton et al. 2010
	Nitrogen input		Redding et al. 2013
	Sedimentation	Lamb et al. 2014; Sheridan et al. 2014	
	Dredging plum	Pollock et al. 2014	
	Winter conditions	Heron et al. 2010	
	Tourism-related impact	Lamb and Willis 2011; van de Water et al. 2015	
YBD	Elevated temperature	Cervino et al. 2004a,b ; Yee et al. 2011; Hauff et al. 2014	
	Chemical pollution		Montilla et al. 2016

1.3.1. Factors affecting the prevalence and spatial distribution of coral diseases

The occurrence of an infectious disease involves the interaction of a susceptible host, the local environment, and a virulent pathogen (Harvell et al. 2002; Work et al. 2008). Environmental factors can influence disease prevalence and spread by hindering the resistance of the host,

increasing pathogen(s) virulence and triggering pathogenic processes (Sutherland et al. 2004). As an example, bacterial-induced bleaching of the coral *Oculina patagonica* in the Mediterranean is highly influenced by seawater temperature. The major effect of increased temperature is induction of bacterial virulence factors, including increased adhesion of the bacterium to the coral surface (Toren et al. 1998) and the production of toxins that inhibit photosynthesis and bleach and lyse the zooxanthellae (Ben-Haim et al. 1999; Banin et al.2001). These complex interactions mean that predicting the prevalence and spread of diseases requires comprehensive analyse of all abiotic (water temperature, quality and light incidence) and biotic (organisms interacting with infected colonies) factors.

In addition to abiotic factors, such as water temperature, light incidence and water quality, biotic factors likewise have the potential to influence the prevalence and spread of coral diseases. In some instances, vectors are fundamental to the transmission and initiation of the disease. For coral diseases, there is increasing (though mostly anecdotal) evidence that coral pathogens are spread by corallivorous fishes, echinoderms, arthropods, annelids and/or molluscs (e.g. Antonius and Riegl 1997; Sussman et al. 2003; Williams and Miller 2005; Rypien and Baker 2009; Nugues and Bak 2009; Raymundo et al. 2009; Gignoux-Wolfsohn et al. 2012; Nicolet et al. 2013; Raymundo et al. 2016). The fireworm *Hermodice caranculata*, for example, is a confirmed winter reservoir and a summer vector of *Vibrio shiloi*, the causative agent of bleaching of a Mediterranean coral (Sussman et al. 2003). Other organisms, such as corallivorous butterflyfish, have a more ambiguous role in disease transmission and have mostly been observed feeding selectively on infected coral tissue (Cole et al. 2009; Chong-Seng et al. 2011). Such behaviour may spread diseases to adjacent healthy colonies, either via feeding, water-borne contamination (i.e. release of infected tissue into the water column) and/or feeding-related injuries that provide an entry site for pathogens (Raymundo et al. 2009). In contrast, the abundance of some diseases, such as *Porites* tissue loss and *Montipora* white syndrome, were found to be negatively correlated with corallivorous fish abundance (Williams et al. 2010), highlighting the need to clarify the role of abiotic factors in disease prevalence and spatial

distribution. By establishing the speed and distance of pathogen spread through a population in areas where vectors are present *versus* absent, it can be deduced whether spread is facilitated by specific vectors, or via direct contact (McCallum et al. 2003).

1.3.2 Factors influencing rates of tissue loss due to coral diseases

Rate of tissue loss and the factors influencing it, are seldom investigated in coral disease research (Table 1.4), especially in natural settings, since the acquisition of such knowledge requires extensive and intensive monitoring of infected colonies. However, such knowledge is essential to evaluate the impact of diseases on coral populations. Elevated temperature is the most commonly investigated factor influencing disease progression and rates of tissue loss, however the predominance of temperature studies in the literature does not imply that other factors, such as nutrient enrichment, sedimentation or pollution, would not have similar impacts on specific coral diseases. More importantly, the combination of all of the aforementioned factors, working in synergy, is likely to increase disease progression rates and cause extensive damage to coral populations. For example, warmer seawater has been correlated with increased BBD progression rate; however, the progression rate of lesions was also greatly influenced by light levels, with increased BBD virulence under strong light and highest progression rate under both strong light and warmer water (Boyett et al. 2007, Sato et al. 2011). Similarly to prevalence, disease progression rate has the potential to be influenced by biotic factors. By selectively feeding on the disease lesion, corallivorous fish might not only increase disease transmission, but also slow the progression of disease by removing infectious microorganisms from the lesion (Cole et al. 2009). While such selective feeding on lesions has been observed for two coral diseases thus far, BBD and BrB (Cole et al. 2009; Chong-Seng et al. 2011, Nicolet et al. 2013), the quantitative impacts of feeding on disease progression have not yet been studied.

To understand coral disease dynamics in their natural environment, all biotic (e.g. fish and invertebrate communities) and abiotic (e.g. water temperature, water quality and sedimentation) factors should be taken into consideration, and their effects on both disease prevalence and rate

of tissue loss should be evaluated. Furthermore, such investigations should be conducted for individual diseases and not overall coral disease prevalence. Indeed, diseases with different pathogens and hosts are unlikely to have common spatial/temporal patterns or environmental associations. Exploring coral disease spatial patterns and complex disease associations with biotic and abiotic factors requires comprehensive field and laboratory experiments, as well as statistical techniques that effectively address the complexity of disease ecology.

1.4 Study objectives and thesis structure

The overarching objective of this thesis is to understand the aetiology of coral diseases and gain insights into the effects of diseases on coral populations. More specifically, I investigated the links between environmental factors and disease prevalence on coral reefs, and evaluate the role that corallivores play in transmission or control of these diseases. To do this, I used a comprehensive series of observations, laboratory experiments and field studies to examine interactions among coral-feeding fishes and invertebrates, coral disease dynamics and the relative importance of multiple environmental stressors in order to determine if the outcomes of coral-vector-pathogen-environment interactions are positive or negative for corals. Doing this, I develop an empirical understanding of spatial and ecological processes that underpin coral disease dynamics in the reef system. This body of work is presented as four distinct data chapters, each of which has specific, but complementary, objectives, as follows.

Chapter 2 – Evaluating the role of corallivorous fish in promoting or reducing progression rates of coral disease

This chapter provides the first empirical investigation of the effects of corallivory on coral disease progression rates. Previous studies offer conflicting results in this regard, with corallivorous fish reducing disease progression rates in laboratory experiments (black band disease: Cole et al. 2009) but not in the field (brown band disease: Nicolet et al. 2013). Extending previous work on disease predation in natural (Chong-Seng et al. 2011, Nicolet et al. 2013) and experimental (Cole et al. 2009) conditions, this chapter provides critical information

on fish-coral-disease interactions. In this chapter, I combine field and laboratory experiments **to understand the impact of selective fish feeding on disease lesions and the rate of coral tissue loss (disease progression rate)**. First, I determined natural levels of corallivory on disease lesions in the field, and the fish species involved in the process. Subsequently, I compared progression rates of black band disease lesions under different levels of predation pressure.

Chapter 3 – Coral-feeding fish as potential vectors of coral disease

Many terrestrial diseases have vectors, spreading the pathogen(s) through the host population. In the case of coral diseases, even though many vertebrate and invertebrates are known to directly feed on healthy and infected coral tissue, very little is known about their potential to transmit diseases in the coral population. Understanding transmission mechanisms of infectious diseases is imperative for disease control and forecasting disease impacts. In this chapter, I sought **to resolve whether corallivores act as coral disease vectors, and understand the biological mechanism through which transmission occurs**. I undertook a comprehensive series of laboratory and field experiments to determine under which conditions corallivores act as vectors of both black band and brown band disease, and how these syndromes are likely to spread among corals on reefs.

Chapter 4 – Spatio-temporal distribution patterns of coral diseases within Acropora assemblages

Quantifying the spatial distribution of disease prevalence and incidence is fundamental for establishing disease cause(s), origin and spread (Mayer 1983). To date, few studies have investigated spatial patterns of coral diseases on reefs, and while coral disease literature has grown significantly in the last decades, the underlying processes that shape disease dynamics are still missing for many disease types. Here, I monitored the prevalence and spatial distribution of all naturally occurring diseases on reefs around Lizard Island over a period of 18 months **to resolve coral disease contagiousness and transmission mechanisms by analysing**

spatio-temporal distribution patterns of naturally occurring coral diseases. This chapter provides the most comprehensive information to date on the spatiotemporal patterns of four Indo-Pacific coral diseases. In addition to detailed investigation of whether or not these diseases are contagious, I compared, for the first time, the spatial distribution of diseases with the spatial distributions of corallivorous invertebrates (*Drupella* spp. snails and crown-of-thorns starfish) in order to determine the involvement of these potential vectors in the transmission of each disease.

Chapter 5 – Environmental factors affecting occurrence and virulence of coral diseases

Natural and human-induced environmental stressors are assumed to influence disease dynamics by weakening host resistance, promoting the virulence of pathogens, triggering the pathogenic process and/or increasing the transmission rate of diseases (Sutherland et al. 2004). However, the dynamics of different diseases are likely to be influenced by different factors (abiotic and biotic), and to react to the same factors in different ways. This chapter **provides critical information required to fully understand disease dynamics, by quantifying how disease prevalence and progression rates vary in response to environmental conditions**. I monitored naturally occurring diseases over an 18-month period, recording disease prevalence and progression rates, alongside various environmental stressors (including water temperature, light level, dissolved nutrients, particulate concentration) and investigated correlations between disease dynamics and each of these potential drivers. This chapter provides detailed information on the effects of specific environmental variables on the ecology of five major Indo-Pacific coral diseases and, combined with the evidence provided in previous chapters, give a comprehensive understanding of coral disease dynamics *in situ*.

CHAPTER 2 – EFFECTS OF CORAL-FEEDING FISHES ON PROGRESSION RATES OF BLACK BAND DISEASE

2.1 Abstract

Selective feeding on coral disease lesions by fishes potentially affects the rate of coral tissue loss (disease progression rate). Black band disease (BBD) is a common coral disease, and although its microbiology has been studied extensively, its aetiology and transmission patterns remain unresolved. Here I use both aquaria and field experiments to determine if feeding on BBD lesions by corallivorous fishes influences disease progression rates. Although selective predation on lesions was observed, I found no evidence that removal of pathogens by fish reduced progression rates of BBD either in a controlled laboratory setting or in the natural environment. Variability in disease progression rates in the field was explained by inherent variation among coral colonies (24%) and among sampling days (38%) rather than by predation treatment (<0.1%). Variation in BBD progression rates over time likely reflects seasonal changes in light intensity and water temperature, as both were positively correlated with progression rates, but selective predation had little impact on overall progression of the disease. Disease progression rate was also significantly correlated with the width of the disease band, suggesting that microgradients within the complex microbial community of the band influence rates of tissue loss. Results highlight that natural variation in pathogen virulence and host resistance play a greater role in BBD dynamics than selective feeding by corallivorous fishes.

2.2 Introduction

Diseases in marine ecosystems are major drivers of population dynamics and can severely reduce biodiversity and influence community composition (Harvell et al. 2002; Plowright et al. 2008; Maynard et al. 2015). In the past few decades, numerous outbreaks of marine diseases have affected a wide range of organisms, including corals (Willis et al. 2004; Harvell et al. 2007; Sokolow 2009), sea urchins (Feehan and Scheibling 2014; Yeruham et al. 2015), sea stars

(Maynard et al. 2015; Eisenlord et al. 2016), abalone (Ben-Horin et al. 2013), shrimp (Escobedo-Bonilla et al. 2008; Lafferty et al. 2015), fish (Oldham et al. 2016) and marine mammals (Burek et al. 2008; Burge et al. 2014). As an example, sea star wasting disease has reduced adult populations of the ochre starfish (*Pisaster ochraceus*) to one-quarter of pre-outbreak numbers and induced a shift in their population size structure towards smaller size classes (Eisenlord et al. 2016). Since ochre starsfish prey primarily on mussels, a reduction in starfish numbers releases mussels from predation pressure, which results in mussels overgrowing other primary space holders and changes the composition of rocky intertidal communities for generations (Eisenlord et al. 2016).

Increasing frequency of disease outbreaks among marine organisms (Harvell et al. 2004; Precht et al. 2016) is often attributed to climate change, especially increasing ocean temperatures (Ben-Horin et al. 2013; Burge et al. 2014; Randall et al. 2014; Maynard et al. 2015; Eisenlord et al. 2016; Groner et al. 2016). On coral reefs, increased seawater temperature has been linked to both increased prevalence (i.e., the number of diseases cases at a particular time) and progression rate (i.e., speed at which disease causes tissue loss) for numerous diseases (Sato et al. 2009; Zvuloni et al. 2009; Vargas-Angel 2010; Williams et al. 2014). According to a recent study that modelled coral disease susceptibility under climate change projections, temperature-induced increases in coral disease prevalence and progression rate will lead to coral disease becoming one of the major drivers of coral decline in the near future (Maynard et al. 2015). However, numerous other biotic and abiotic factors can influence prevalence and progression rates, such as nutrient enrichment (Kuntz et al. 2005; Kline et al. 2006; Voss and Richardson 2006; Bruno et al 2007; Kaczmarsky and Richardson 2011), light intensity (Boyett et al. 2007; Sato et al. 2011), injury and breakage (Miller and Williams 2007; Nicolet et al. 2013; Lamb et al. 2014), sedimentation (Frias-Lopez et al. 2002; Haapkyla et al. 2011), proximity of algae (Haas et al. 2011; Barott and Rohwer 2012; Casey et al. 2014) and coral cover (Bruno et al. 2007; Hoff 2007; Williams et al. 2010; Aeby et al. 2011). Whereas studies over several decades (i.e., since the first disease-temperature study conducted by Antonius in 1981 on black band

disease) reveal consistent patterns in the role of environmental factors in disease onset and progression, studies of other biological factors, such as the role of corallivores in disease progression, have so far shown ambiguous results. Such results highlight the need for a better understanding of coral disease dynamics under natural levels of predation, and under exposure to natural fluctuations in environmental conditions.

Coral-feeding fishes and invertebrates have the potential to influence coral disease dynamics through the continual removal of coral tissue, causing the coral to redirect energy towards tissue repair (Gochfeld 2004) and potentially lowering its resistance to pathogens. Indeed, predation by corallivores can hinder the recovery of corals after stressful events like bleaching (Rotjan et al. 2006). Tissue damage caused by coral predators may provide increased opportunities for pathogens to overcome the inherent resistance and defences of individual colonies, thereby representing a site for new infections (Raymundo et al. 2009). Alternatively, in some cases invertebrates can be beneficial and defend corals against pathogens. For example, feeding by *Cymo* crabs on white syndrome lesions has been shown to debride lesions and slow rates of disease progression (Pollock et al. 2013). Similarly, corallivorous fishes have been observed to feed selectively on infected coral tissue (Cole et al. 2009; Chong-Seng et al. 2011). Such behaviour could slow the progression of disease by removing infectious microorganisms (Cole et al. 2009) but it may also spread diseases to adjacent healthy colonies, either via pathogen transport associated with feeding, water-borne contamination (i.e. release of infected tissue into the water column) and/or feeding-related injuries that provide an entry site for pathogens (Raymundo et al. 2009). Corallivorous fishes have been documented to feed selectively on specific infection sites for both black band disease and brown band disease (Cole et al. 2009; Chong-Seng et al. 2011, Nicolet et al. 2013). Nevertheless, the role of corallivores in promoting or supressing coral diseases remains a subject of debate (Aeby and Santavy 2006; Cole et al. 2009; Chong-Seng et al. 2011).

Black band disease (BBD) is a common coral disease known to infect corals worldwide (Garret

and Ducklow 1975; Antonius 1985; Korrubel and Riegl 1998). The disease is characterized by a dark polymicrobial mat that progresses across the host coral colony, killing coral tissue and exposing white skeleton (Richardson 2004). The pathogenicity of BBD derives from the anoxic and sulphide-rich microenvironment created by the synergistic effects of a consortium of cyanobacteria, sulphur cycle-related bacteria, and other heterotrophic microorganisms present in the disease mat (Sato et al. 2013). Although the ecology and microbiology of BBD has been studied extensively (Antonius 1985; Frias-Lopez et al. 2002; Voss and Richardson 2006; Sato et al. 2009, 2010, 2013), its aetiology, transmission patterns and overall *in situ* dynamics remain largely unknown. In a study at Lizard Island, Chong-Seng et al. (2011) reported that eight species of corallivorous fish (six of which were chaetodontids) and four species of non-corallivorous fish selectively feed on BBD lesions *in situ*, and speculated that these reef fish could be disease vectors, transmitting pathogens to neighbouring corals (see also Aeby and Santavy 2006, Raymundo et al. 2009). Cole and co-workers (2009) suggested that intense selective feeding could slow the progression of BBD and, at very high levels of predation pressure, even stop progression of the disease. If so, corallivorous fishes may simultaneously transmit BBD and regulate progression of established infections. However, neither of these studies quantified the impact of selective feeding on progression rates of BBD. Therefore, manipulative experiments, conducted under both field and laboratory conditions, are required to resolve the effect of corallivorous fish on BBD dynamics.

To determine the effect of predation on disease progression, various factors known to influence progression rates must be considered. Many environmental variables (water temperature, light intensity, sedimentation and nutrient enrichment due to higher rainfall) have seasonal fluctuations that are known to influence both coral disease prevalence and progression rate (e.g. Harvell et al. 1999, 2002; Kline et al. 2006; Lafferty 2009; Zarlenga et al. 2014). Seawater temperature is undoubtedly the most well-studied seasonally-varying factor and has been shown to affect both the prevalence and progression rate of over 15 diseases around the world (Antonius 1981; Willis et al. 2004; Bruno et al. 2007; Haapkyla et al. 2010; Maynard et al.

2015). Recently, light intensity has been shown to account for a large proportion of seasonal variability observed in BBD dynamics, a seasonality previously attributed to water temperature variation (Boyett et al. 2007; Sato et al. 2011). Apart from abiotic factors, recent studies have also found that intraspecific variation among coral hosts can explain up to 70% of variation in disease dynamics *in situ* (Rodriguez and Croquer 2008; Nicolet et al. 2013). Such differences among colonies of the same species may arise as a consequence of the ecology of the disease (e.g., some diseases have increased virulence over time as microbial communities develop; Glas et al. 2012), but might also be influenced by the health status and natural resistance of the coral host prior to infection (i.e., a coral genotype effect). However, innate resistance of corals is seldom accounted for in coral disease ecology, often being treated as random variation that obscures treatment effects. Understanding and quantifying this natural variation in disease susceptibility among coral colonies is critical for understanding the effects of coral disease on coral population dynamics.

The purpose of this study was to determine the effects of selective predation by coral reef fishes on progression rates of BBD (i.e. the rate of disease-related coral tissue loss), using a combination of laboratory and field experiments. In the field, the impact of natural predation on BBD progression rate was evaluated by comparing progression rates of BBD on coral branches that were caged (i.e. protected from coral-feeding fishes) *versus* exposed to naturally occurring fish assemblages. In aquaria, I experimentally tested whether high levels of predation pressure by *Chaetodon plebeius* (Chaetodontidae) enhance or inhibit disease progression. To account for the potential influence of environmental covariates on disease progression, I recorded water temperature and light intensity using *in situ* data loggers.

2.3 Methods

2.3.1 Study site

This study was conducted between March (end of austral summer) and June (beginning of austral winter) 2013 at Lizard Island (14˚40’08’’S 145˚27’34’’E), a mid-shelf island on the

northern Great Barrier Reef, Australia. Lizard Island has an extensive and highly diverse reef system, with well-developed fringing reefs on the exposed reef front, a lagoon, and a mosaic of patch reefs on the sheltered (north-west) side of the island. To locate a study site with a high abundance of BBD, I completed extensive surveys across 12 reefs, including exposed reef fronts, lagoon and back reef habitats, in March 2013 using timed-swims (3 replicates of 10 min, ~ 100 m^2, per reef). Disease prevalence, especially of BBD and growth anomalies, was highest on staghorn colonies of *Acropora muricata* at Trawler reef, a shallow reef on the northern part of the lagoon, which was subsequently selected for the field experiment.

2.3.2 Field Experiment: BBD progression and predation by resident fish communities

To test whether natural predation by reef fishes influences progression rates of BBD, I conducted a controlled caging experiment in the field. A total of 15 colonies of *Acropora muricata* showing similar signs of BBD infection were tagged using cable ties secured on exposed skeleton below the disease band. All 15 colonies were located at the same site, at depths between 2 and 4 m depending on the tide, and separated by a maximum of 40 m from each other. Disease progression along infected branches was monitored every two days for 10 days by taking pictures of diseased branches including a ruler as a scale. Since virulence of BBD pathogens and migration patterns of cyanobacteria that dominate the BBD microbial consortium are known to vary with light intensity (Sato et al. 2011), all photographs were taken during the middle of the day (between 1100 h and 1500 h) to avoid additional variation in lesion progression rate and width. Initially, one lesion per colony was monitored, but when lesions progressed past bifurcation points on branches, newly diseased branches were considered separately from the original branch to avoid overestimating progression rates. As a consequence, the number of diseased branches per colony varied from one to six, producing an unbalanced dataset in which the total number of branches increased over time.

At the same time as progression rate was measured, rates of natural predation on disease bands and the identity of disease-consuming fishes were estimated using digital underwater video

cameras (GoPro Hero 2). Cameras were placed 30 to 60 cm away from BBD-infected colonies and focused on coloured bands bordering disease lesions adjacent to seemingly healthy coral tissue. All 15 colonies were filmed before caging during five replicate 30-min-long video recordings. After 10 days, branches of five colonies were caged using 0.5 x 0.5 cm wire mesh to prevent predation on the infected coral tissue without reducing water flow, five colonies were left uncaged, and branches on the remaining five colonies were only partially caged to control for the presence of caging material around the coral without preventing predation (branches enclosed by a frame but with wire mesh on only two sides of the cube; Figure 2.1). After caging, disease progression was monitored every two days for another 15 days using the same methodology. All pictures were taken using an underwater camera (Panasonic DMC-TZ15), and BBD progression rate (standardized to centimetres per day) was measured on each diseased branch using the image analysis program ImageJ (1.44o, Java 1.6.0_29, public domain). Each infected branch was photographed every second day and care was taken to ensure that images taken on successive days were taken from the same angle and distance from the band to minimise this potential source of variation in the data. Progression rate was estimated by measuring the linear distance the disease band moved along coral branches over time (over the total of 25 days of the experiment), by measuring from the tag (cable tie on exposed skeleton) to the margin of healthy tissue. Band width was measured in a similar fashion, from the interface between the skeleton and black band to the margin of the healthy tissue.

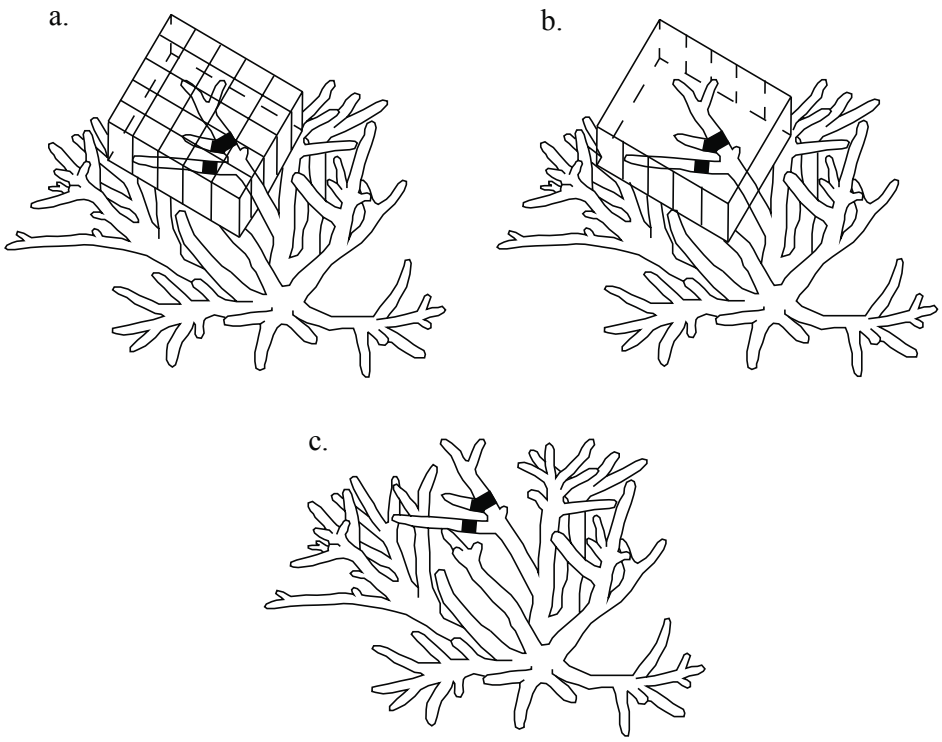


Figure 2.1: Visual representation of the three treatments, (a) cage, (b) cage control, and (c) control. Thick black bands on the illustration represent the disease.

Environmental conditions (mainly light and temperature) through the course the experiment were recorded by GBROOS loggers located at several locations in the northern part of the lagoon, where temperature and light were logged every five minutes. Data were acquired from the GBROOS website (http://data.aims.gov.au/gbroos/ on Jan 2015). The averages of all light readings from sunrise to sunset (0600 to 1900 hrs) were used for daily light values; daily temperature values were an average of day and night seawater temperatures (24h). All diseased colonies used in this study were within a 50m-area of the reef; all larger-scale environmental factors (i.e. seawater temperature, light and fish assemblages) are thus expected to be the same.

2.3.3 Laboratory Experiment: BBD progression and predation by Chaetodon plebeius

To closely monitor corallivore impact on disease progression, aquarium trials were conducted at Lizard Island Research Station using *Chaetodon plebeius* (Chaetodontidae), the corallivore that interacted most frequently with BBD lesions in the field. Individuals of *C. plebeius* (total length 5 to 8 cm) were collected from multiple reefs (at least 1 km away from Trawler Reef) and were

caught using a 5 x 1.5 m barrier net and hand nets. To minimise the potential effects of fish size and potential previous exposure to BBD, fishes of different sizes and from different reefs were mixed together in the aquarium experiment. Healthy nubbins of *A. muricata* were collected from the lagoon, and the absence of BBD lesions confirmed under a dissecting microscope (Olympus SZX7). Fish and coral nubbins were allowed to acclimate to laboratory conditions for 48 h prior to the experiment. *C. plebeius* were fed healthy coral branches (renewed every three days) and coral nubbins were fed every day at dusk with brine shrimp (*Artemia salina* nauplii) hatched in 0.5 μm filtered and UV sterilized seawater. After the acclimation period, heavily diseased nubbins of *A. muricata* were collected from the reef and placed directly in experimental aquaria (each 120 x 40 x 50 cm), supplied with flow-through seawater filtered to 0.5 μm and UV sterilized.

To determine the impact of predation levels on BBD progression rates under controlled aquarium conditions, healthy coral nubbins, diseased coral nubbins and fish were randomly allocated to one of three experimental (high, medium and no predation) and two control treatments (Figure 2.2). The high predation treatment comprised a healthy nubbin, a diseased nubbin and four *C. plebeius*. The medium predation treatment comprised an infected nubbin with two *C. plebeius* in one half of the tank and two *C. plebeius* and a healthy nubbin separated by a divider in the other half of the tank; this maintained the total number of fishes per tank at four to control for nutrient input due to fish presence, as nutrient enrichment can increase BBD virulence (Kuta and Richardson 2002; Richardson and Ragoonath 2008). Fish feeding behaviour on experimental diseased nubbins was measured every second day using underwater videos (GoPro Hero 2). The no predation treatment comprised a diseased nubbin on one side of the tank separated by a divider from four fish and a healthy nubbin on the other side of the tank. Plexiglas dividers were perforated to enable water to flow normally between the two compartments but prevent the fish from moving freely. All fish in experimental treatments were fed, as outlined above, with non-experimental healthy nubbins that were renewed every three days to maintain fish health (four non-experimental nubbins per *C. plebeius* pair, renewed every

three days over the duration of the trial, in addition to the one experimental healthy nubbin per tank). Control treatments comprised: 1) a healthy and an infected nubbin in a tank without direct contact (control for BBD progression rate in the absence of fish), and 2) one healthy nubbin in a tank to test for pathogen presence in the aquarium system (water control) (Figure 2.2).

In combination, the five treatments comprised one trial (seven aquaria per trial: two aquaria for each of the high and medium predation treatments, one aquarium for the no predation treatment and for each of the BBD progression and water controls). Seven replicate trials were conducted in total, spread out over two months because of space limitations that precluded running the complete design simultaneously. Each replicate trial was run for six days to allow sufficient time to detect an effect of selective feeding on disease progression. Moreover, after a week most diseased nubbins had little to no remaining tissue. Progression rate was quantified in the same manner as in the field, using an underwater camera (Panasonic DMC-TZ15) and with a ruler as scale. Pictures were taken every day to better estimate progression rates. In total, the experiment ran for 42 days (6 days x 7 trial replicates) and between each replicate trial, all diseased corals, healthy nubbins and fish were changed to avoid pseudo-replication. New fish, freshly caught from the reef, replaced 'used' fish (after a 48 h acclimation period) whenever possible. A total of 80 *C. plebeius* were used to run the seven replicate trials.

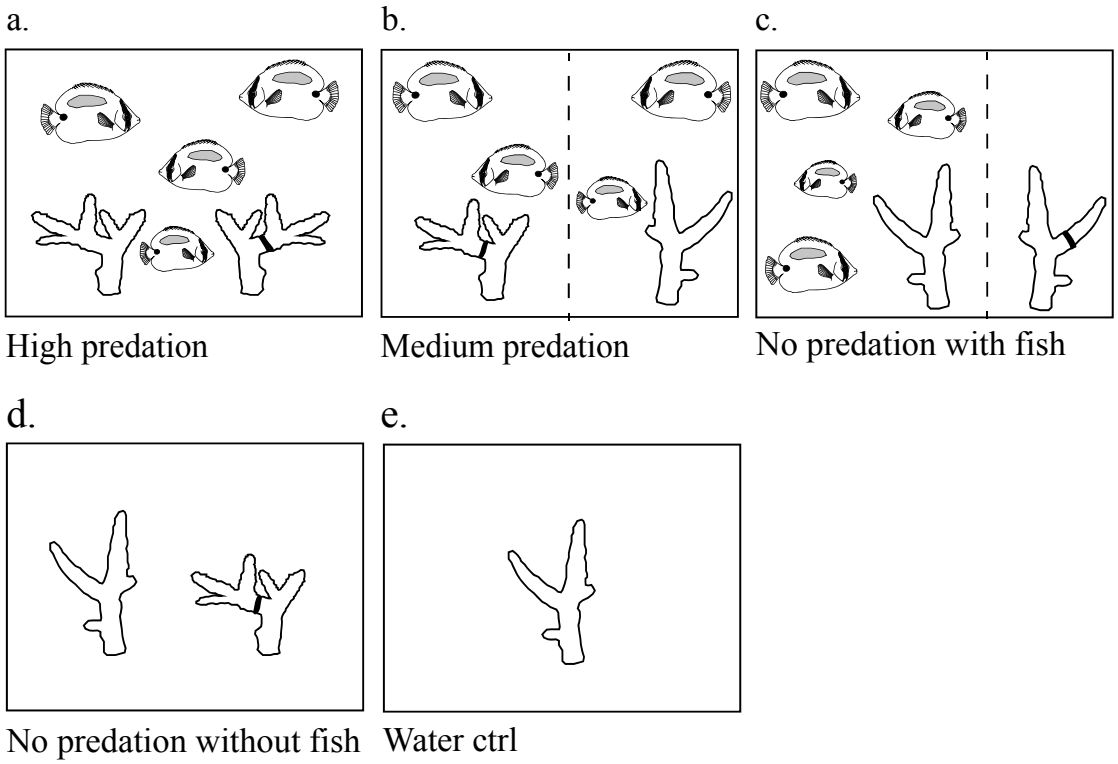


Figure 2.2: Laboratory experimental design to assess the effect of various predation levels on black band disease progression rate. Each replicate contained three treatments (a) high, (b) medium, and (c) no predation with fish present; and two controls (d) no fish control, and (e) water control.

2.3.4 Video analysis and statistical analysis

Analyses of *in situ* video recordings enabled identification of all species of fish that took bites from coral tissues within the field of view, as well as quantification of bite rates and qualitative observations of feeding behaviour. Similarly, fish feeding behaviour and bite rates during the laboratory study were determined from video analysis. In both cases, the number of bites taken by each fish was recorded in two categories: bites on the disease band (comprised of pathogens and necrotic tissue) and bites on apparently healthy coral tissue. All bites that were visually estimated to be between 1-5 mm above the disease band were considered diseased tissue, as this region typically comprises pathogens, mucus and necrotic coral tissue (i.e. non-healthy tissue). The observer only counted bites when the mouth of the fish and the food source were clearly visible; hence it is likely that the counts slightly underestimated actual bite rates. Predation data

on both diseased and healthy corals were standardized to bites min^{-1} for both field and aquarium experiments.

Progression rate data from the 15 colonies of *Acropora muricata* monitored in the field were graphically and statistically analysed to tease apart major influences on black band disease progression rates, including inter-colony variation, seasonal variation, and predation effects. The relationships between progression rate and both experimental stage (before and after caging) and band width (cm) were investigated using a linear mixed-effects model using the function 'lme' in the 'nlme' package in RStudio (Version 3.0.2 – © 2013 RStudio, Inc.). Experimental stage (before and after caging) was treated as a fixed effect and band width as a continuous covariate, while colony was included as a random effect. The width of the disease band at each time point was measured and included in this analysis because it was hypothesised to be positively correlated with disease progression rate. Variation in residuals was heterogeneous (residuals increasing with fitted values) and, hence, an additional argument 'weights' was included in the mixed-effects model using band width as a covariate. To account for repeated measurements of colonies over time (days of experiment, 1 to 25 as a factorial variable), which violates the independence assumption of the linear mixed-effects model, a 'correlation' argument was also added to model the auto-correlation between residuals of different time points. After various trials of auto-correlation models for residuals, the ARMA structure ($p = 2$, $q = 0$) was selected using the AIC criterion (see Appendix A for all R codes). The effect of treatment (cage, cage control and control) on disease progression rate was tested in a separate analysis, only including the dataset after caging. In this model, caging treatment was treated as a fixed effect and band width as a continuous covariate and a generalised least squares analysis was run including a correlation argument for repeated measurements ('gls' using the 'nlme' package).

In addition, given the combination of fixed, random and repeated factors included in the analysis, a variance components analysis was used to assess how much of the observed variance

in progression rates was explained by each factor (i.e. experimental stage, caging treatment, band width, colony, and time (i.e., days of experimental exposure: 1 to 25)). These values were acquired by running a linear mixed-effects model, with progression rate as the response variable, and time, band width and colony as random effects. Time and experimental stage were combined because stage (before/after caging) also captures variation through time. The standard deviations of each random effect (time, treatment, band width, and colony) were extracted from the model summary, squared to calculate variances, then each expressed as a percentage of the total variance. Lastly, a generalised least squares model was used to test the average progression rate across all colonies, for each day of the experiment, against averages of both light, water temperature readings and the interaction between the two. The residuals of the linear regression were observed to increase with the values of light, hence, the argument 'weights' was included in the final generalised least squares model (see Appendix A for all R codes).

In the aquarium experiment, lesion progression rates were highly variable and non-normally distributed. A non-parametric Kruskal-Wallis test comparing mean progression rates among predation levels in the different treatments revealed that mean predation rates did not differ significantly between the medium and high predation treatments (Kruskal-Wallis test, $\chi^2 = 0.05$, df = 1, p = 0.83). Thus, progression rates for the two treatments were pooled and tested against predation rate (i.e. predation present vs absent) in the final model. The linear mixed-effects model was used to investigate the effect of predation on progression rate, where predation (as a continuous variable, bites min^{-1}) was included as a fixed factor, band width as a continuous covariate, and experimental replicate as a random factor. Again, the arguments 'weights' and 'correlation' were added to the model to deal with heterogeneity and repeated measures over time. Finally, a linear mixed-effects model was used to test the effect of time (1 to 42 days of experimental exposure; treated as a factorial variable) and band width on BBD progression rate in the absence of predation (experimental replicate treated as a random factor). All statistical analyses were performed with RStudio (Version 3.0.2 – © 2013 RStudio, Inc.). Linear mixed-effects models and generalised least squares models were computed using the nlme package and

the multiple comparison test (kruskalmc) using the 'pgirmess' package. The Kruskal-Wallis, Kolmogorov-Smirnov and Pearson's correlation tests were calculated using the 'stats' package.

2.4 Results

In the field study, ten fish species were observed feeding on BBD lesions during 37.5 h of video recording. *Chaetodon plebeius* was responsible for more than half (52.7%) of the total number of bites taken from disease lesions, followed by *Chaetodon lunulatus* (22.3%), *Chaetodon rainfordi* (9.2%) and *Chaetodon aureofasciatus* (5.6%). The other 6 fish species were *Chaetodon baronessa*, *Chaetodon trifascialis*, *Labrichthys unilineatus* (juvenile), *Oxymonacanthus longirostris, Pomacentrus amboinensis* and *Pomacentrus grammorhynchus*.

2.4.1 Field Experiment: Among-colony variation in disease progression

Mean progression rates of BBD lesions varied significantly among the 15 replicate coral colonies in the field experiment (Kruskal-Wallis test $\chi^2 = 121.75$, df = 14, $p < 0.001$; Figure 2.3a). Mean progression rate was 0.79 cm day^{-1} (SE ± 0.05), but ranged from 0.005 to 5.2 cm day^{-1} among colonies. Intra-colony variation in mean progression rates were also observed, with standard errors of colony means ranging from 0.037 to 0.49. Band width differed significantly among colonies (Kruskal-Wallis test $\chi^2 = 146.14$, df = 14, $p < 0.001$), varying from 0 (e.g., at the end of the experiment when very little live tissue remained on branches) to 4.9 cm, with a mean of 0.5 cm (SE ±0.02) across all colonies (Figure 2.3b).

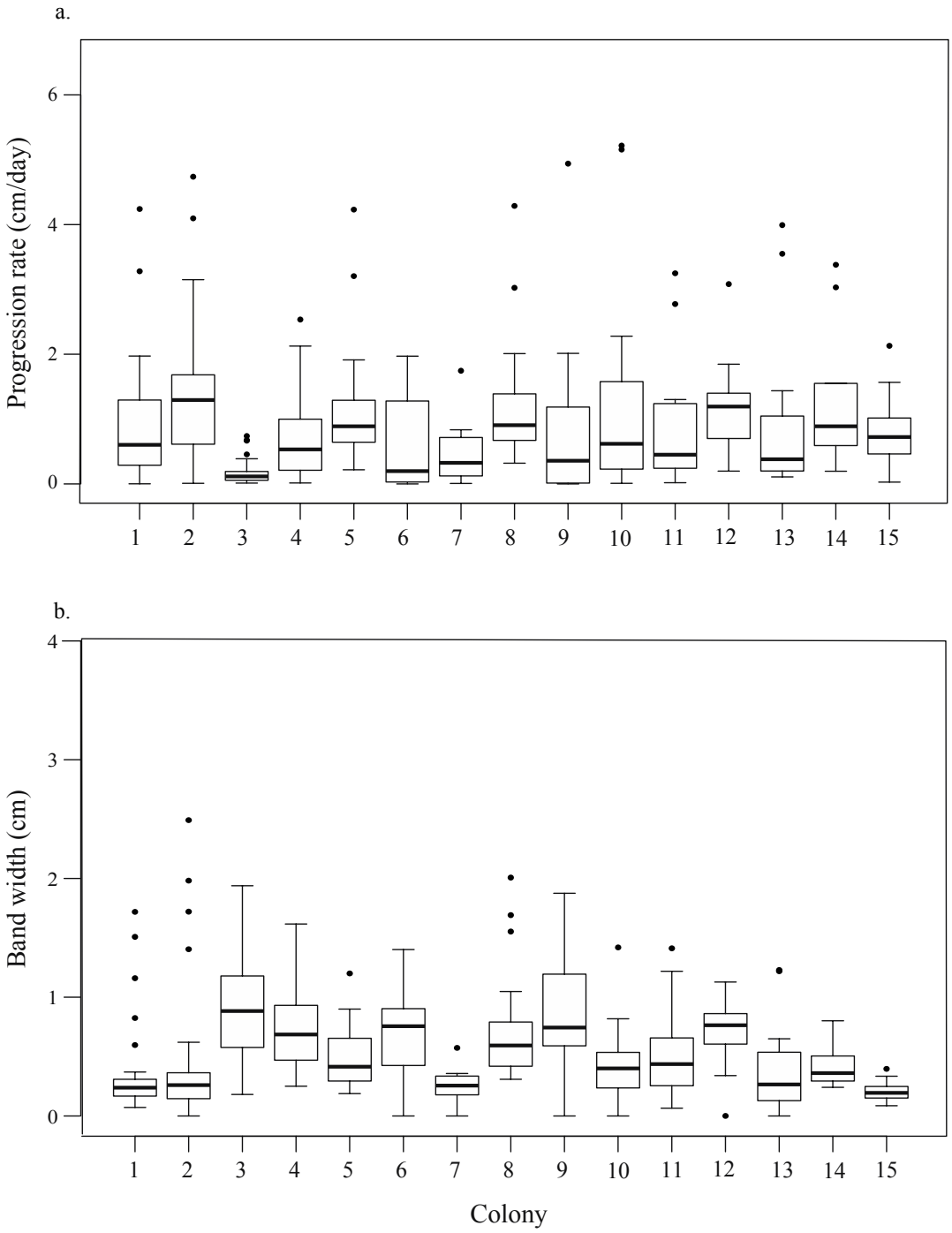


Figure 2.3: Natural variation in black band disease (a) progression rate (cm day^{-1}) and (b) band width (cm) across the 15 colonies of Acropora muricata before the onset of the caging experiment. Thick lines inside boxes represent the median (or second quartile), while the lower and upper lines of boxes represent quartiles 1 and 3. The box itself is the inter-quartile range. The whiskers show 1.5 times the inter-quartile range and individual points on the graph show data outside the range of the whiskers.

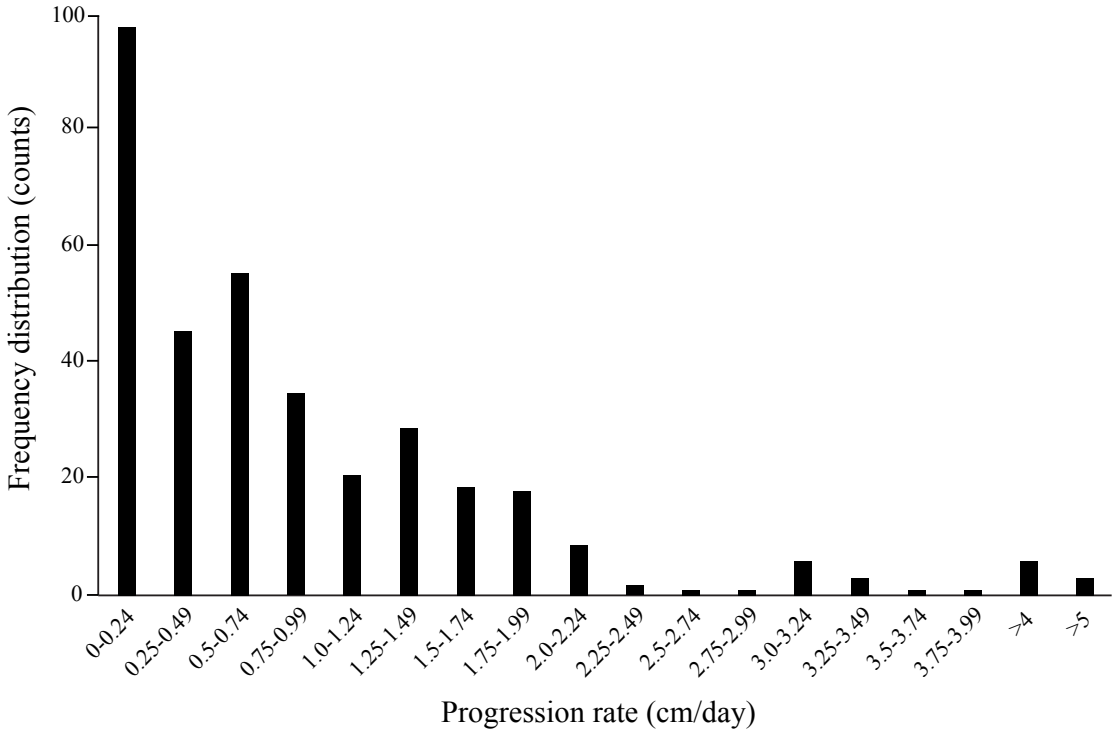


Figure 2.4: Frequency distribution of black band disease progression rate (cm day^{-1}) across the 15 colonies of Acropora muricata before the onset of the caging experiment.

Only 3 (from n = 606) observations had band widths greater than 3 cm; these were considered outliers and removed from subsequent analyses. When considering all the data (before and after caging), there was a significant positive relationship between band width and disease progression rate (linear mixed-effects model, band-width: denDF = 589, F = 13.98, $p < 0.01$; slope = 0.33, Figure 2.4). Moreover, correlation coefficients were positive for most of the colonies (13 out of 15) when considered individually, supporting the interpretation that band width and progression rate are positively associated under natural conditions.

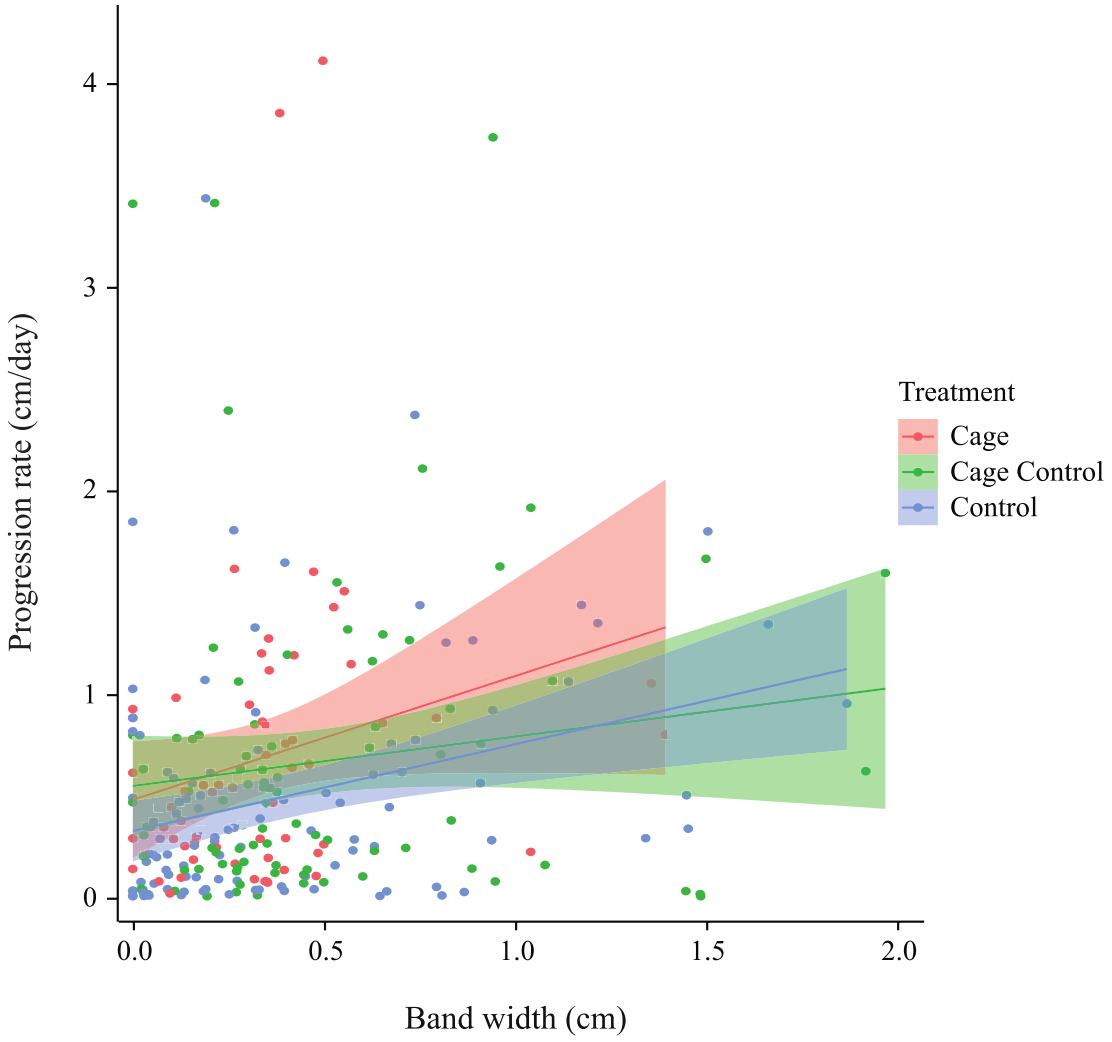


Figure 2.5: Illustration of the correlation between progression rate (cm day^{-1}) and band width (cm) for each treatment in the field experiment. Shaded areas show the 95% confidence interval.

Progression rate also varied significantly through time. Mean BBD progression rate was significantly faster in the summer months before caging (0.92 cm day^{-1} SE ± 0.05) compared with in cooler months after the onset of the caging experiment (0.61 cm day^{-1} SE ± 0.04; linear mixed-effects model, Experimental stage: denDF = 589, F = 12.63, $p < 0.001$, Figure 2.5).

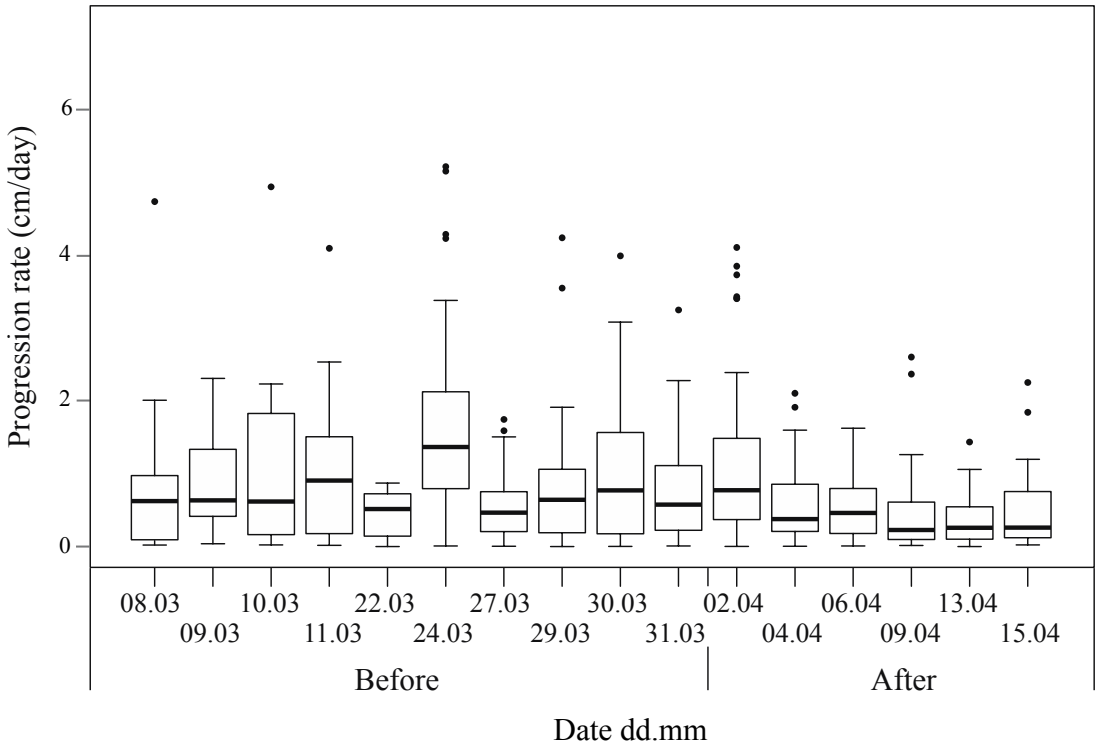


Figure 2.6: Progression rates of black band disease over time in the field. Dates before and after the onset of the caging experiment are separated by a vertical line on the x-axis. Thick lines inside boxes represent the median (or second quartile), while the lower and upper lines of boxes represent quartiles 1 and 3. The box itself is the inter-quartile range. The whiskers show 1.5 times the inter-quartile range and individual points on the graph show data outside the range of the whiskers.

2.4.2 Field Experiment: Effect of predation on BBD progression rate

Overall, mean predation rate before caging was 0.46 bites min^{-1} (SE ± 0.14), but rates ranged widely from 0 to 6.6 bites min^{-1}. After caging, progression rate was independent of predation level (generalized least squares, treatment: numDF = 2, F = 2, p = 0.11, Figure 2.6) but positively correlated with band width (generalized least squares, band width: numDF = 1, F = 12, $p < 0.001$).

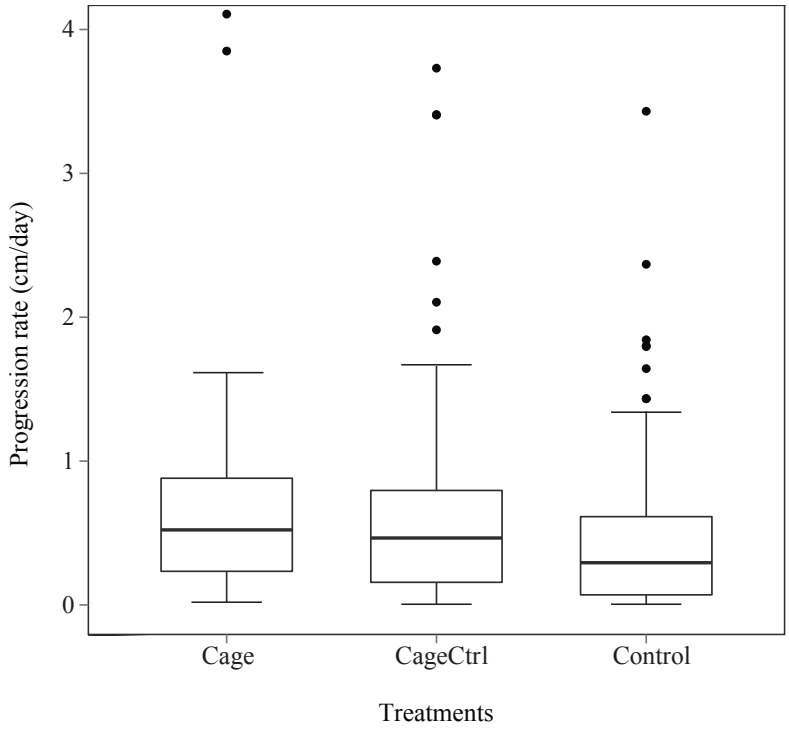


Figure 2.7: Mean black band disease progression rate (cm day^{-1}) compared among three caging treatments; cage, cage control and control. Thick lines inside boxes represent the median, the lower and upper lines of boxes represent quartiles 1 and 3 and the box itself is the inter-quartile range. The whiskers show 1.5 times the inter-quartile range and individual points represent data outside that range.

Variance components analyses revealed that the variability in BBD progression due to caging treatment (cage, partial cage control and uncaged control) was negligible (<0.1%); the greatest variability in disease progression was primarily due to inter-colony variation (~24%), variance over time (i.e., days of experimental exposure; ~38%), and changing band width over time for each colony (~38%). The high variability over time (~38%) is likely due to fluctuating or seasonally varying environmental factors, especially during March and April when seawater temperatures cool down relatively rapidly. Indeed, when average progression rates (across all colonies) for each day of the experiment were tested against water temperature and light intensity readings from the field, both light and water temperature were significantly and positively correlated with BBD progression, i.e., disease progression rate declined with declining light and temperature readings (generalized least squares, light: df = 1, F = 34.76, p < 0.001; water temperature: df = 1, F = 12.76, p = 0.004, Figure 2.7).

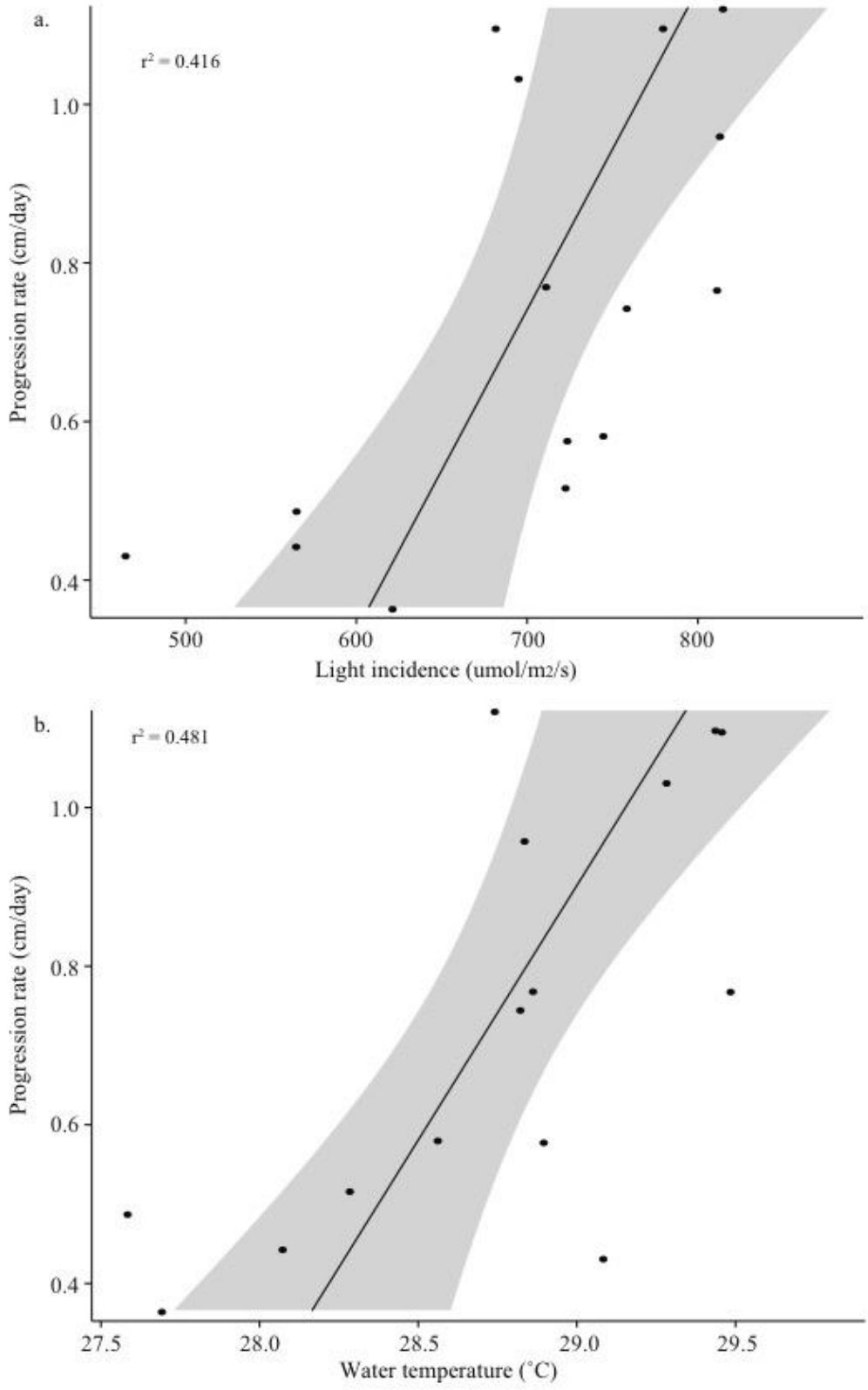


Figure 2.8: Relationship between black band disease progression rate and (a) light intensity (PAR in umol m^{-2} s^{-1}) and (b) water temperature (˚C) in situ. Progression rates are averages per day (n=15 colonies). Light incidence values are averages of readings between 0600 and 1900 hrs each day; water temperatures are daily averages of day and night readings (24h).

2.4.3 Laboratory Experiment: Effect of predation on progression rate

In the aquarium study, mean predation rate was 1.76 bites min^{-1} (SE ± 0.19) per nubbin, which was 3.5-fold greater than mean predation rate recorded in the field, and ranged from 0 to 10.3 bites min^{-1}. Over the entire duration of the experiment, *C. plebeius* was observed to take more bites from the disease band (14323 bites) than from healthy tissue (13417 bites), despite the lesion representing less than 10% of tissue available on the branch. Mean disease progression rate in aquaria with predation was 0.36 cm day^{-1} (SE ± 0.03), but ranged from 0.002 to 1.32 cm day^{-1}. Due to high variation in fish predation rates within treatments, and the lack of a clear difference in predation between medium and high predation treatments, data were analysed using predation as a continuous variable. In this model, disease progression rate was significantly positively correlated with predation rate (bites min^{-1}), but not band width (cm) (Figure 2.8, linear mixed-effects model using only progression data under predation; predation: denDF = 150, F = 8.16, p = 0.005; band width: denDF = 150, F = 0.04, p = 0.8). In the absence of predation (i.e., in the no predation treatment), lesion progression rates reached a higher maximum (3.02 cm day^{-1}) and mean (0.56 cm day^{-1}) than under predation. No significant difference was found in the frequency distribution of the disease progression data with or without predation (Kolmogorov-Smirnov, D=0.25, p=0.69), meaning that the difference in progression rate with and without predation was not significant. Disease progression rate in the absence of predation varied significantly over time (linear mixed-effects model, time: denDF = 44, F = 2.09, p = 0.008), increasing during the cooler months. Light and temperature were not systematically recorded in the laboratory experiment but were approximately equivalent to conditions in the field because tanks were kept under natural irradiance, and ambient seawater was pumped directly from the Lizard Island lagoon. Lastly, an overall model including mean progression and predation rate for both colonies in the field and replicate aquarium in the laboratory experiment did not find a significant correlation between predation and progression rate (One-way ANOVA, df = 1, F = 0.38, p = 0.54).

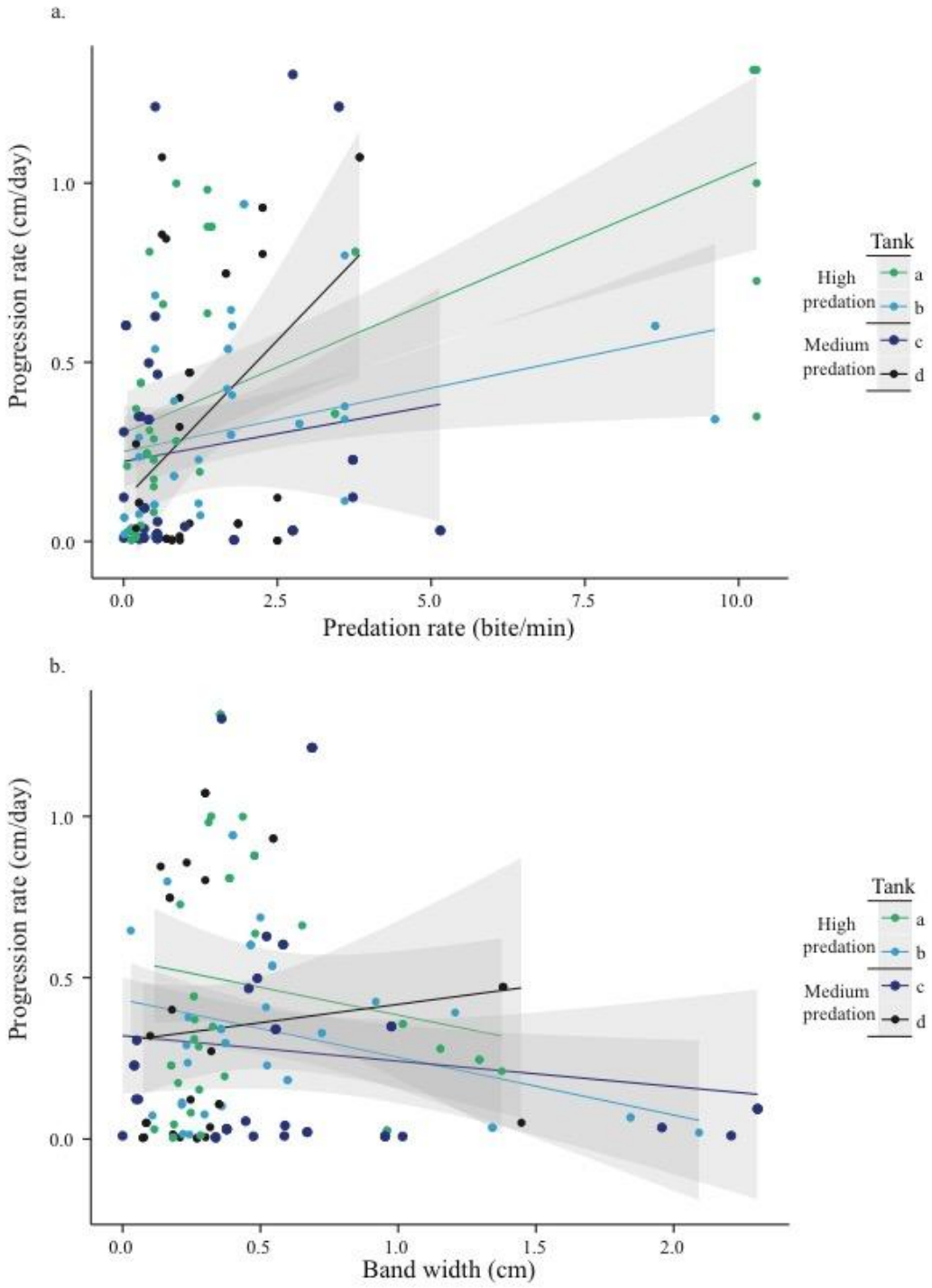


Figure 2.9: Correlation between black band disease progression rate (cm day^{-1}) in aquaria and either (a) Chaetodon plebeius predation rate (bites min^{-1}), or (b) band width (cm). Each aquarium is represented by a specific shade of grey to help visualise the correlations accounting for variation per aquarium. Aquaria a and b were the high predation treatments with four fish feeding on the disease, while aquaria c and d had two fish feeding on the nubbin (medium predation treatments).

2.5 Discussion

I found no evidence that selective predation by corallivorous fishes led to declines in the progression rates of BBD. Rather, high levels of selective predation on disease lesions by the butterflyfish *Chaetodon plebeius* were correlated with increased progression rates of BBD in aquaria. These results do not support inferences from previous studies (Cole et al. 2009) that predation by corallivorous fishes might suppress coral diseases. Although mean progression rates were highest in the complete absence of predators, they were not significantly higher than mean progression rates in predation treatments. Similarly, in field experiments, predation treatments had no effect on progression rate and explained less than 0.1% of the overall variance in BBD progression rates. Results from both the field and laboratory experiments were consistent; predation rate did not explain variation in progression rates of BBD found among corals and among sampling days. Variation throughout the course of the study (among days) was likely due to fluctuating environmental factors, as BBD progression rates in the field were found to significantly decrease with decreasing water temperature and light intensity.

Chaetodon plebeius was the predominant fish species recorded to feed on *Acropora* colonies infected with BBD during this study. In a previous study on Lizard Island reefs, Chong-Seng et al. (2011) reported *Neoglyphidodon melas* and *Chaetodon baronessa* to be the predominant corallivores targeting BBD. Inconsistencies between the two studies may simply reflect differences in study sites; the Trawler beach site in our study is a shallow back reef that typically harbours a different fish community with a lower diversity than the crest site where the 2011 study was mainly located. These contrasting results are unlikely to indicate seasonal variation in fish feeding activity, as Chong-Seng et al. (2011) also conducted their experiment during the austral summer (2008/2009). Despite differences in the species of corallivore studied, Chong-Seng et al. (2011) reported very similar average rates of predation on BBD by butterflyfishes (0.31 bites min^{-1}) to those found here (0.46 bites min^{-1}).

Fish predation on disease lesions is highly selective, often accounting for half of the bites taken on diseased colonies, despite lesions typically representing <10% of the available coral surface area (Chong-Seng et al. 2011, this study), but the reason why fish actively target BBD lesions is unknown. Such selective predation may be related to increases in the density of microbial communities, increased mucus production, or because pathogens have inactivated the corals' nematocysts making the tissue more palatable to fish. Selective feeding by predators on disease lesions is uncommon in the animal kingdom, except for cases of specialised host-parasite interactions (cf. Aeby 1991, 1998, 2002, 2003, 2007). In these latter cases, predation on encysted parasites is required for transitions among alternate host species, leading to morphological modifications designed to encourage selective feeding on encysted parasites, which affect predation dynamics for infected corals. Otherwise, to my knowledge, only one other case of selective predation on a disease lesion has been reported; the grasshopper *Melanoplus differentialis* was found to prefer sunflower leaf tissue infected with the rust fungus, *Puccinia helianthii*, to healthy tissue from the same plant, potentially because the fungus modifies the plants' chemistry facilitating ingestion by the grasshopper (Lewis 1984). However, as very few studies of reef fish corallivory report bite rates per colony as a function of coral health (but see laboratory and field experiments of Dirnwoeber and Herler 2013 and Gochfeld 2010, respectively), it is difficult to distinguish between the alternative possibilities - that disease lesions attract predators to the infected colony (increasing overall predation on diseased colonies), or disease lesions simply focus predation on diseased tissue (releasing healthy tissue from predation). These two alternatives could have very different impacts on overall coral health and the likelihood that infected colonies will survive.

In the field study of BBD progression, the proportion of variance explained by colony (~24%), time (~38%) and band-width (~38%) was far greater, in each case, than the variance explained by caging treatment and, by extension, predation (<0.01%). Thus, inter-colony variation, environmental variation over the study period, and changes in the width of the disease band had stronger impacts on disease progression rate than fish feeding behaviour. Collectively, these

findings suggest that any effects of natural levels of predation on disease lesions in the field are largely overshadowed by other biotic and abiotic factors. However, high variability among and between colonies, in addition to a relatively small sample size (n = 5 colonies per treatment), could have reduced statistical power. Finding more than 15 coral colonies of the same species infected with the same disease at the same time and on the same reef proved to be impossible, even after monitoring the reef over a two-year period, thereby constraining the sample size. Nonetheless, regardless of the sample size, the impacts of natural sources of variation among colonies and over time are likely to remain greater than any effect of fish feeding behaviour. This low impact of fish predation on rates of black band disease progression might be specific to branching corals. Mounding or encrusting species might show a different relationship with corallivory and BBD progression rate, potentially because their growth forms attract a distinct fish community. Further studies are needed to clarify the impact of corallivory on disease dynamics using other coral and fish species.

The results of this study do not support the hypothesis that selective feeding on disease bands removes pathogenic organisms, thereby reducing BBD progression rates (e.g. Cole et al. 2009). Rather, our study shows that high numbers of corallivores in aquarium experiments (and abnormally high rates of predation on disease lesions) are linked to increased rates of BBD progression. Injuries caused by high predation pressure are known to reduce coral health (Gochfeld 2004, Rotjan et al. 2006), in which case, fish feeding behaviour could indirectly enhance rates of BBD progression found in aquarium experiments. Alternatively, the positive correlation between fish predation and disease progression in aquarium experiments could be a consequence of fish being attracted to more virulent disease bands because of the greater amounts of mucus, dying tissue and bacteria on these nubbins. In this scenario, virulence of the band affects fish feeding behaviour, and it would be the pathogen consortium, rather than corallivory, that drives BBD progression rates. A feedback loop, in which disease bands attract fish feeding, which then provides further entry wounds for pathogens, is the most likely explanation, but further controlled studies are required to tease apart these relationships.

In the aquarium experiment, bite rates did not differ significantly between treatments with high and medium densities of fish. This could be because fishes excluded each other from the food source in the high-density treatment, resulting in only two fishes feeding on the band at any one time –a hypothesis supported by the video footage collected in this study. Direct competitive exclusion has been studied extensively in many taxa, usually at the species level, where two species limited by the same resource cannot coexist (Armstrong and McGehee 1980). At the within-species scale, aggressive or competitive behaviour between individuals targeting the same resource is also common across taxa (e.g. crayfish: Bovbjerg 1970; chipmunks: Brown 1971; birds: Murray 1971; marsupials: Dickman 1986). Diets of chaetodontids, especially hard coral feeders, typically overlap by 30 to 50% but sometimes by up to 70% (e.g. Pratchett 2005). When dietary overlap is not minimised by spatial or temporal partitioning, intense competition can occur, with frequent aggressive interactions between conspecifics and congenerics (Berumen and Pratchett 2006, Blowes et al. 2013). This aggressive behaviour could result in competitive exclusion, whereby few individuals can feed simultaneously within the same exact position on the same coral colony, thus preventing excessive predation rates on disease lesions even at elevated densities of corallivorous fishes.

Variation among coral colonies in both BBD progression rate and disease lesion size is likely due to a combination of both intrinsic factors (e.g., genotypic differences in disease susceptibility or differences in colony condition; Pisapia et al. 2014), and extrinsic factors (e.g., differences in the specific micro-habitat and recent disturbance history for colonies). This inter-colony variation remained after the onset of the caging experiment, with variation among colonies over time accounting for ~25% of the total variance. These results are consistent with other studies, which have also found that colony typically accounts for the greatest amount of variability in rates of disease progression. For example, Rodriguez and Croquer (2008) reported that variability within and among colonies explained 52 and 48% of the total variance, respectively. Similarly, variability among colonies over time explained 73% of the total

variance in progression rates of brown band disease (Nicolet et al. 2013). As all colonies of *Acropora muricata* used in the field experiment co-occurred within 50 m of each other, they experienced similar ranges in environmental factors like wave action, coral cover, water temperature, salinity, water quality and light intensity. Only microhabitat variation, such as the presence of territorial damselfish that influence disease dynamics by harbouring potential BBD pathogens (Casey et al. 2014), could be an alternative explanation for among-colony variation observed in this study. Benthic primary producers around colonies have also been found to alter microbial processes by modifying biochemical cycling in their surrounding environment (Haas et al. 2011). The close proximity of algae releasing dissolved organic carbon into their surroundings could promote bacterial growth and increase the virulence or likelihood of infection in neighbouring coral colonies (Kuntz et al. 2005; Kline et al. 2006; Haas et al. 2011). Understanding how these fine-scale processes influence coral health, and how much this explains among-colony variation, requires further study.

Disease progression rate was temporally variable in both the field and aquaria, which may be attributable to changing environmental factors. Progression significantly decreased over time, regardless of caging treatment or colony level variation, and this response was correlated with decreases in light intensity and water temperature. BBD prevalence and rate of related tissue loss have previously been linked to seasonal fluctuations in water temperature and light intensity (Boyett et al. 2007; Sato et al. 2011). A manipulative experiment testing both factors reported that BBD progression rate is greatest (0.52 cm day^{-1}) at high temperatures (30.5˚C) and high light intensities (Sato et al. 2011). However, when taken separately, high temperature under low light did not significantly increase BBD progression rate, whereas high light treatment in cool water significantly enhanced BBD associated tissue loss (Sato et al. 2011). In the present study, both factors were strongly correlated with BBD progression rate and with one another, such that no inference can be made about whether one or both factors underlie these patterns.

Positive correlations between the width of the disease band and BBD progression rate, both in the field and in aquaria (in the absence of predation), highlight that characteristics of the pathogenic consortium are also likely to contribute to variation in BBD progression rates. A wider, more complex band comprises more pathogens and is likely to break down coral tissue faster. The correlation was consistent for all 15 colonies in the field, although the strength of the correlation varied. To my knowledge, this is the first time that a correlation between disease band width and disease progression rate has been found. It is possible that the two factors, progression and lesion size, are not directly correlated but instead are the product of another aspect of black band dynamics. For example, Glas and co-workers (2012) found that biogeochemical microgradients within the complex microbial community of the band, particularly through the creation of an anoxic and sulphide-rich environment, are responsible for disease virulence. Because the microbial community within the band changes over time, mean progression rate and potentially the width of the band increase as the community becomes more vertically stratified. However, although microbial community complexity and stratification are responsible for pathogen virulence, coral tissue loss is a mere by-product of this process. Consequently, reducing the disease band width, for example via fish predation, would not impact progression rate of the disease. Further research is needed to tease apart factors underlying virulence of the BBD microbial community and rate of coral tissue loss, and how both can be moderated.

In conclusion, this study shows that corallivorous fish have limited potential to suppress the progression of black band disease on the common staghorn coral *A. muricata*. Instead, variation in progression rate was driven by characteristics of the coral host (e.g. genotypic differences in disease susceptibility or health), the pathogens (e.g. successional stage of the microbial community), or the environment (e.g. seasonal variation in light or temperature). The precedence of inter-colony variability in explaining progression rate variability highlights that some colonies are naturally more resistant to black band disease and can impede progression of the disease and minimise tissue mortality. Such genotypic variation is commonly acknowledged

in studies of coral immunity (Pinzon et al. 2014, Toledo-Hernandez and Ruiz-Diaz 2014, Palmer and Traylor-Knowles 2012) but is often disregarded in coral disease ecology research, or only regarded as random variation concealing targeted factors. Overall, the potential of both small-scale (e.g. benthic primary producer community composition) and large-scale processes (e.g. water quality, light and temperature) to influence coral disease dynamics highlights the need for multi-factor studies in order to better predict and manage the impact of BBD on coral reefs.

CHAPTER 3 –POTENTIAL ROLE OF COMMON CORALLIVORES AS VECTORS OF CORAL DISEASE

3.1 Abstract

Infectious diseases that are not limited by host density, such as vector-borne diseases, have the potential to drive population declines and extinctions of host species. This study explores the vector potential of the corallivorous snail *Drupella* sp. and a corallivorous butterflyfish *Chaetodon plebeius* for two virulent coral diseases on Australia's Great Barrier Reef, black band (BBD) and brown band (BrB) disease. *Drupella* transmitted BrB to healthy corals in 40% of cases immediately following feeding on infected corals, and even in 12% of cases 12 and 24 hour following feeding. However, *Drupella* was unable to transmit BBD in either delayed or direct transmission treatments. In a field experiment, approximately equivalent numbers of caged and uncaged coral fragments (n = 96) became infected, indicating that corallivorous fish were unlikely to have been responsible for the transmission. In a controlled aquarium experiment, corallivorous fish did not transmit either disease, even following extended feeding directly on both infected and healthy nubbins. These results indicate that polyp-feeding fishes are unlikely to be vectors of bacterial coral diseases, possibly because their feeding creates small lesions that are too shallow for pathogens to invade coral tissues. In contrast, corallivorous invertebrates create deeper feeding scars that often expose coral skeletons, that may become sites of new disease infections. A review of the literature highlighted that all known vectors of coral disease -only 4 species identified thus far- are corallivorous invertebrates that remove coral tissue and expose underlying skeleton. Given that the feeding ranges of invertebrate corallivores are generally small, these results have implications for the spatial distribution of diseased colonies on reefs, and for the rate of spread of disease between colonies.

3.2 Introduction

Infectious diseases, defined as health disorders caused by pathogenic biological agents, affect all living organisms, with detrimental consequences for host species and ultimately for ecosystem function and biodiversity (Daszark et al. 2000; Jones et al. 2008; Plowright et al. 2008). Until the late 1970s, it was generally thought that "well-adapted" pathogens would not harm their hosts (McCallum 2012), and modelling studies suggested that pathogens would be lost before host populations went extinct, because pathogens would drive their hosts below a density threshold critical for disease persistence (Anderson and May 1992). Consequently, the role of infectious disease as a driver of host population dynamics has been underappreciated, and diseases have rarely been considered to contribute to species extinctions (McCallum 2012). However, some diseases are transmitted as a function of the proportion of infected *versus* uninfected individuals in the population ('frequency-dependent') regardless of the density of individuals (Smith et al. 2009). When a disease is density-dependent, transmission increases as population density increases because of the increased probability of contact between infected and susceptible individuals. For example, in airborne diseases like viral influenza, the likelihood of an individual becoming infected depends on the number of individuals per unit area (i.e., population density). When a disease is frequency-dependent, transmission increases as the proportion of infected individuals increases regardless of density. Vector-borne pathogens and sexually-transmitted diseases are commonly frequency-dependent, and their prevalence can continue to increase even when host density is low, leading to disease-mediated population declines and extinctions (Thrall et al. 1993; Boots and Sasaki 2003). The same is true when pathogens remain viable outside of their hosts, in a 'reservoir', or when pathogens are able to infect multiple hosts, both of which release pathogens from the dynamics of a specific one host-one pathogen system (Fenton and Pedersen 2005, Pedersen et al. 2007).

The potential for coral-eating predators (corallivores) to be effective vectors for pathogens, independently of host density, is a cause for concern, given drastic declines in coral populations over the past 50 years (Carpenter et al. 2008; Osborne et al. 2011; De'ath et al. 2012). Such

declines have previously been attributed to overfishing, pollution, coastal development and climate change (Burke et al. 2011), with only limited losses attributed to infectious diseases. More recent studies, however challenge the general view that marine diseases only marginally impact coral reefs. For example, on the Great Barrier Reef, disease is estimated to have caused equivalent coral losses to bleaching between 1995 and 2009 (Osborne et al. 2011). Moreover, a study modelling reef degradation (Maynard et al. 2015), predicted that "increases in the prevalence and severity of coral diseases will be a major future driver of decline and changes in coral reef community composition", given projections that rising sea temperatures are likely to increase both pathogen virulence and host susceptibility. Such projections are consistent with evidence that most infectious diseases emerge due to changes in host-pathogen interactions following environmental and/or ecosystem modification (Daszak et al. 2001). For instance, a recent and devastating sea star wasting disease is thought to have emerged because of warming sea temperatures (Eisenlord et al. 2016).

For most of the 22 coral diseases reported from the Caribbean (Weil 2004) and the 17 described in the Indo-Pacific (Willis et al. 2004; see Table 1.1, Section 1.2.1), coral pathogens affect multiple coral species, raising the possibility that disease prevalence can be independent of individual host abundance. For example, black band disease affects at least 40 coral species on the Great Barrier Reef (Willis et al. 2004; Page and Willis 2006). Moreover, some coral pathogens have reservoirs and vectors that maintain pathogen loads, even when host population densities are low. The coral disease white pox, for example, is caused by the pathogen *Serratia marescens*, which survives and remains virulent within the corallivorous snail *Coralliophila abbreviata* (Sutherland et al. 2011), enabling the snail to infect new coral colonies. Numerous coral diseases have either complex epidemiology or unconfirmed infectious agent(s) (see Chapter 1), but express characteristic disease signs. However, studies of disease vectors can be undertaken even before a pathogen has been formally identified, and are particularly critical in cases where vaccination and quarantine programs are difficult or impossible, such as for coral populations. Malaria, for example, is a well-known disease that is managed primarily by vector

control via insecticide spraying and/or mosquito habitat reduction (Caraballo and King 2014). Ultimately, a good understanding of which species are vectors of diseases, and the timeframes and biological processes involved in disease transmission, is required to establish effective disease management practices for syndromes with known or unknown pathogens.

Black band disease (BBD) and brown band disease (BrB) are among the most conspicuous and widespread coral diseases found on the Great Barrier Reef (GBR), Australia (Willis et al. 2004; Page and Willis 2006), and although previous studies suggest that vectors, such as butterflyfish, marine snails and crown-of-thorns starfish, might contribute to their transmission, the evidence to date has been conflicting. In a few studies (Sussman et al. 2003; Williams and Miller 2005; Sutherland et al. 2011; Nicolet et al. 2013), corallivorous vectors, predominantly gastropods, have been confirmed to actively transmit pathogens to new hosts within coral populations. However, in most cases it appears that corallivores enhance disease spread through indirect processes, promoting pathogenic infections by weakening the host and/or creating an entry point for pathogens (Raymundo et al. 2009). For example, the crown-of-thorns starfish, *Acanthaster planci*, produces large feeding scars that can be the origin of BrB infections (Nugues and Bak 2009; Katz et al. 2014). Observations that corallivorous fishes feed selectively on infected coral tissues led to speculation that they transmit coral diseases (Cole et al. 2009; Chong-Seng et al. 2011). However, evidence from experimental tests on the role of butterflyfishes in coral disease transmission (e.g., Aeby and Santavy 2006; Nicolet et al. 2013) has been equivocal. While the presence of fish in one aquarium experiment increased the transmission rate of BBD, possibly due to nutrient enrichment of the water (Aeby and Santavy 2006), no study has explicitly demonstrated transmission of coral pathogens via corallivorous fish (Aeby and Santavy 2006; Nicolet et al. 2013).

In this chapter, I evaluate the effects of predation by the gastropod *Drupella* (Muricidae) and coral reef fishes (Chaetodontidae) on the transmission rates of two common coral diseases on the GBR: black band disease (BBD) and brown band disease (BrB). Both aquarium and field-

based experiments were used to provide a better understanding of the vector potential of *Chaetodon plebeius* and *Drupella* sp. Aquarium experiments were designed to explicitly test the hypothesis that corallivores directly transmit coral diseases by feeding successively on infected and uninfected corals. Building on results of a previous study, which demonstrated that the gastropod *Drupella* sp. is capable of transmitting BrB to corals (Nicolet et al. 2013), the duration of the vector potential of *Drupella* was investigated by testing if BrB and BBD pathogens can survive within the mollusc for up to 24h and subsequently infect corals. The potential of corallivorous reef fish to transmit BrB and BBD was also tested in the field: a) under natural rates of butterflyfish predation (uncaged treatment), and b) in the absence of predation (caged treatment). To synthesise new insights into whether and how vector-borne diseases circumvent density-dependent infection dynamics that prevent species extinctions, I review existing knowledge of coral disease vectors and their potential to amplify coral disease impacts on coral population dynamics. The review of the literature will also provide an insight into whether the potential for disease transmission is stronger in vertebrate or invertebrate corallivores.

3.3 Methods

3.3.1 Study site and study species

All experiments took place on Lizard Island (14˚40’08’’S 145˚27’34’’E), a mid-shelf island on the northern Great Barrier Reef, Australia. At Lizard Island, populations of the staghorn coral *Acropora muricata* have highest disease prevalence, especially of BBD and BrB (Chong-Seng et al. 2011; see Chapter 2). This locally abundant species was thus selected as the experimental coral.

3.3.2 Aquarium set-up and maintenance of experimental animals

Experimental studies were conducted in flow-through aquaria at Lizard Island Research Station in March-June 2013 (BBD experiments) and January-March 2014 (BrB experiments). Different aquaria were used for disease transmission experiments using the corallivorous butterflyfish

Chaetodon plebeius (120 x 40 x 50 cm aquaria) and the gastropod *Drupella* sp. (30 x 30 x 50 cm aquaria) due to the different requirements of these animals. All aquaria were supplied with flow-through seawater filtered to 0.5 µm and UV sterilized. *C. plebius*, *Drupella* sp.*,* and nubbins of the coral *Acropora muricata* used in these experiments were all collected from within the Lizard Island lagoon. *C. plebeius* was used as it interacted most frequently with BBD and BrB lesions in video recordings at the study location (see Chapter 2). Adult and sub-adult *C. plebeius* (5 to 8 cm total length) were collected using a 5 x 1.5 m barrier net and hand nets. Healthy nubbins of *A. muricata* were collected from the lagoon, and the absence of BBD lesions or BrB ciliates confirmed under a dissecting microscope (Olympus SZX7, 50x magnification). Fish and coral nubbins were allowed to acclimate to aquarium conditions for 48 h prior to the experiment. *C. plebeius* were fed healthy coral branches (renewed every 3 days) and coral nubbins were fed every day at dusk with brine shrimp (*Artemia salina* nauplii) hatched in 0.5 µm filtered and UV sterilized seawater. *Drupella* snails were collected from rubble and *Acropora* thickets by hand using laboratory gloves, avoiding snails on infected colonies. All snails were placed in a holding tank (120 x 40 x 50 cm aquarium) containing diseased corals (either BBD or BrB) for a 3-day exposure period. After the acclimation period for both the fish and the snails, heavily diseased (disease band wider than 0.5 cm) nubbins of *A. muricata* were collected from the reef and placed in experimental tanks as described below.

3.3.3 Aquarium experiment: Vector potential of the butterflyfish, Chaetodon plebeius

To test whether *C. plebeius* is capable of transmitting BBD and/or BrB, and to explore mechanisms by which potential transmission occurs, multiple fishes were placed in aquaria with and without access to diseased and healthy coral nubbins. Three experimental treatments distinguished between active *versus* passive transmission mechanisms (Figure 3.1): (a) both healthy and diseased nubbins fully accessible to *C. plebeius* (4 fish per tank), testing for direct vectored transmission through successive feeding on diseased and then healthy nubbins (active transmission); (b) diseased nubbins accessible to *C. plebeius* (4 fish per tank) but healthy nubbins protected from predation by a semi-permeable tank divider, testing for passive

transmission of pathogens due to dislodgement during feeding on diseased tissues and/or enhanced nutrients (passive transmission with feeding); and (c) neither diseased nor healthy nubbins accessible to *C. plebeius*, which were separated from the nubbins by a semi-permeable tank divider, testing whether the presence of the fishes increased transmission, possibly due to increased nutrient levels (passive transmission without feeding). Controls for these treatments comprised diseased and healthy nubbins in the absence of fish (Figure 3.1), as follows: (d) a healthy and an infected nubbin in a tank without direct contact (passive transmission control), (e) a diseased nubbin cable-tied to a healthy nubbin to test if BBD and BrB pathogens can infect corals in an aquarium setting (pathogen infectivity control), and (f) a single healthy nubbin in a tank to test for pathogen contamination in the aquarium system (water control).

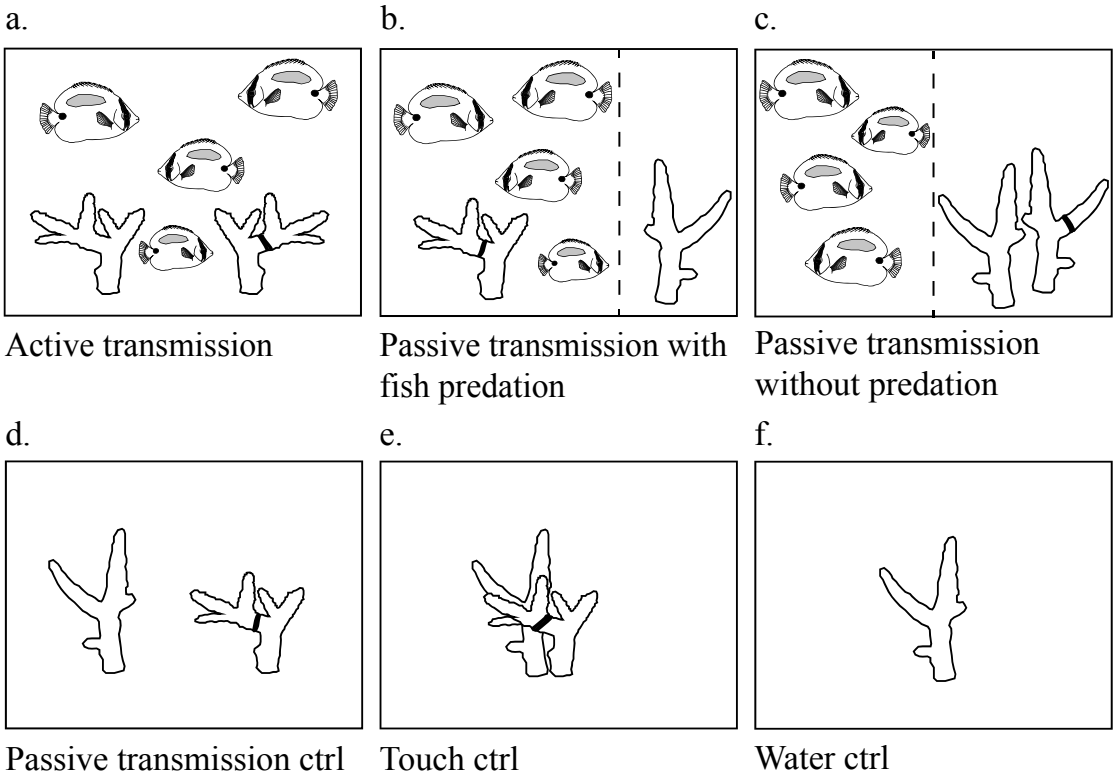


Figure 3.1: Experimental design assessing the effect of predation by the butterflyfish Chaetodon plebeius on black band and brown band disease transmission rates in an aquarium setting. Each replicate trial contained three treatments (a) active transmission, (b) passive transmission with fish predation, and (c) passive transmission without fish predation. The experiment also included three controls: (d) passive transmission without fish, (e) pathogen infectivity control, (f) seawater system control.

Due to space limitations in the aquarium system, only one set of the 3 experimental and 3 control treatments (hereafter referred to as a trial) could be conducted at any one time. Each trial ran for 6 days to allow enough time to detect the appearance of disease on healthy coral nubbins. Trials were replicated through time, i.e., 7 replicate trials for the BBD experiment (total of 8 aquaria per trial: 2 aquaria for each of the active and passive transmission with feeding treatments, 1 aquarium for the passive transmission without feeding treatment, and 1 aquarium for each of the passive transmission, pathogen infectivity, and water controls), and 5 replicate trials for the BrB experiment (again 8 aquaria per trial). The uneven design of the experiment (n = 2 for treatments with fish *versus* n = 1 for the treatment without fish) was accounted for in the statistical analyses. New fishes freshly caught from the reef replaced "used" fishes (after a 48h acclimation period) whenever possible. A total of 40 *C. plebeius* were used to run the 7 replicates of the BBD trial in Mar-Jun 2013, and another 30 *C. plebeius* for the 5 replicates of the BrB trial between Jan-Mar 2014.

3.3.4 Aquarium experiment: Vector potential of the gastropod Drupella sp.

After the 3-day period of exposure to either BrB or BBD, during which snails were observed to feed on diseased tissues, *Drupella* were placed in a holding tank for varying periods of time to determine how long pathogens might be retained and remain viable on/in the snail. Three experimental treatments were established to test the potential of *Drupella* to act as a vector for BrB and BBD (Figure 3.2): (a) "No delay", where 3 snails were placed in the holding tank for 5 seconds, then directly placed in an experimental tank at the base of a healthy nubbin; (b) "12h delay", where 3 snails were placed in contact with a healthy nubbin after spending 12h in the holding tank; and (c) "24h delay", where 3 snails were placed in contact with a healthy nubbin after 24h in the holding tank. Due to the discontinuous nature of feeding activity of the snails, immediate feeding could not be guaranteed; therefore, "12h" and "24h" represent minimum delays between pathogen exposure and first feeding on coral hosts. Three controls for these experimental treatments comprised healthy and diseased nubbins in the absence of *Drupella* (Figure 3.2), as follows: (d) a pathogen infectivity control, comprising a diseased nubbin cable-

tied to a healthy nubbin; (e) an injury control, comprising a healthy nubbin mechanically injured with a sterilised scalpel blade, resulting in a 100 x 50 mm area where tissue was removed but the skeleton only minimally damaged to simulate a *Drupella* feeding scar without exposure to the pathogen; and (f) a water control, comprising a single healthy nubbin in a tank to test for pathogen contamination in the aquarium system. Each trial comprised 6 tanks (1 tank per experimental or control treatment), and was replicated 8 times for the BrB experiment, and 6 times for the BBD experiment due to time and space constraints.

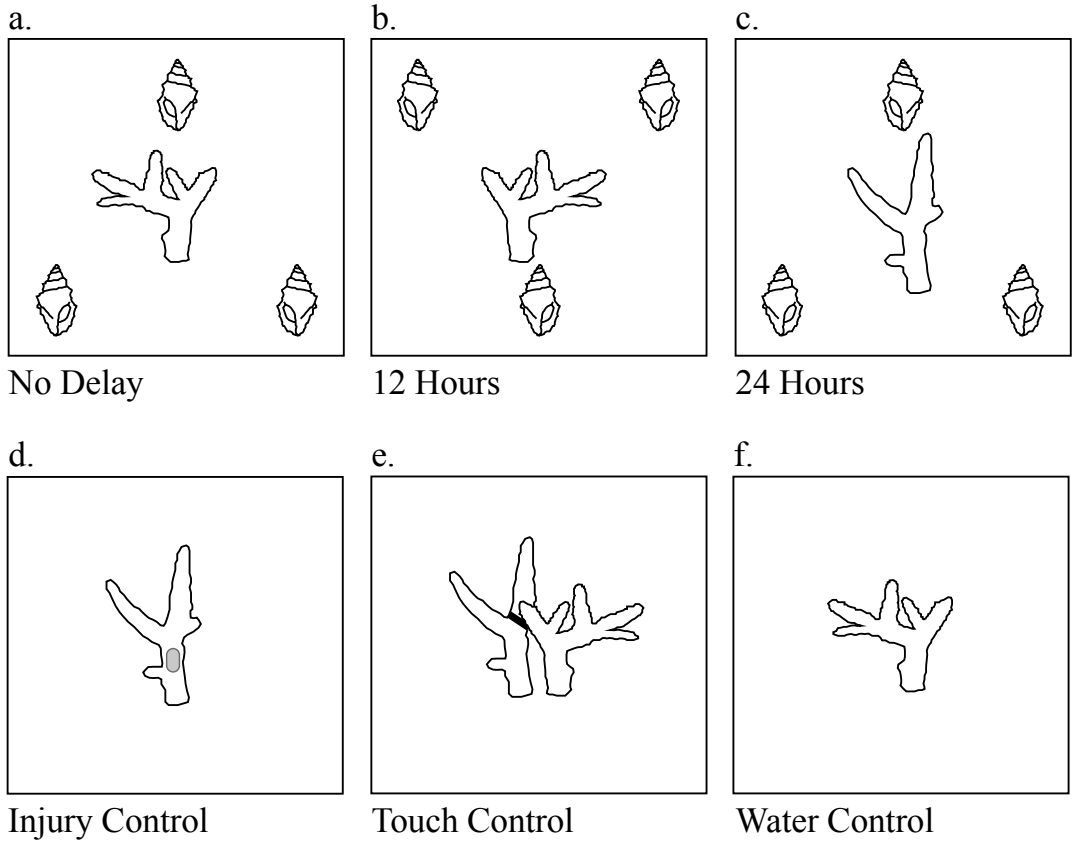


Figure 3.2: Experimental design to assess the vector potential of Drupella spp. snails for both black band (BBD) and brown band disease (BrB) in aquaria. Prior to the experiment, the snails were fed nubbins heavily infected with either BrB or BBD for 3 days. Then, three individual snails were allocated to one of three treatments (a) no delay treatment, where snails were rinsed for 5sec in filtered seawater before being introduced to the experimental tank, (b) 12h delay treatment, where snails were kept in a holding tank for 12h before being placed in the treatment tank, (c) 24h delayed treatment, where treatment snails were held in a similar fashion for 24h. An injury (d), touch (e) and water control (f) were also added.

All *Drupella* snails were removed 48h after the '24h delayed transmission' treatment was initiated, which was enough time to observe the presence or absence of snail feeding scars. All nubbins (both experimental and control) were monitored for another 3 days to allow any macroscopic signs of diseases to become visible. In total, experiments ran for 48 days for BrB (6 days per trial x 8 replicate trials) and 36 days for BBD (6 days per trial x 6 replicate trials). Between each replicate trial, all diseased, healthy and injured nubbins, and snails were replaced by new specimens collected from the field and acclimatised or exposed accordingly. A total of 72 *Drupella* were used for the BrB experiment (9 *Drupella* per trial x 8 trials between Jan-Mar 2014), and 54 *Drupella* were used for the BBD experiment (9 *Drupella* per trial x 6 trials between Mar-Jun 2013).

3.3.5 Field experiments: Vector potential of in situ assemblages of corallivorous fish

Field experiments testing the potential of *in situ* assemblages of corallivorous fish to transmit BBD and BrB were conducted in February 2009 at two Lizard Island sites: Horseshoe Reef on the western (leeward side) of the island, and a sheltered lagoon site between Palfrey and South Islands (Figure 3.3). Nubbins of *Acropora muricata* (n = 96), approximately 10 cm long, were collected from healthy colonies on reefs on the north-west side of the island. One nubbin was attached to each corner of 24 concrete breezeblocks (39 cm x 18 cm) that had been conditioned by immersion in seawater for several weeks. Modelling clay was used to mount nubbins in plastic bottle tops attached to the concrete blocks with epoxy cement. Thus, each block contained four healthy experimental nubbins, one on each corner, for a total of 24 blocks and 96 experimental nubbins. An additional stressor treatment was originally added by bleaching half of the experimental nubbins using freshwater, however bleaching treatment had no effect on disease transmission and thus methods and results are not presented or discussed. To test if predation by corallivorous fish enhances transmission of BrB or BBD to nearby nubbins, half of the healthy nubbins (2 on each block) were individually caged using plastic mesh with 1x1 cm openings (Figure 3.3).

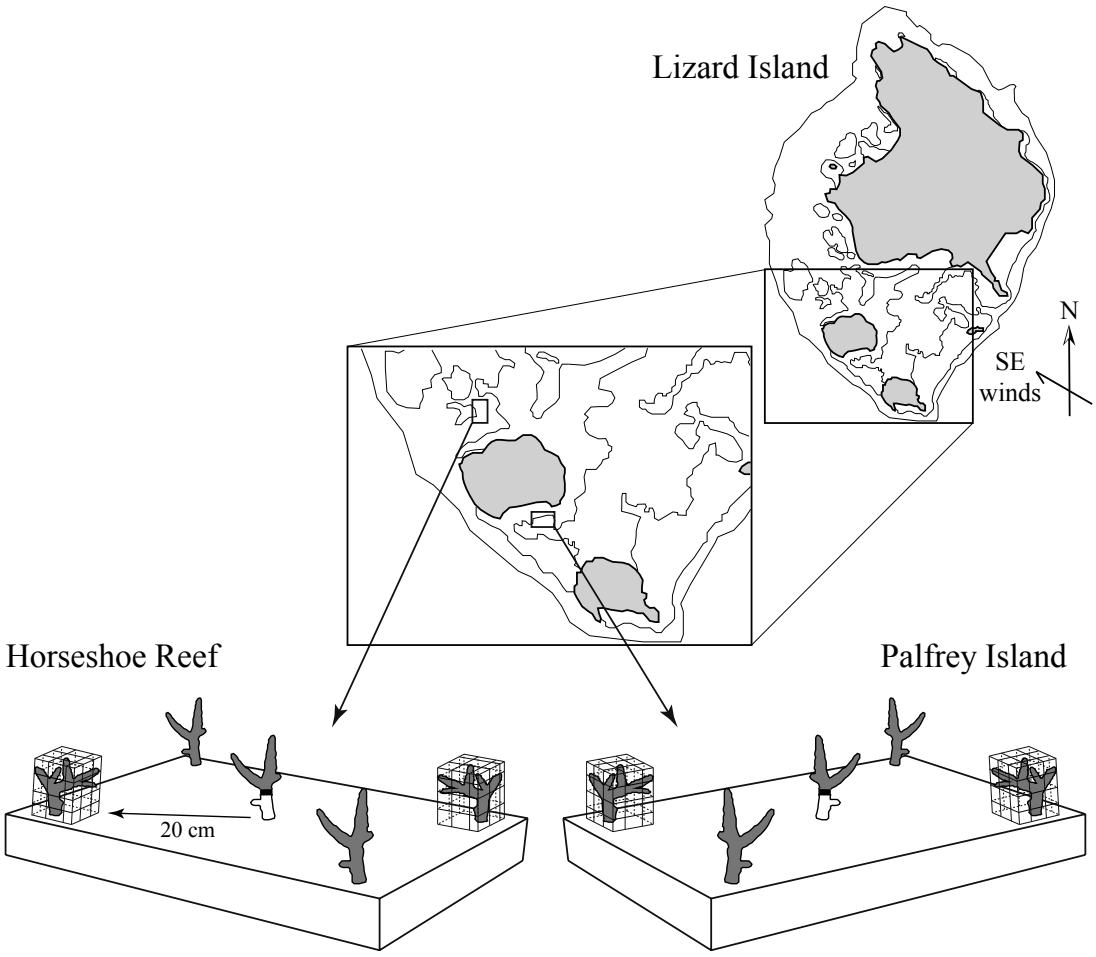


Figure 3.3: Study sites and experimental design for the field experiment at Lizard Island, with arrows illustrating north and the prevailing wind direction. Insets show the two experimental sites, Horseshoe Reef and South Palfrey Island, and experimental nubbins, either caged or uncaged, and location of the diseased nubbin in the middle of the block.

Blocks were deployed at the two reef sites and set 1-2 m apart at depths of 3-4 m. Half of the blocks were placed among the reef matrix at Horseshoe Reef (i.e., 12 blocks) and the other half within the sheltered lagoon between Palfrey and South Islands (i.e., 12 blocks). Both sites are similarly sheltered from the prevailing southeast trade winds, and both had relatively high densities of corallivorous fishes known to target diseased corals (Chong-Seng et al. 2011). Once blocks were positioned on the reef, an infected branch of *A. muricata* was mounted in the centre of each block (6 BrB-infected and 6 BBD-infected nubbins at each site), 20 cm away from uninfected branches. In summary, each experimental block held 5 nubbins (1 diseased nubbin, and 4 healthy nubbins, 2 of which were caged and 2 uncaged; Figure 3.3). Blocks were

surveyed every 2 days for 7 days to record the incidence of new infections. On day 2, video recordings (30 minutes per block) were also made to confirm that the cages effectively prevented corallivorous fishes from feeding on caged coral branches. At the end of the experiment, nubbins were brought back to the research station and were observed with a dissecting microscope (Olympus SZX7, 50x magnification) for signs of infection.

3.3.6 Statistical analysis

Disease transmission data from the *Drupella* experiment were analysed using generalized linear models, where "infection status" (binomial: infected or healthy) was the response variable, and the factor in the model was either "treatment" (5 levels) or "vector" (two levels: *Drupella* vs controls). The "treatment" factor levels were the three experimental treatments (no delay, 12h and 24h) and the two control levels (injury and water controls). The transmissibility control was excluded from the analysis because it was not directly related to corallivore vector potential, and was included only to ensure disease transmission was possible in tanks. For the model testing the "vector" factor, all transmission treatments were pooled together (*Drupella* level), and injury and seawater system controls were combined (control level). A likelihood ratio test was run on each model to compute p values (see Appendix B for all R codes). No transmission was recorded in the experimental treatments involving chaetodontids, either for BBD or BrB, and thus, the dataset was not formally analysed.

Data from the field experiments were analysed to test whether site of block deployment (Horseshoe vs. Palfrey), disease type (BrB *versus* BBD) and caging treatment (caged *versus* uncaged) influenced the incidence of infection using a generalised linear mixed model. The variable "status" referred to infection status and was treated as a binomial response variable in the analysis (infected or healthy, where infected indicates ciliate presence on nubbins). The random factor "block" was added to control for potential variation between replicates.

All statistical analyses were performed with R (version 3.0.2, R Development Core Team 2013). The generalized linear model used to analyse the data from the aquarium experiment was from the 'stats' R package, while the R package 'lme4' was used to run the generalized linear mixed model to allow for random effects.

3.3.7 Literature Review

To review the literature on coral disease vectors to date, the search term "coral disease vector" was used to collect all relevant publications recorded in the ISI Web of Science database between 1965 and August 2016. Next, the broader literature was explored using cited references in relevant publications and additional search engines (e.g. Google scholar) to ensure the relevant publications were identified. Studies from this set of papers (n = 53 publications) were screened and only included if they focussed on biological vectors of coral diseases; studies on algal reservoirs of pathogens and non-biological vectors (e.g., ballast waters, dust) were not included. A total of 22 studies met the criteria for inclusion in this review.

3.4 Results

3.4.1 Aquarium experiment: Potential of corallivorous fish as disease vectors

Chaetodon plebeius did not transmit either BBD or BrB over the time frame of this experiment. The only instances of disease transmission in these experiments occurred in pathogen infectivity controls, whereby 100% of seemingly healthy nubbins placed in direct contact with infected nubbins exhibited conspicuous evidence of disease within 6 days (5/5 nubbins for BrB and 7/7 for BBD), demonstrating that pathogens were active and infectious, and confirming that the duration of the experiment was sufficient for macroscopic signs of disease to become visible. No disease transmission was detected for either BBD or BrB in any of the other butterflyfish treatments, or in the passive transmission treatment or the water controls.

3.4.2 Aquarium experiment: Potential of Drupella as a disease vector

Brown band disease experiment: *Drupella* snails transmitted BrB in 3 out of 8 replicates (40%) in the "No delay" treatment, and in 1 out of 8 replicates (12.5%) in both the "12h delay" and "24h delay" treatments (Figure 3.4). High rates of transmission (7/8) occurred in the pathogen infectivity treatment, though there was no transmission observed for water or injury control nubbins. This demonstrates that new infections in the treatment tanks were a result of ciliates carried by *Drupella* snails and not caused by potential pathogens in the seawater system colonising feeding injuries. A generalized linear model and subsequent likelihood ratio test comparing "treatments" against "controls" (where treatments were pooled together and compared against grouped controls, excluding the touch control) revealed that the presence of *Drupella* significantly increased infection rates of BrB relative to controls (Analysis of deviance table; Vector, $DF_{resid} = 38$, $p = 0.02$, Figure 3.4). When *Drupella* treatments were compared against each other, infection rates did not differ among the 'No delay', '12h delay' and '24h delay' treatments (Analysis of deviance table; Treatment, $DF_{resid} = 21$, $p = 0.38$), suggesting that snails do not lose their vector potential over a 24h period. One nubbin in the seawater control treatment bleached and died, but no signs of ciliates or other pathogens were observed on the nubbin.

Black band disease experiment: BBD was never transmitted in any of the "No delay", "12h delay" or "24h delay" *Drupella* treatments. All injury and seawater control nubbins remained healthy throughout the experiment, while 5 out of 6 pathogen infectivity controls became infected. One nubbin in the "12h delay" treatment became infected with BrB ciliates, even though the snail was exposed to BBD. The ciliates were unlikely to have come from the filtered seawater system since the injured and seawater controls remained healthy; instead, they may have been present on the snails since initial collection from the field (up to 3 days prior).

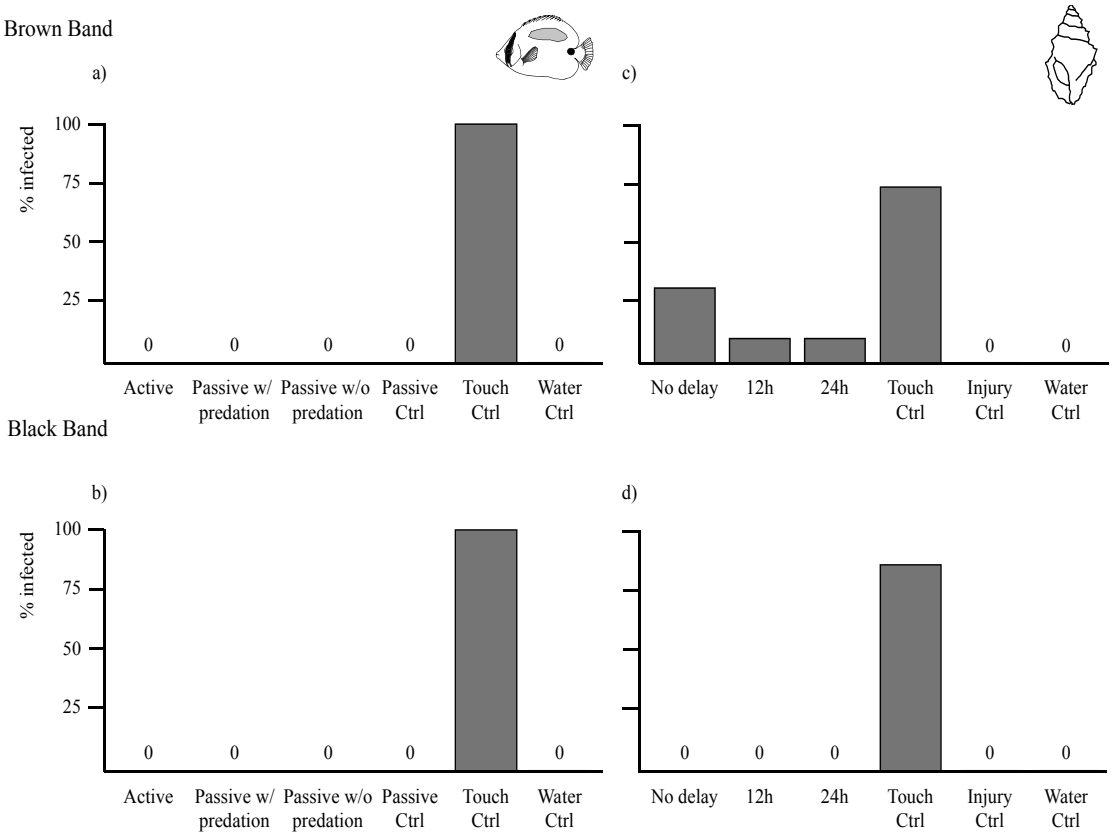


Figure 3.4: Percentage of experimental fragments that became infected in transmission experiments (boxes represent proportions of 100%). Two potential vectors were tested: Chaetodon plebeius as a vector for (a) brown band disease, and (b) black band disease, using 3 treatments (active, passive with fish predation, passive without predation) and 3 controls (passive, touch and water control); and Drupella as a vector for (c) brown band disease, and (d) black band disease, using 3 treatments (no delay, 12h delay and 24h delay) and 3 controls (touch, injury and water control).

3.4.3 Field experiment: Potential of corallivorous fish as disease vectors

In the field, 55% of experimental branches of *A. muricata* (n = 96 branches) became infected with either brown band, black band or skeletal eroding band disease (another ciliate-related coral disease) during the 7-day observation period. Of the branches that developed new infections, slightly more than half (28 out of 51) were caged and, therefore, protected from feeding by corallivorous fishes. Feeding observations on day 2 confirmed that several different species of corallivorous butterflyfishes (*Chaetodon aureofasciatus*, *C. baronessa*, *C. lunulatus* and *C. plebeius*) visited the experimental blocks, and fed on both the infected branches at the centre of the block and the uncaged branches on opposite corners of each block. Video footage

confirmed that butterflyfishes were unable to access caged branches. Despite obvious differences in visitation and feeding by corallivorous butterflyfishes, caging had no effect on whether or not branches became infected (glmer; Caging, $z = -0.66$, $p = 0.51$; Figure 3.5). The number of infections was significantly higher at Palfrey Island than Horseshoe Reef (glmer; Site, $z = 2.74$, $p = 0.006$, Figure 3.5).

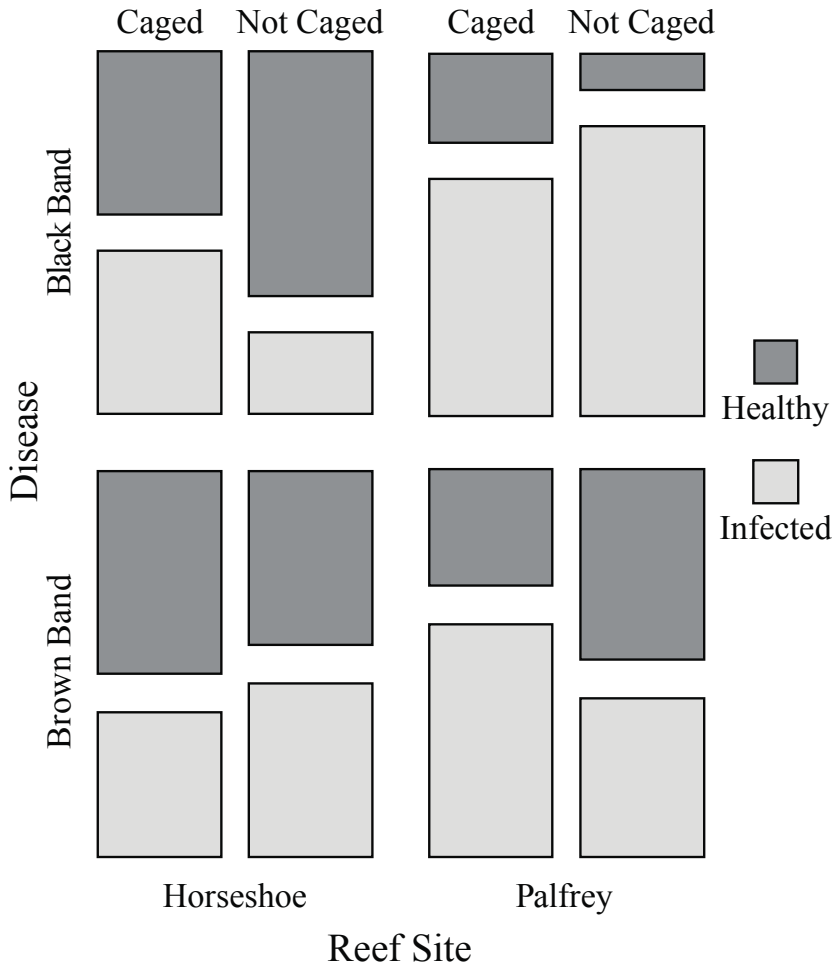


Figure 3.5: Table plot illustrating the proportion of healthy and infected (pathogen present) nubbins as a result of caging treatment, disease type and reef site.

3.4.4 Literature Review

The present review synthesises the role of corallivores in amplifying the impact of diseases on coral populations by acting as vectors and facilitators of infections (Table 3.1). To date, 22 peer-reviewed publications have assessed the effectiveness of putative biological vectors of coral diseases and only seven have experimentally determined a corallivore to be a disease vector.

One unique disease, *Porites* trematodiasis, causes swollen nodules on corals (Aeby 1991, 1998, 2002, 2003, 2007) as a consequence of infection by the trematode *Podocotyloides stenometra*, which requires multiple intermediate hosts (a mollusc, *Porites* corals, and the corallivorous fish, *Chaetodon multicinctus*) to complete its complex life cycle (Aeby 2003). Of the remaining studies, only 6 have successfully identified a pathogen within the vector's body, or have shown that vectors transmit disease in controlled experiments (Sussman et al. 2003; Williams and Miller 2005; Rypien and Baker 2009; Sutherland et al. 2011; Gignoux-Wolfsohn et al. 2012; Nicolet et al. 2013). The majority of the studies reviewed found a correlation between disease onset and the presence of, or predation by, a corallivore but did not demonstrate a causal link (Antonius and Riegl 1997; Dalton and Godwin 2006; Miller and Williams 2007; Cole et al. 2009; Nugues and Bak 2009; Raymundo et al. 2009; Chong-Seng et al. 2011; Katz et al. 2014; Raymundo et al. 2016). Other controlled experiments found that the corallivore tested did not transmit coral disease (Aeby and Santavy 2006; Gignoux-Wolfsohn et al. 2012; Nicolet et al. 2013; Pollock et al. 2013). The majority of publications on this topic have been conducted in the Caribbean region, where the most successful vector is the marine snail *Coralliophila abbreviata*, which has been shown to transmit three diseases (Williams and Miller 2005; Sutherland et al. 2011; Gignoux-Wolfsohn et al. 2012). The close relative, *Coralliophila violacea*, was also shown to cause tissue loss (resembling white syndrome) in *Porites* in Guam after feeding on infected and healthy colonies, although the vector potential of the snail remains equivocal since secondary infections remain likely due to the experimental design (Raymundo et al. 2016). In the Indo-Pacific, *Drupella* snails, and potentially the crown-of-thorns starfish, are the most likely candidates as coral disease vectors (Antonius and Riegl 1997; Nugues and Bak 2009; Nicolet et al. 2013; Katz et al. 2014). While multiple studies have reported that the feeding scars of crown-of-thorns starfish can become the origin of coral infections, no experiment has yet demonstrated COTS to actively carry coral pathogens from one colony to the next. Except in the case of trematodiasis, all corallivores experimentally proven to transmit coral diseases are invertebrates: *Hermodice carunculata* (Polychaeta), *Cyphoma gibbosum* (Gastropoda), *Coralliophila abbreviata* (Gastropoda), *Drupella* sp. (Gastropoda)

Table 3.1: List of peer-review publications aimed at testing the effect of potential vectors on coral disease transmission; listed by main finding, disease type, vector organism, pathogen species, transmission mechanism and source.

Finding	Disease	Vector	Pathogen	Mechanism	Source
Vector transmitted parasite	Trematodiasis	*Chaetodon multicinctus*	*Podocotyloides stenometra*	*P. stenometra* has a complex life cycle involving a molluscan first intermediate host, *Porites* coral as the second intermediate host, and coral-feeding fish as the final host	Aeby 1991, 1998, 2002 2003, 2007
Pathogen detected within the vector's body	*Vibrio shiloi* bleaching	*Hermodice carunculata*	*Vibrio shiloi*	Worms contained viable *V. shiloi* bacteria and transmitted bleaching to healthy *Oculina patagonica*	Sussman et al. 2003
	Aspergillosis	*Cyphoma gibbosum*	*Aspergillus syndowii*	*A. syndowii* was found to survive through the digestive track of the snail. Viable spores and hyphae in snail feces.	Rypien and Baker 2009
	Acroporid serratiosis	*Coralliophila abbreviata*	*Serratia marcescens*	Bacterial strains from *C. abbreviata* successfully infected *Acropora palamata* in aquaria	Sutherland et al. 2011
Vector transmitted disease in controlled laboratory experiments	Unknown disease	*Coralliophila abbreviata*	Unknown	Snails feeding on infected colonies transmitted disease to healthy nubbins	Williams and Miller 2005
	White band disease	*Coralliophila abbreviata*	*Vibrio* and *Rickettsiales* bacteria	Snails collected from the field transmitted WBD to healthy nubbins in aquaria	Gignoux-Wolfsohn et al. 2012
	Brown band disease	*Drupella* sp.	*Philaster guamensis*	Snails collected on infected colonies in the field transmitted BrB to healthy nubbins in the laboratory	Nicolet et al. 2013
Correlation between disease onset and either presence of or predation by vector	Coral diseases	*Drupella cornus*	Various	Correlation between abundance of snail and diseases prevalence	Antonius and Riegl 1997
	Unknown disease	*Phestilla* sp.	Unknown	One nudibranch was placed on 7 coral fragments and progressive coral tissue mortality followed predation	Dalton and Godwin 2006
	Unknown disease	*Hermodice carunculata*	Unknown	*H. carunculata* commonly observed feeding on disease margin	Miller and Williams 2007
	Coral diseases	Chaetodontids	Various	Correlation between chaetodontids density and coral disease prevalence	Raymundo et al. 2009

	Black band, brown band disease	Chaetodontids	*Philaster guamensis,* Bacterial consortium	*Chaetodon aureofasciatus, C. baronessa, C. lunulatus, C. plebeius* and *C. trifascialis* selectively targeted disease lesions over adjacent healthy coral tissues.	Cole et al. 2009, Chong-Seng et al. 2011
	Brown band disease	*Acanthaster planci*	*Philaster guamensis*	Feeding scars of crown-of-thrones starfish became the origin of BrB infections	Nugues and Bak 2009, Katz et al. 2014
	Porites white syndrome	*Coralliophila violacea*	Unknown	Feeding scars of *C. violacea* became the origin of PWS.	Raymundo et al. 2016
Corallivore not found to transmit disease in controlled experiments	Black band disease	*Chaetodon capistratus*	Complex bacterial consortium	Feeding behaviour of the fish did not increase *Phormidium corallyticum* transmission	Aeby and Santavy 2006
	White band disease	*Coralliophila caribaea*	*Vibrio* and *Rickettsiales* bacteria	*C. caribaea* feeding behaviour did not transmit WBD in aquarium-based infection experiment	Gignoux-Wolfsohn et al. 2012
	White syndrome	*Cyamo melano-dactylus*	Unknown	Transplanting crabs from infected colonies onto healthy corals does not result in disease transmission.	Pollock et al. 2013
	Brown band disease	*Chaetodon aureofasciatus*	*Philaster guamensis*	The fish neither aided nor hindered the transmission of BrB from infected to uninfected corals	Nicolet et al. 2013

3.5 Discussion

This study demonstrates that *Drupella* snails transmit the virulent coral disease, brown band disease (BrB), both immediately, and for at least 24 h, after feeding on diseased coral nubbins. The vector potential of *Drupella* declined, although not significantly, following 12h and 24h delays between disease exposure and introduction to healthy nubbins. Survival of BrB ciliates within the snail for 24 hours, and potentially longer, would facilitate disease transmission both within and between coral colonies. Considering the rapid progression rates reported for BrB (over 4 cm day^{-1}; Katz et al. 2014), the ease with which *Drupella* transmits the disease, and the potentially high abundance of *Drupella* spp, (single aggregations up to 3000 snails per m^2; Moyer et al. 1982), the disease is capable of causing substantial coral mortality. The potential of vector-transmitted pathogens to drive significant population declines and even extinctions of host species suggests that managing *Drupella* outbreaks will be crucial to controlling potential disease outbreaks in coral populations. In contrast, *Drupella* did not transmit BBD and butterflyfish did not transmit either disease in the laboratory. Consistent with the laboratory study, predation by butterflyfish in the field experiment had no effect on the incidence of new infections.

In contrast to BrB, I found that *Drupella* snails did not transmit BBD, most likely because of the complexity of the BBD pathogenic community. Whereas BrB is caused by one to two species of ciliates (Bourne et al. 2008; Lobban et al. 2011; Sweet and Bythell 2012), BBD is characterised by a complex microbial community that develops over time from a cyanobaterial patch to a complex polymicrobial BBD mat (Sato et al. 2010, 2016). Hence, *Drupella* might not have the potential to carry all of the required microbes, in the right proportions, to establish BBD in a new host. While BBD is prevalent on reefs around the world, experimental and observational studies conducted with a range of potential vectors (*Drupella* sp*., Chaetodon capistratus*) have never found BBD to be vector transmitted (Williams and Miller 2005; Aeby and Santavy 2006; Nicolet et al. 2013; present study). Although one study suggested that *Chaetodon* butterflyfishes (specifically, *C. capistratus*) contribute to transmission of BBD (Aeby and Santavy 2006), that

study reported transmission in the presence of fishes, whether or not fish had access to nubbins and, therefore, does not directly confirm these fish to be vectors of the disease. Direct transmission of BBD via corallivory is thus unlikely, potentially due to the complexity of the microbial community causing BBD.

A synthesis of all results from both field and laboratory experiments demonstrates that corallivorous butterflyfishes play little or no role in the transmission of either BBD or BrB. This conclusion is supported by aquarium experiments, where none of the experimental corals in butterflyfish treatments became infected. Even under high predation pressure, associated with four fishes feeding directly on both diseased and healthy nubbins for six consecutive days, corallivorous fishes were never found to initiate BBD or BrB. Similarly, in field experiments, I found no difference in the proportion of new infections between caged *versus* uncaged coral nubbins. This suggests that corallivorous butterflyfishes are ineffective vectors of coral diseases, which is supported by previous studies that found that butterflyfish feeding behaviour did not increase either BrB or BBD transmission rates (Aeby and Santavy 2006; Nicolet et al. 2013). However, chaetodontids do play an indirect role in the dynamics of one parasite infection, *Porites* trematodiasis, by being an intermediate host in the life cycle of the trematode (see Aeby 1991, 1998, 2002, 2003, 2007). For infectious diseases like BrB and BBD, however factors such as environmental conditions or inherent variability in susceptibility among corals are likely to influence transmission rates far more than the presence or absence of corallivorous fishes.

The inability of polyp-feeding butterflyfishes to successfully transmit pathogens directly could be related to their specialised feeding mode, as these fishes rarely remove enough tissue to expose coral skeletons (Cole et al. 2008). Many studies have emphasised the role of deep tissue injury and exposed skeleton in the spread of diseases, particularly BrB (Nugues and Bak 2009; Nicolet et al. 2013; Katz et al. 2014), BBD (Aeby and Santavy 2006) and skeletal eroding band (SEB: Page and Willis 2008; Lamb et al. 2014). In aquarium experiments testing for coral

disease transmission, only corals with injuries exposing underlying skeleton became infected, regardless of experimental setting or disease type (BrB: Nicolet et al. 2013; Katz et al. 2014; SEB: Page and Willis 2008; BBD: Aeby and Santavy 2006). These results lead to the conclusion that corallivores which inflict deep feeding scars, such as those caused by many invertebrates, are better candidates than butterflyfishes as vectors of coral diseases. Additional studies quantifying the depth of feeding scars from different invertebrate and vertebrate corallivores, and verifying disease transmission, are needed to test this hypothesis.

A review of the coral disease literature highlights the paucity of confirmed vectors. Only three studies have detected coral disease pathogens within the bodies of vectors (Sussman et al. 2003; Rypien and Baker 2009; Sutherland et al. 2011), and three additional publications have demonstrated vector transmission in controlled experiments (Williams and Miller 2005; Gignoux-Wolfshin et al. 2012; Nicolet et al. 2013; Table 3.1). To date, only four vectors have been confirmed to transmit a total of six coral pathogens (Sussman et al. 2003; Williams and Miller 2005; Rypien and Baker 2009; Sutherland et al. 2011; Gignoux-Wolfsohn et al. 2012; Nicolet et al. 2013). Of the four confirmed coral disease vectors, the fireworm *Hermodice carunculata* acts as a winter reservoir and a summer vector of *Vibrio shiloi*, a bacterium causing bleaching (Sussman et al. 2003). *H. carunculata* was also observed feeding on disease lesion during an outbreak of a 'white disease' in the Caribbean (Miller and Williams 2007). The remaining three vectors are corallivorous gastropods (Table 3.1). The Caribbean snail *Coralliophila abbreviata* (accepted species name now *C. galea*) has successfully transmitted various bacteria responsible for acroporid serratiosis (*Serratia marcescens*), white band disease (*Vibrio* and *Rickettsiales* bacteria) and an unknown type of white syndrome (Williams and Miller 2005; Sutherland et al. 2011; Gignoux-Wolfsohn et al. 2012). Interestingly, a close relative, *Coralliophila caribaea*, was unable to transmit white band disease in the same laboratory conditions (Gignoux-Wolfsohn et al. 2012). Feeding scars of a snail from the same genus, *Coralliophila violacea*, were also found to be the origin of *Porites* white syndrome, this time in the Indo-Pacific (Raymundo et al. 2016). Another mollusc, *Cyphoma gibbosum*, is a

successful vector of the fungus *Aspergillus syndowii* that affects gorgonian corals (Rypien and Baker 2009). Finally, *Drupella* spp. transmitted BrB in a precursor to this study (Nicolet et al. 2013), as well as in the current study, and is the only confirmed vector in the Indo-Pacific. Although only 4 invertebrate vectors have been confirmed, a comprehensive review identified 314 invertebrate species that feed directly on coral tissue, including 4 *Drupella* species, 10 *Coralliophila* species and 12 echinoderm species (starfish and sea urchins) (Stella et al. 2011). Many of these species leave deep feeding scars and, considering the limited research on coral disease vectors and the extensive number of corallivorous invertebrates, the importance of vectors in coral disease transmission is likely underestimated.

Frequency-dependant diseases in terrestrial ecosystems have had devastating consequences for their host populations, driving many species to extinction (e.g., van Riper et al. 1986, McCallum 2012). Most coral diseases affect multiple coral species (Weil 2004; Willis et al. 2004), and vectors can maintain pathogen loads independently of host populations (e.g., Sussman et al. 2003). Consequently, coral disease have the potential to inflict significant losses on coral populations because pathogens are not constrained to decline as host density declines. Managing diseases in the natural environment requires knowledge of disease transmission mechanisms, aetiology and pathogenesis, but such knowledge is currently limited for coral diseases (Porter 2016). Even when the pathogen has been identified, diseases affecting corals are challenging to manage or treat directly due to the complexity of the holobiont and the nature of the marine environment. The results of this study show that invertebrate vectors that create relatively deep feeding scars are the most likely vectors of coral diseases, whereas disease transmission by corallivorous butterflyfishes would occur only rarely on coral reefs. Effective control of invertebrate corallivores (e.g., Rivera-Posada and Pratchett 2012) that are known to either cause (*Drupella* sp.) or facilitate (crown-of-thorns starfish) disease transmission would help to minimise the spread and prevalence of coral diseases.

CHAPTER 4 – ANALYSES OF SPATIOTEMPORAL DISTRIBUTIONS OF FOUR CORAL DISEASES AFFECTING *ACROPORA* SPECIES REVEAL DIFFERING MODES OF TRANSMISSION AND LEVELS OF CONTAGIOUSNESS

4.1 Abstract

Spatial and temporal distributions of disease prevalence and incidence provide insights into the cause, origin, and transmission mechanisms of diseases. In this study, spatiotemporal patterns in the occurrence of four major diseases affecting corals on the Great Barrier Reef were examined through repeated surveys of quadrats over the course of 1.5 years. For black band disease (BBD) and skeletal eroding band (SEB), incidences of disease were mostly aggregated (i.e., in 78% and 66% of cases, respectively), suggesting that these two diseases are contagious. In contrast, random spatial distributions found for white syndromes (WS) in more than 75% of analyses (n = 9) suggest that this group of diseases is not contagious. The lack of a clear pattern in the distribution of brown band disease (BrB) in analyses of quadrats over the 1.5 years suggests that multiple interacting factors culminate in BrB disease signs. Furthermore, the spatial distribution of most diseases (WS, SEB and BrB) was independent of the distribution of feeding scars created by *Drupella* snails and crown-of-thorns starfish. BBD, however aggregated around feeding scars in 43% of cases, suggesting that physical injury of the coral host might play a role in the transmission of BBD. The lack of relationship between scars and other diseases (WS, SEB or BrB) might result from either a lack of impact of injury on disease onset, or an absence of the diseases agent(s) at the time the injuries were created. Understanding which diseases are contagious, and which are facilitated by tissue injury, is critical to managing infectious diseases to prevent them from becoming key drivers of coral loss in the future.

4.2 Introduction

Quantifying the spatial distributions of diseases, as well as their prevalence and incidence, is fundamental to establishing the cause(s), origins and spread of disease (Mayer 1983). Concurrent knowledge of temporal dynamics in the patterns of disease occurrence, particularly when combined with knowledge of ambient physical-environmental factors, provides further insights into disease origins, transmission mechanisms, rate of pathogen spread through populations, and epidemic potential (reviewed in McCallum et al. 2003; Cottam et al. 2013). For example, analyses of spatiotemporal patterns of Ebola cases spread across eight countries enabled researchers to track the origins of the 2014 outbreak to a two-year-old boy, who died in the village of Meliandou in Guinea most likely from contact with infected wild bats (World Health Organization 2015, 2016). Spatiotemporal data can also be used to monitor spill-overs of disease from domestic to wild animal populations (and vice versa), and can provide clues about potential reservoirs of pathogens in different host species. For instance, knowledge of the location and timing of Canine Distemper Virus (CDV) incidence and spread, indicated that a viral infection affecting lions in Serengeti National Park originated in dog populations of nearby villages, but that other wild carnivores acted as disease reservoirs after dog populations were vaccinated (Viana et al. 2015).

In spatial analyses, distributions of disease cases are often illustrated as point patterns on maps, enabling patterns to be characterised as random, aggregated or uniform at specified spatial scales. Statistical analyses of such spatial patterns have been used to test hypotheses about pattern-generating processes as far back as the late 1940s (Moran 1948, Geary 1954), and early disease mapping since the cholera outbreak in London in 1854 (Snow 1854). Spatial clustering over short time scales and small spatial scales can be an important indicator that an infectious disease is contagious, here defined as a disease capable of being transmitted from one individual to another (Marshall 1991, Dorland 2000). Combined analyses of spatio-temporal patterns in disease and environmental data can indicate whether infections develop as a consequence of an environmental stressor affecting the virulence of a commonly present agent. For example,

statistical analysis of the distribution of New Zealand kelp die-off indicated that mass mortalities were attributable to light deprivation associated with plankton blooms rather than a contagious disease (Cole and Syms 1999). Such examples highlight the critical importance of analysing spatial distributions of disease cases to elucidate their aetiologies.

The dynamics of diseases affecting marine organisms are very different from those affecting terrestrial plants and animals (McCallum et al. 2004), a factor that has significant implications for spatial distributions of marine diseases. Firstly, animal diversity is much greater in the marine environment (34 phyla) than in terrestrial environments (9 phyla), creating the potential for more diverse host-parasite interactions and multiple reservoirs for pathogens (McCallum et al. 2004). Secondly, long-distance dispersal of propagules is prevalent in the marine realm, thus marine populations are often more 'open' than terrestrial ones (Kinlan and Gaines 2003). This is important because the spatial scales at which populations might be considered open or closed can differ for pathogens and hosts, and such spatial dynamics influence the spread of diseases within and between areas (Kuris and Lafferty 1992). Lastly, rates of spread of marine diseases are extremely rapid, reaching up to 10,000 km $year^{-1}$, whereas only a few epidemics have reached rates of spread of 1,000 km $year^{-1}$ in terrestrial systems (McCallum et al. 2003). Given these differences between terrestrial and marine environments, additional studies are required to assess the extent to which spatial analyses can contribute to understanding disease transmission in the ocean.

In marine environments, coral diseases have increased dramatically over the last 40 years, becoming important contributors to coral loss (Harvell et al. 2002; Precht et al. 2016). To date, over 25 coral diseases have been identified worldwide (Weil 2004, Willis et al. 2004; Woodley et al. 2016), however the aetiology, transmission patterns and ecology of most diseases are largely unknown. Spatial patterns in the incidence or prevalence of most coral diseases are largely unquantified, with most such studies focusing on black band disease (BBD: Edmunds 1991, Kuta and Richardon 1996, Zvuloni et al. 2009), and only a few quantifying patterns for

other diseases (summarized in Table 4.1). In a study of BBD on mound-shaped corals, new cases were found to cluster around pre-existing infections, suggesting that BBD is indeed contagious (Zvuloni et al. 2009). The absence of potential disease vectors in the study area suggested that pathogens are most likely transmitted via the water column over short distances. Whereas tracking BBD cases is relatively straightforward for small, discrete mound-shaped colonies, it is inappropriate for large thickets of staghorn corals that do not have distinct boundaries between colonies, such as in the genus *Acropora*, one of the most susceptible groups of corals on the GBR (Willis et al. 2004). Other studies have used field-based surveys, often conducted along replicate belt transects, to deduce spatial patterns in the occurrence of diseases on staghorn corals at a scale of 10s to 100s of meters (e.g., Lentz et al. 2011). However, such studies yield limited conclusions about disease contagiousness because transmission is likely to occur at smaller spatial scales (< 5 m). In addition, coral reefs are spatially complex environments where coral colonies are, themselves, clustered on hard substratum around patches of sand and other topographic features. Consequently, analytical methods that account for the potentially aggregated spatial distribution of hosts are required to tease apart whether clustering of disease cases represents contagiousness or simply reflects the underlying spatial distributions of hosts. Such analyses have only rarely been applied to studies of coral diseases (see Jolles et al. 2002; Zvuloni et al. 2009, 2015; Muller and van Woesik 2012 Table 4.1).

Table 4.1: Overview of articles assessing the spatial distribution of coral diseases, presented here by disease, analytical method, main finding and source.

Disease	Method	Main finding	Contagious	Source
Acropora white syndrome	Three 40 m transects in which quadrats (1 m^2) were photographed. To analyse spatial aggregation, the number of affected colonies per transect was compared with a random (Poisson) distribution.	The frequency distribution of white syndrome colonies within each transect (40 m^2) did not differ significantly from a random distribution.	NO	Roff et al. 2011
Aspergillosis	Location, height, and disease status of all sea fans were recorded in a 10 x 20 m quadrat. The Ripley's K function used took the underlying distribution of sea fans as its null distribution to find disease distribution independent of host distribution.	The sea fan distribution was aggregated over a wide range of spatial scales. Diseased fans were significantly more aggregated than the total fan population at a spatial scale of 2 - 4.5 m at 1 site out of 3 study sites.	MAYBE	Jolles et al. 2002
Black band disease	Surveys using 10 m radius circle (total area 314 m^2). The frequency distribution of the number of surveys with 0-5 BBD colonies was tested for goodness of fit to a random (Poisson) distribution.	The frequency distribution of the number of surveys with 0-5 BBD colonies was not significantly different from a Poisson distribution.	NO	Edmunds 1991
	Follows Edmunds (1991). The frequency distribution of the number of replicate sites with 0 to 4 or more BBD infections was tested for goodness of fit to a random (Poisson) distribution.	The frequency distribution of the number of replicate sites with 0 to 4 or more BBD infections was significantly different from a Poisson distribution. Indices of dispersion indicate a clumped distribution.	YES	Kuta and Richardson 1996
	One 10x10 m plot. The Ripley's K index was used as well as a modified Ripley's K function to test whether newly infected colonies (NIC) develop in proximity of previously infected colonies (PIC)	Corals infected by BBD during the first season were found to be aggregated on small spatial scales of 0.2–1.2 m. Additionally, NICs appeared to be significantly closer to PICs than would be expected by chance.	YES	Zvuloni et al. 2009
Dark spot disease	Fifteen permanent belt transects of 2 x 30 m. Every coral colony was identified and examined for DSD. Paired-Quadrant Variance method (PQV -could be influenced by the spatial distribution of the coral) and the Nearest-Neighbor sampling method used to assess the distribution of the DSD.	The PQV analysis shows evidences of a clumped distribution of the disease. The Nearest-Neighbor analysis for the affected ramets suggested a clumped distribution of the disease among ramets within a genet on the reef.	YES	Gil-Agudelo and Garzón-Ferreira 2001
	10 x 10 m quadrats at randomly distributed points	Among sites, dark spot disease clustered only in one	NO	Muller and

	within different habitat types in three locations. Total of 253 100 m^2 of reef area surveyed. Ripley's K function used for between sites comparison and Euclidean distance used for within sites comparisons.	time point, with clusters ranging from 1.5 to 3 km in radius. No clusters detected within sites.		van Woesik 2012
White plague	Morisita's index of dispersion	Clumped distribution of infected colonies	YES	Richardson et al. 1998
	10 x 10 m quadrats at randomly distributed points within different habitat types in three locations. Total of 253 100 m^2 of reef area surveyed. Ripley's K function used for between sites comparison and Euclidean distance used for within sites comparisons.	White-plague disease only clustered once, with clusters ranging from 1 to 4.5 km in radius. Analysis could not be run for within sites comparisons.	NO	Muller and van Woesik 2012
	Spatiotemporal index based on Ripley' s K – function tests the spatial relations between newly-infected corals (NICs) and previously infected corals (PICs).	NICs appeared to form aggregations around PICs over distance scales of up to 4.5 m	YES	Zvuloni et al. 2015
White band disease	Two sets of spatial data: a 'transect-level' data set of WBD presence or absence and a 'colony-level' data set that weighted each transect by the number of *Acropora palmata* present. Ripley's K and 'Difference function' (D) used to examine the spatial distribution of WBD with respect to environmental heterogeneity caused by the presence of the underlying coral population.	The 'transect-level' analyse revealed spatial aggregation in all transects containing WBD. The 'colony level' data revealed that colonies with WBD present had fairly random spatial distributions at distances<2.1 km, becoming more dispersed at distances >2.1 km. When compared with the underlying corals distribution, the spatial distribution of the WBD colonies was significantly more dispersed.	NO	Lentz et al. 2011
Yellow band disease	Individual locations were displayed as XY coordinates. Use of a generalization of the Ripley K function to allow for heterogeneous point processes	Coral colonies (diseased and healthy) were highly spatially clustered, compared with expected complete spatial randomness. However, within the overall clustered spatial distribution of colony hosts, YBS affected distribution was less clustered than expected.	NO	Foley et al. 2005
	10 x 10 m quadrats at randomly distributed points within different habitat types in three locations. Total of 253 100 m^2 of reef area surveyed. Ripley's K function used for between sites comparison and	Among sites, yellow band disease clustered only in two time points, with radius between 1 and 4 km. Whitin sites, individuals of *Montastraea annularis* spp. complex with yellowband disease were significantly closer than the average distance between	?	Muller and van Woesik 2012

	Euclidean distance used for within sites comparisons.	all colonies within the species complex

The presence, body size, and mobility of potential disease vectors can also influence spatial patterns in the distribution of disease cases within a population, as well as affecting overall levels of disease prevalence among populations, however the role of vectors has never been explicitly considered in spatiotemporal analyses of coral diseases. As shown in Chapter 3, there are four known invertebrates that serve as vectors for five coral diseases: the fireworm *Hermodice carunculata* (Sussman et al. 2003), and the gastropods *Cyphoma gibbosum* (Rypien and Baker 2009), *Drupella* sp. (Nicolet et al. 2013, Chapter 3), and *Coralliophila abbreviata* (Wiliams and Miller 2005, Sutherland et al. 2011, Gignoux-Wolfsohn et al. 2012) are vectors for *Vibrio corallilyticus* induced bleaching, aspergillosis, brown band disease, acroporid serratiosis and white band disease, respectively. In the Indo-Pacific region, only the gastropod *Drupella* has been identified as a vector of coral diseases (Nicolet et al. 2013, Chapter 3). While these studies have confirmed that corallivorous vectors actively transmit pathogens to new hosts within coral populations, other studies have identified an array of organisms that can enhance disease spread through indirect processes. These "disease enhancers" promote pathogenic infections by weakening the host and/or creating an entry point for pathogens (Raymundo et al. 2009). For example, the crown-of-thorns starfish, *Acanthaster planci*, is known to produce large feeding scars that may represent initiation sites for brown band infections (Nugues and Bak 2009, Katz et al. 2014). Regardless of whether these invertebrate corallivores directly transmit disease pathogens to new hosts, or simply increase the susceptibility of corals to infection, they are expected to influence disease spatiotemporal distributions at small spatial scales and, therefore, should be considered in studies of disease dynamics.

Herein, I quantify spatiotemporal patterns in the occurrence of four major diseases affecting corals on the Great Barrier Reef through repeated surveys of quadrats over the course of 1.5 years. My goal was to use analyses of spatiotemporal patterns in disease distributions to gain new insights into the aetiologies of black band disease (BBD), brown band disease (BrB), skeletal eroding band (SEB), and white syndromes (WS). To account for the spatial distributions of coral hosts in these analyses, only live coral colonies were used to define space

available for disease development. Given that there are few barriers to disease transmission in marine environments and heterogeneity in environmental conditions is limited at the spatial scale of these analyses, I assumed that distributions of disease cases within the host populations at this spatial scale would provide an indicator of the contagiousness of the disease. Feeding scars of corallivores, specifically the gastropod *Drupella* and *Acanthaster planci* (crown-of-thorns starfish), were also recorded to explicitly account for their feeding activities in spatiotemporal analyses of these four diseases.

4.3 Methods

4.3.1 Study site

This study was conducted over 18 months, with sampling conducted in four distinct time periods; March-June 2013, October-November 2013, January-March 2014, and July-August 2014. Sampling was undertaken at Lizard Island (14˚40'08''S 145˚27'34''E), a mid-shelf island on the northern Great Barrier Reef (GBR), Australia. Lizard Island is an extensive and highly diverse reef system, with a lagoon and a mosaic of patch reefs on the sheltered (north-west) side of the island. In March 2013, I completed extensive surveys on 12 sheltered reefs, including exposed reef fronts, lagoon and back reef habitats, to locate sites with high prevalence of coral diseases. Disease prevalence was highest at three sites on Trawler reef, a shallow reef in the northern part of the lagoon, and two sites at Horseshoe Reef, a shallow reef on the southwest part of the island (Figure 4.1). These 5 sites had high coral cover, mainly of corymbose and staghorn species of *Acropora*, and were subsequently selected for the field experiment.

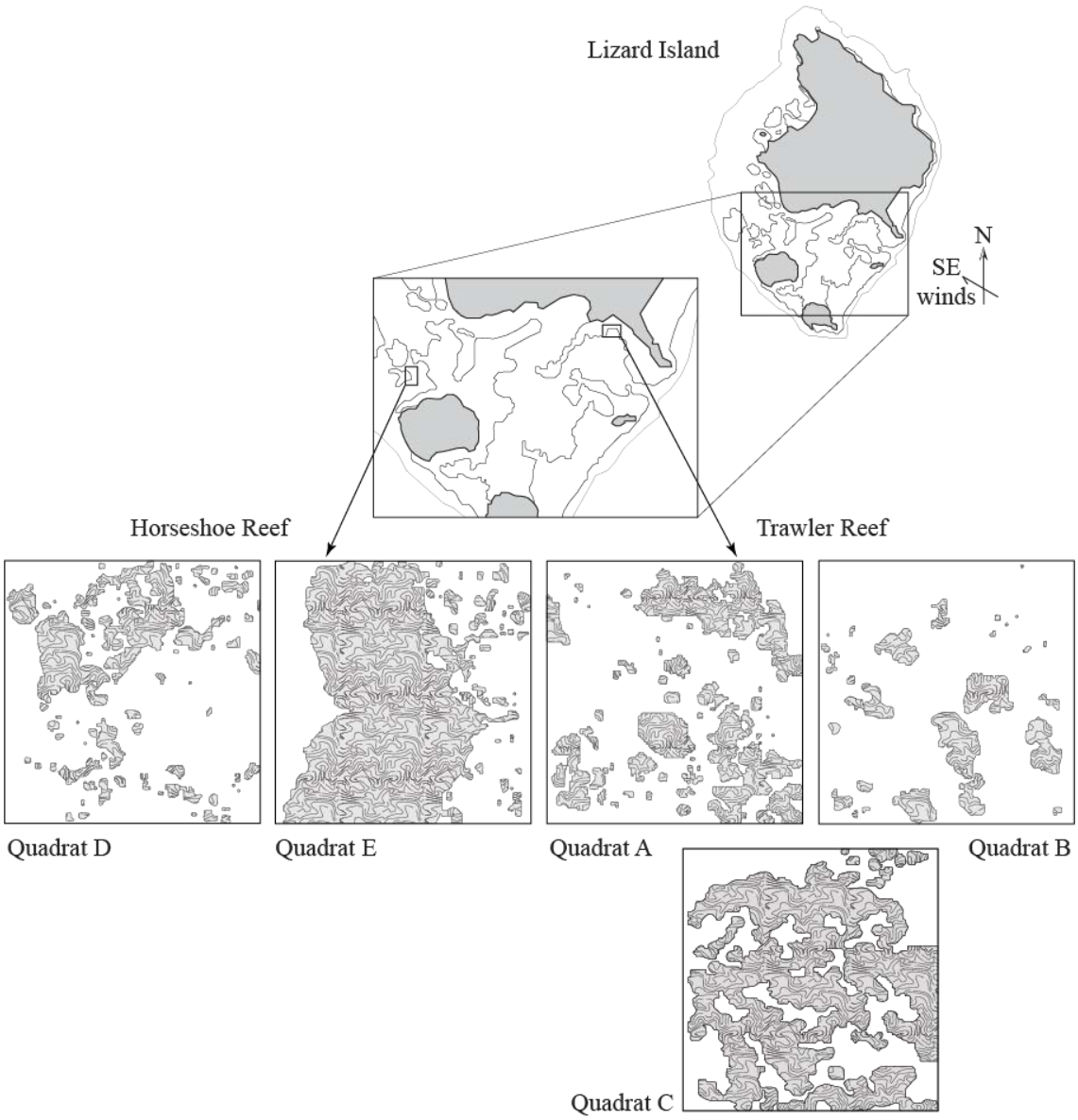


Figure 4.1: Location of study sites at Trawler and Horseshoe reefs, where five quadrats were established. Grey shading inside quadrats represents living corals, predominantly branching Acropora.

4.3.2 Reef Mapping: Spatial distribution of coral diseases

To explore spatiotemporal dynamics for each of the four coral diseases (BBD, BrB, SEB, WS), five 10 x 10 m quadrats were established: 3 quadrats on Trawler Reef, and 2 on Horseshoe Reef (Figure 4.1). According to previous studies, 10 x 10 m quadrats are large enough to capture coral-disease clusters (Jolles et al. 2002, Zvuloni et al. 2009, Muller and van Woesik 2012). The locations of all coral colonies and substratum types in each of the quadrats (5 x 100 m^2) were mapped using photography. Pictures were meshed together in Adobe Illustrator and substratum

maps were created by outlining every coral colony (hard and soft), rock, sandy area and the position of giant clams. All corals present in the 5 quadrats (quadrats A to E) were surveyed every 3 days (except when weather conditions did not allow for safe boating and diving activities) within each of the 4 time periods, and an X-Y coordinate was allocated to every infected branch based on the maps. In all 5 quadrats, species of *Acropora* were the most prevalent coral type and were the most susceptible to infection. Disease type and location were recorded over a total of 20 weeks: 7 weeks in autumn (24th of April to 3rd of June 2013), 3 weeks in spring (28th of October to 13th of November 2013), 7 weeks in summer (16th of January to 6th of March 2014), and 3 weeks in winter (23rd of July to 11th of August 2014). Within each sampling period, pictures of every infected branch were taken every 3 days to estimate progression rates (standardized to centimeters per day) using the image analysis program ImageJ (1.44o, Java 1.6.0_29, public domain). Lastly, each branch or colony preyed upon by either *Drupella* snails or crown-of-thorn starfish (COTS) was likewise allocated an X-Y coordinate and surveyed for subsequent disease onset.

4.3.3 Statistical analysis: Spatial analysis of coral disease

Substrate maps for each quadrat were imported into ArcGIS (ArcMap) and transformed into grids. Each grid cell was 10 cm^2 and designated as either "live hard coral" or "other". For each quadrat, only grid cells designated as "live hard coral" were used for disease clustering analyses, as they represented the sum total of space available for disease development (Figure 4.1). The centroid of each grid cell was assigned X-Y coordinates in the same manner as coordinates were assigned to infected branches. Data were then imported into R (Rstudio), where they were used to create a window (see glossary below) for each quadrat. Thus, each quadrat (A to E) had a specific window in R, within which X-Y coordinates of all infected and injured branches were added (as a point pattern) and clustering analysis of disease was conducted using the spatstat package (see R code in Appendix C).

Glossary of modelling terms (based on Baddeley and Turner 2005):

Point Pattern: Is an arrangement of points in an area. Each point represents an object or event (here a diseased branch). A point pattern dataset gives the location (X-Y coordinates) of objects occurring in a study region.

Marks: Points may have extra information, called marks, attached to them. A mark is an "attribute" of the point. A mark variable could be categorical (e.g. species or disease status) or continuous (e.g. tree diameter).

Multitype Point Pattern: Is a point pattern where points have categorical marks, resulting in points of different "types". Here, points in the study area represent the occurrence of different disease types, or the position of potential vectors of diseases.

Window: Is a region in two-dimensional space that represents the study area. Here, the window has been modified to include only areas of hard coral cover.

Intensity: Is the average density of points, from which the expected number of points per unit area can be deduced. Intensity may be constant ('uniform') or may vary within the window ('non-uniform').

Interaction: Is the stochastic dependence between the points in a point pattern. It results from the distance between the points, which defines the observed pattern. We expect dependence to be strongest between points that are close to one another. In a study area, interactions result in one of three distribution patterns: random, regular or clustered.

To characterise spatial patterns in the distributions of BBD, BrB, SEB and WS cases, the Ripley's K index (Ripley 1981, 1987) was used. Ripley's function $K(r)$ is here defined as the expected number of infected corals within a radius *r* from an arbitrary infected coral. Because the area under study (window) is finite, portions of the circles of radius *r* might fall partially outside the window. An edge correction is thus added to the function to reduce bias. Ripley's K, in the spatstat R package is calculated as:

$$\widehat{K}(r) = \frac{a}{n(n-1)} \sum_i \sum_j I(d_{ij} \leq r) e_{ij}$$

where *a* is the area of the window, *n* is the number of data points, and the sum is taken over all ordered pairs of points *i* and *j* in the point process (as per glossary). Here d_{ij} is the distance between the two points, and $I(d_{ij} \leq r)$ is the indicator that equals 1 if the distance is less than or equal to *r* (radius) or 0 if the distance between *i* and *j* is greater than *r*. The term e_{ij} is the edge correction. To assess the spatial distribution of the observed points, the estimate of $\widehat{K}(r)$ is compared with the value of K for a completely random (Poisson) point process, expressed as

$K(r) = \pi r^2$. Values $K(r) > \pi r^2$ suggest spatial clustering, meaning that there are more observed points within a radius *r* than would be expected under random processes alone.

In order for comparisons between observed point patterns and expected random point patterns to be meaningful, tests were conducted using the *Monte Carlo* principle. One thousand simulations of the theoretical K function for a completely random (Poisson) point process were generated inside each modified window. The estimated $\widehat{K}(r)$ for each of these simulations was computed as: $\widehat{K}^{(j)}(r)$ for $j = 1, \ldots, 1000$. These simulations of estimated curves allow for the creation of the pointwise upper and lower confidence envelopes for statistical hypothesis testing:

$$L(r) = \min_j \widehat{K}^{(j)}(r)$$

$$U(r) = \max_j \widehat{K}^{(j)}(r)$$

For any fixed value of *r*, the probability that $\widehat{K}(r)$ lies outside the envelope for the simulated curves is calculated. This enables a test of statistical significance by visualising whether the observed pattern falls within the 95% envelope of the randomly distributed point pattern.

The above function was computed for each disease type (BBD, BrB, SEB, WS) during each field observation period (i.e., Apr-Jun 2013, Oct-Nov 2013, Jan-Mar 2014, Jul-Aug 2014) and for each quadrat (quadrats A to E). All analyses were conducted using the spatstat package in RStudio (Version 3.0.2 – © 2013 RStudio, Inc.), and *Monte Carlo* simulations were generated using the modified window of available space for each quadrat. To generate a *Monte Carlo* simulation appropriate to the observed point pattern, the same intensity (number of points) as the relevant spatiotemporal replicate was used to run the simulation. For example, the point pattern illustrating BBD distribution in the period April to June 2013 in quadrat A had 12 data points. Thus, one thousand *Monte Carlo* simulations for 12 points randomly distributed within

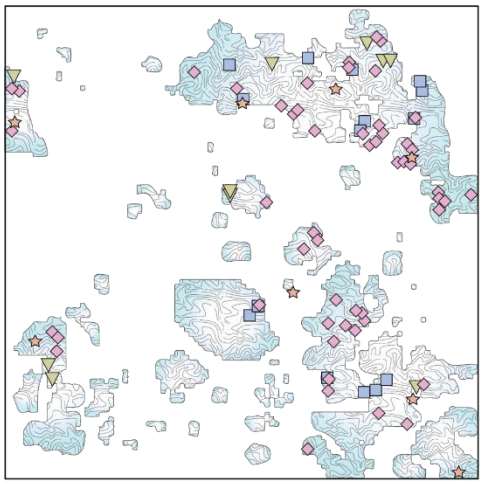
the modified window for quadrat A were generated, and the resulting envelope was tested against the observed point pattern of BBD (see R script in Appendix C).

A slightly modified formulation of $K(r)$ was used to test whether diseases clustered significantly around feeding scars of *Drupella* snails and COTS. The "cross-type" (type *i* to type *j*) K function of a multitype (marked point pattern) point process $K_{ij}(r)$ is derived by counting, for each point of type *i*, the number of type *j* points lying closer than *r* units away. Here, for each feeding scar, the number of infected branches lying closer than *r* units away was calculated. Mathematically, it is defined that $\lambda_j K_{ij}(r)$ equals the expected number of additional random points of type *j* within a distance *r* of a typical point *i*. Here, λ_j is the intensity of the type *j* points, i.e. the expected number of points of type *j* per unit area. If the process of type *i* points are independent of the process of type *j* points, then $K_{ij}(r)$ would equal πr^2. Deviation between the empirical K_{ij} curve and the theoretical curve πr^2 suggests dependence between the points of type *i* and *j*.

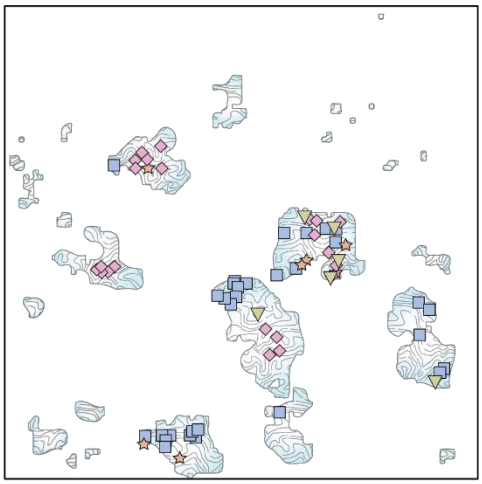
4.4 Results

A total of 592 branches showing signs of infectious diseases (presence of a disease band) or compromised health (tissue loss without band, paling/bleaching, discoloration or presence of foreign bodies such as algae and cyanobacteria) were tagged across all five quadrats and surveyed in four survey periods (20 weeks of surveys in total), spread over a 1.5 year period (March 2013 – August 2014). A subset (23.6%) of these branches (140 out of 592) did not develop a clear disease band and showed no signs of disease progression (non-active), thus they were excluded from subsequent statistical analysis. Of the remaining infected branches, 136 (~30 %) were infected with WS, 122 branches with BBD (~25 %), 104 with SEB (~23 %) and 90 with BrB (~20 %). Additionally, 197 branches showed signs of predation by either *Drupella* snails or COTS.

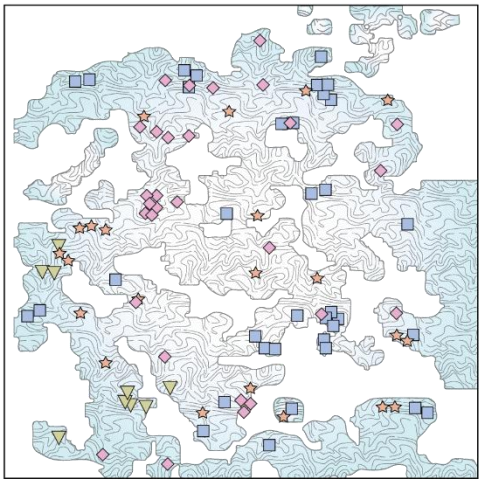
Taken together, there were more occurrences of diseases (all 4 disease types combined) in warmer sampling periods, when seawater temperatures ranged between 26 and 30ºC (i.e., 145 and 122 branches were infected in Mar-Jun 2013 and Jan-Mar 2014, respectively), than in cooler sampling periods when seawater temperatures were between 24 and 27ºC (98 and 87 branches were infected in Jul-Sep 2014 and Oct-Nov 2013, respectively). BBD was especially prevalent in warmer months (62 branches infected out of 122 in the first Mar-Jun field trip) compared with cooler (Jul-Sept) months (lowest number of BBD cases recorded: 16 out of 122 branches). Highest prevalence of WS was also recorded during the Mar-Jun trip (43/136 branches); the lowest prevalence was recorded in Oct-Nov (27/136). In contrast, SEB exhibited the lowest prevalence in the warmer Mar-Jun period (16/104 branches) and the highest prevalence in the cooler Jul-Sep period (35/104). Prevalence of BrB, like that of SEB, was relatively low across all sampling periods, varying from a maximum of 35 branches (out of 90) in Jan-Mar to a minimum of 15 (out of 90) in Oct-Nov. Interestingly, both SEB and BrB mostly occurred in quadrat E and were rarely seen in other quadrats (SEB: 65 infected branches out of a total of 104 branches infected with SEB; BrB: 60/90 in quadrat E).



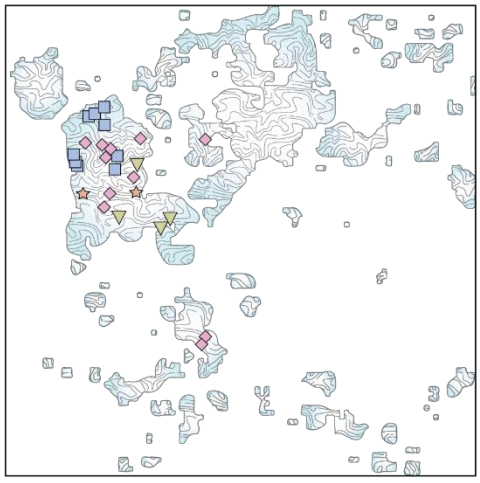
Quadrat A



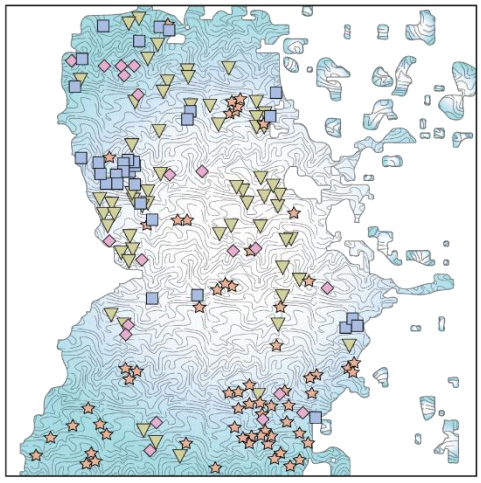
Quadrat B



Quadrat C



Quadrat D



Quadrat E

Figure 4.2: Illustration of the five quadrats with total disease cases observed over the 20 weeks of monitoring. Blue squares represent black band disease cases, green triangles brown band, orange stars skeletal eroding band, and pink diamonds represent white syndrome cases.

Clustering analyses were conducted separately for each of the four diseases (BBD, BrB, SEB and WS), in each quadrat (5 quadrats), and for each of the four sampling periods (March-June 2013, October-November 2013, January-March 2014, and July-August 2014), giving a total of 80 separate analyses. However, due to the low prevalence of most diseases, spatial analyses could only be implemented for a subset of sites and survey periods. Ripley's K function was used to detect spatial clustering only when the number of infected branches per disease was greater than 5 in a given quadrat. As a result, 9 spatiotemporal datasets could be analysed for BBD, 9 datasets for WS, 6 datasets for SEB and 5 datasets for BrB. Due to the size of the modified windows and the edge correction used with Ripley's K, all clustering observed in this study occurred at scales of between 0 and 2.5 m. Cases of BBD were found to cluster within the available space in 7 out of the 9 analyses. All analyses found BBD to cluster between 0 and 1 m, and in some cases, the clustered distribution was retained at scales of up to 2.5 m (3 out of 7 analyses). In contrast, the distribution of WS cases was found to be random (no clustering detected) in 7 out of 9 spatiotemporal datasets. SEB was highly clustered in quadrat E in all sampling periods (4 datasets) but not otherwise (random distributions detected in 2 datasets). Again, all analyses found SEB to cluster between 0 and 1 m, and in some cases, the clustered distribution was retained at scales of up to 2.5 m (2 out of 4 datasets). Lastly BrB showed no consistent pattern, with clustering detected in 2 datasets, but a random distribution observed in 3 datasets.

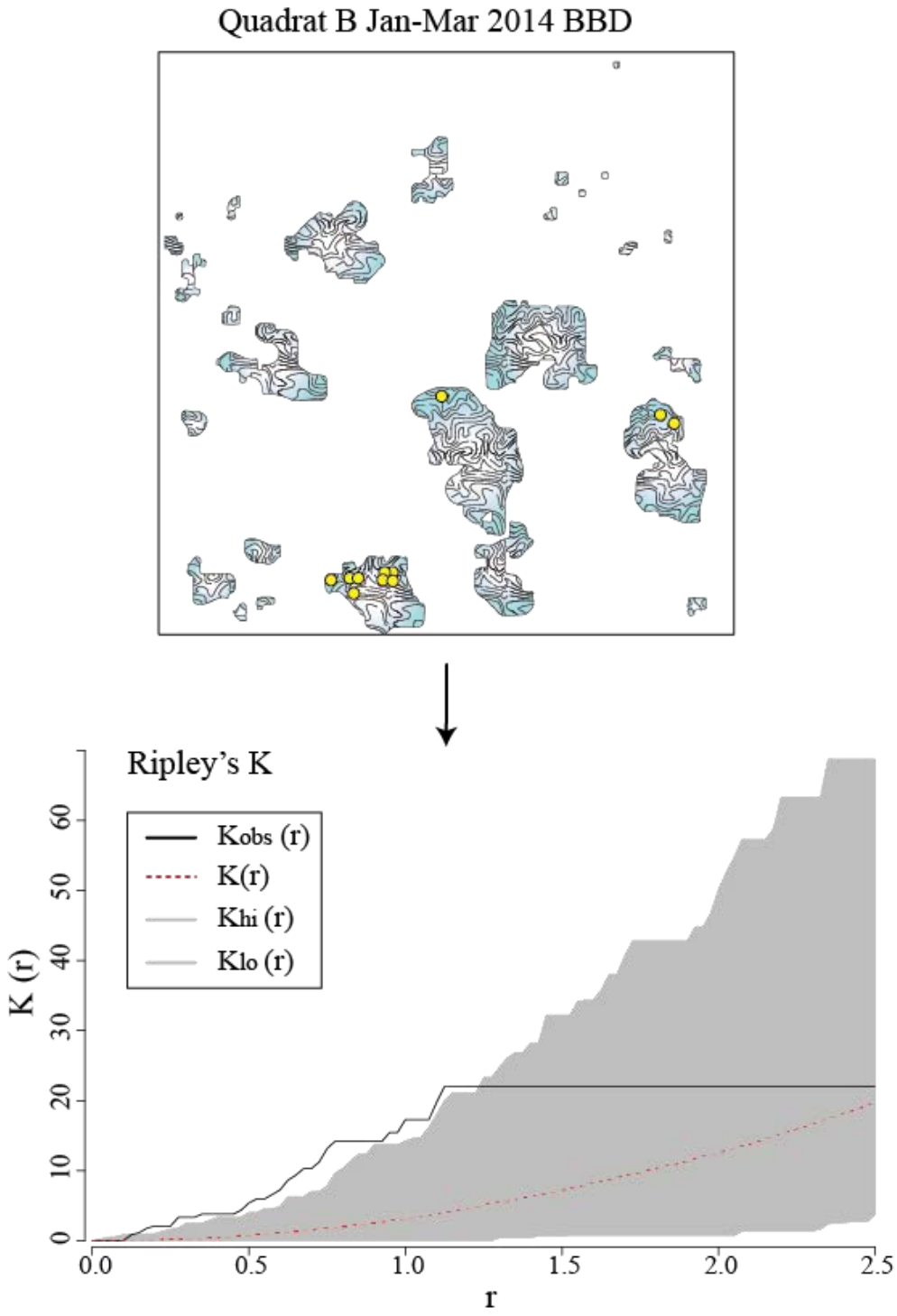


Figure 4.3: An example of results produced for Ripley's K analysis, here for the distribution of black band disease in quadrat B in the January to March 2014 period. Yellow circles represent black band disease (BBD) infected branches. Blue patterns represent coral cover. The red dotted line (Kr) represents the expected shape of K if the distribution of BBD cases was completely random and the grey shaded area shows the 95% confidence interval around the expected values generated by Monte Carlo simulation. The black line (Kobs) is the observed distribution of BBD in this period. Here the spatial distribution is significantly clustered under ~1.2 m (black line above grey area), but random above ~1.2 m (black line inside grey area).

In total, 197 branches showed signs of predation by either *Drupella* snails or COTS. In order to use the K cross function to accurately analyse relationships between feeding scars of corallivores (*Drupella* snails and crown-of-thorns starfish) and disease distributions, both the number of infected branches and the number of branches preyed upon by *Drupella* or COTS had to be higher than 5. As a result, the association between BBD and scars could be investigated in 7 spatiotemporal datasets. Eight spatiotemporal datasets were available for the analysis of the association between scars and WS, 6 for SEB and 5 for BrB. BBD clustered around feeding scars in 3 out of 7 datasets, while the distribution of WS cases was independent of the distribution of scars in all but one dataset (7 out of 8). SEB was never found to cluster around feeding scars (n = 6 datasets), and BrB was only correlated once with scars (1 out of 5 datasets).

Interestingly, in all 3 datasets in which BBD clustered around feeding scars, the disease itself was also found to cluster within the space. However, even in spatiotemporal datasets where feeding scars were absent or in very low numbers, BBD still exhibited a clustered distribution within the space (4 replicates). This suggests that BBD has an inherent contagiousness independent of predation by corallivores. In contrast, WS cases were randomly distributed on the reef in almost 80% of datasets, only associating with feeding scars in one dataset (12.5%). Thus, WS seems unlikely to be a contagious disease. Although SEB clustered within the available space in 66% of cases, it was never associated with feeding scars. Similarly to BBD, SEB occurred independently of whether or not the host was previously preyed upon by a corallivore. Lastly, BrB showed no clear patterns with respect to either disease clustering or association with feeding scars. It is interesting to note however, that the one time BrB was clustered around feeding scars, the disease was also found to cluster within the space. It is thus possible that this aggregated distribution of the disease was more a reflection of the distribution of feeding scars, which are inherently aggregated, than a reflection of the contagiousness of the disease.

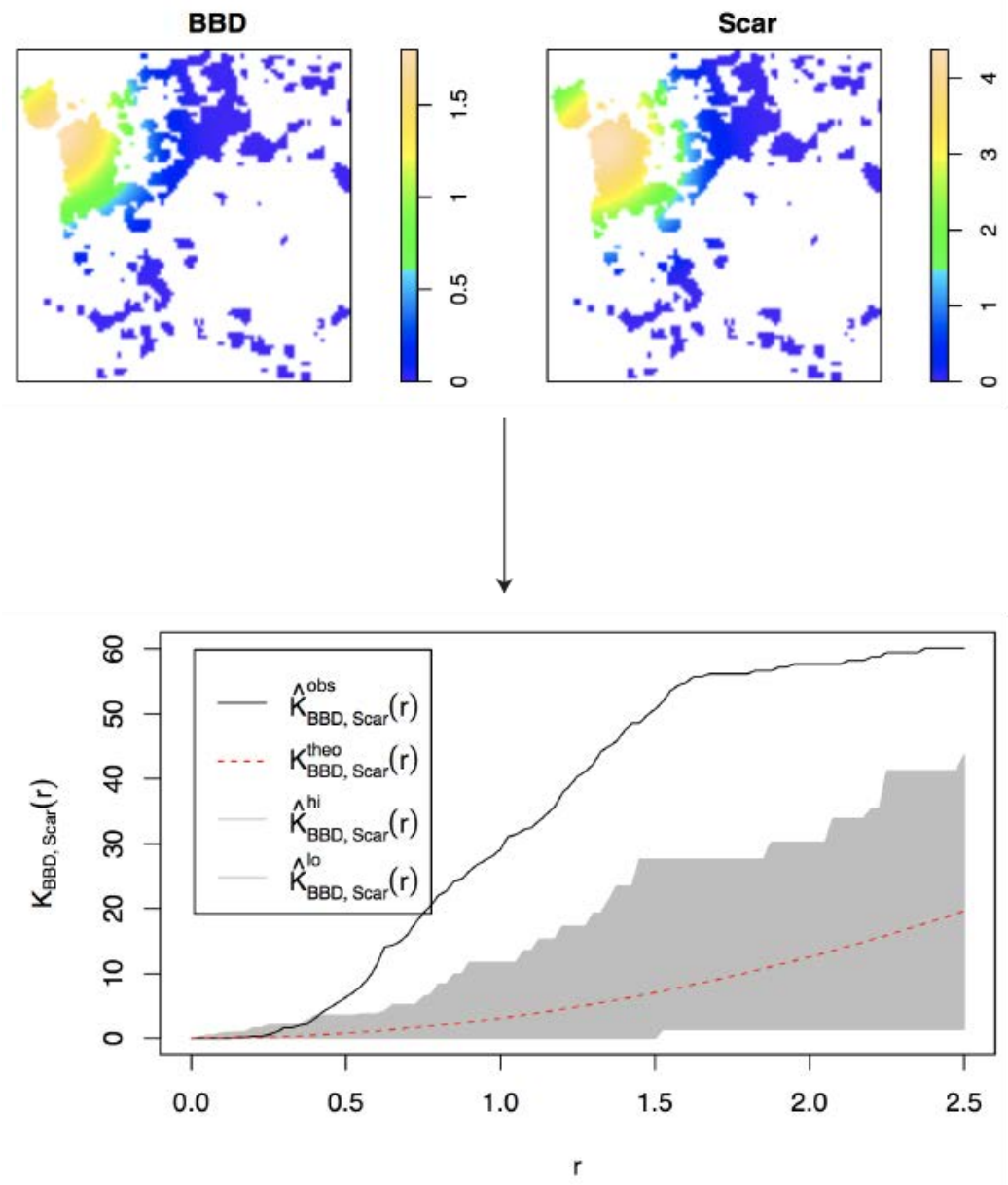


Figure 4.4: An example of results produced for Ripley's K analysis, here for the spatial distributions of black band disease (BBD) cases and feeding scars in quadrat D in the March to June 2013 period. In the top panel, the colour gradient represents the density of either BBD cases or scars, with warmer (yellow) colours representing higher density. All non-white areas depict live coral cover. In the bottom panel, the red dotted line (Kr) represents the expected shape of K if BBD and scars were randomly distributed independently of each other. The grey shaded area shows the 95% confidence interval around the expected values generated by Monte Carlo simulation. The black line (Kobs) is the observed interaction of BBD and feeding scar distributions at this time point. Here the interaction is significant, with BBD spatially clustered around scars at all distances above ~30cm.

4.5 Discussion

Spatiotemporal analyses of four common and virulent coral diseases affecting acroporid populations in the northern Great Barrier Reef revealed differences in their distribution patterns consistent with differences in their contagiousness. While some previous studies have tested for spatial aggregation in the occurrence of multiple coral diseases (see Muller and van Woesik 2012), this is, to the best of my knowledge, the first study to specifically account for the distribution of potential host corals directly in the statistical analysis. The use of Ripley's K within windows modified for the availability of ecologically relevant space (i.e., the presence of live corals), enables robust inferences about the contagiousness of these diseases. Predominantly aggregated patterns for distributions of black band disease (BBD) and skeletal eroding band (SEB) (i.e., in 78% and 66% of datasets, respectively) suggest that these two diseases are contagious. In contrast, random distributions found for white syndromes (WS) in more than 75% of analyses (n = 9 spatiotemporal datasets) suggest that this group of diseases is not contagious. The lack of a clear pattern in the distribution of brown band disease (BrB) in analyses of quadrats over the 1.5 years suggests that a range of factors likely contribute to a web of causation (*sensu* Wobeser 2006) that culminate in BrB disease signs.

Three of the diseases studied here had higher prevalence in austral summer months than in winter; SEB was the only disease that had higher counts in cooler winter months. Earlier studies of coral disease on the Great Barrier Reef (GBR) support this pattern. For example, overall mean prevalence of six diseases was found to be more than 15-fold greater in acroporids in summer compared with winter (Willis et al. 2004). In addition, BBD has been shown to reappear on previously infected colonies in summer and to continue tissue degradation after disease signs had disappeared during winter months (Sato et al. 2009). Many modelling studies have also highlighted a link between high seawater temperatures and WS abundance (Willis et al. 2004, Bruno et al. 2007, Maynard et al. 2011), with some also reporting a potential correlation with bleaching and/or previous mild winter conditions (Heron et al. 2010, Ban et al. 2013). In contrast, seasonal patterns for BrB on the GBR are conflicting, with one study

reporting highest prevalence in winter (Haapkylä et al. 2010) and another reporting highest prevalence in summer (Willis et al. 2004). Higher prevalence of SEB in summer has been reported both on the GBR and in the Caribbean (reviewed in Page et al. 2016), however lack of seasonality has been reported on the southern GBR (Haapkylä et al. 2010). Further studies are required to investigate correlations between environmental factors, coral disease prevalence and virulence, especially for poorly understood diseases such as SEB and BrB (see Chapter 5).

4.5.1 Black band disease (BBD)

Even after accounting for apparent clustering in the distribution of *Acropora* colonies, BBD cases were highly aggregated. Although earlier studies have also reported aggregated distribution patterns for BBD (Kuta and Richardson 1996, Bruckner et al. 1997, Page and Willis 2006, Zvuloni et al. 2009), in all but the latter study (i.e. Zvuloni et al. 2009), the underlying distribution of susceptible corals was not directly taken into account in statistical analyses, hence aggregated disease patterns reported previously could have reflected possible clustered distributions of susceptible coral colonies. In the study by Zvuloni et al. (2009), BBD was surveyed in populations of a massive coral, unlike the current study of thicket-forming species of *Acropora* that have indistinct colony margins, explaining the need to develop the new statistical methods presented here. The present study confirms that BBD behaves as a contagious disease, spreading from one infected colony to neighbouring colonies. Due to edge correction in the Ripley's K function, clustering could only be tested at scales up to 2.5 m, with BBD clustering always occurring at scales from 0 to 1 m, and in some cases, at scales of between 0 to 2 m and over (40% of cases). This is consistent with the scale of spatial clustering of BBD at distances under 2 m reported by Zvuloni et al. (2009).

Previous studies have proposed that the primary mode of transmission is water-borne for BBD, following observations that microbial mats can be dislodged by water motion and transported through the water column (Bruckner et al. 1997, Richardson 2004). This suggestion was supported by a BBD transmission model (Zvuloni et al. 2009), which demonstrated that newly

infected colonies were strongly spatially correlated with the presence of infected colonies close by. Unfortunately, low disease prevalence and incidence prevented investigation of the relationship between newly and previously infected branches in the present study. Evidence presented here, suggests that, in some cases, BBD may be associated with feeding scars caused by either *Drupella* snails or COTS. However, even when feeding scars were absent or in very low numbers, BBD still exhibited clustering (4 spatiotemporal datasets). This suggests that BBD has an inherent contagiousness independent of physical weakening of the coral host, although injuries caused by predation may contribute to the likelihood of infection if the pathogenic consortium is present locally. An experimental study has demonstrated that mechanical injury enhances the susceptibility of corals to BBD when the BBD consortium was placed directly on the injured area, with all injured coral fragments contracting the disease and uninjured corals remaining healthy after exposure to BBD (Aeby and Santavy 2006). However, experimental studies specifically testing whether corallivorous butterflyfish or *Drupella* snails are potential vectors for BBD failed to show increased rates of transmission in the presence of these organisms (see Chapter 3). While corallivores appear to be unable to transport BBD pathogens directly among corals, disruption of a coral's protective barrier by corallivores is likely to increase its susceptibility to disease.

4.5.2 Skeletal eroding band (SEB)

SEB distribution patterns were highly aggregated at Lizard Island, which is consistent with purported contagiousness of this disease suggested by other studies (Winkler et al. 2004, Page and Willis 2008, Lamb et al. 2014, 2015). Notably, my results suggest that the ciliates causing SEB (*Halofolliculina corallasia*) can travel up to 2.5 m to infect new coral colonies. Although SEB was first described from Indo-Pacific reefs in 1999 and its aetiology is now well resolved (Antonius 1999, Antonius and Lipscomb 2001, reviewed in Page et al. 2016), no study has ever investigated spatial patterns in the distribution of this disease. My finding that SEB distribution patterns were not linked to the distribution of feeding scars is supported by an experimental study which showed that *H. corallasia* ciliates readily colonise injuries caused by an airgun but

failed to form band-like aggregations that progressed along coral branches, potentially because corals were otherwise healthy and able to resist infection (Page and Willis 2008). Similarly, ciliates were observed to colonise areas of exposed coral skeleton following feeding by *Drupella*, but did not form the virulent black-grey band characteristic of SEB in a study in the Red Sea (Winkler et al. 2004). Only at high use diving sites, at a location where other environmental factors were contributing to water quality degradation, were injured corals found to be more prone to SEB infection (Lamb et al. 2014). In combination with these previous studies, my results suggest that additional stressors or factors increasing ciliate virulence or weakening disease resistance of the coral host may be required for ciliates to establish after an injury (Page and Willis 2008, Lamb et al. 2014). In the present study, SEB was more prevalent in winter, when feeding scars were few and when environmental factors weakening corals -such as warm temperature anomalies- were absent. In summer, when injuries from feedings scars were most abundant, and when seawater temperature was high, the overall number of SEB cases was low, potentially due to a lack of ciliates in the environment. This might have prevented the detection of a potential correlation between SEB presence and injury.

4.5.3 Brown band disease (BrB)

Spatiotemporal patterns of BrB were unclear, with BrB distributions being aggregated in 40% of datasets but random otherwise. The disease has only been described recently (Willis et al. 2004) and, although it is prevalent throughout the Indo-Pacific (Lamb and Willis 2011, Lobban et al. 2011, Sweet and Bythell 2012, Sweet and Séré 2016), little is known about the dynamics of the disease. BrB was initially attributed to the ciliate *Philaster guamense* (Lobban et al. 2011), later described as *Porpostoma guamense*, and has since also been associated with the presence of *Philaster lucinda* (Sweet and Séré 2016). In an aquarium-based study, inoculation of the ciliates onto non-diseased but injured corals (either mechanically wounded or injured by COTS) resulted in the onset of BrB, whereas none of the uninjured corals became infected after ciliate inoculation (Katz et al. 2014), suggesting that healthy corals can generally resist infection by the ciliates. In the present study, BrB was found to cluster within the space on very rare

occasions, suggesting that the disease is generally not contagious. Moreover, the disease was found to cluster around feeding scars only once, and in analysis of this quadrat for this specific time period, the disease itself was also found to cluster within the space. It is possible that the aggregated disease distribution found in this one analysis was more a reflection of the distribution of feeding scars, which are inherently clustered, than a reflection of the contagiousness of the disease. Alternatively, it is possible that the disease is contagious but the ciliates were not present in the vicinity of feeding scars in other quadrats surveyed in other time periods.

Drupella snails have been shown to be vectors of BrB, even after a 24h delay between exposure to ciliates and contact with corals (Chapter 3; Nicolet et al. 2013). Crown-of-thorns starfish have also been suggested to increase BrB transmission (Nugues and Bak 2009, Katz et al. 2014), but although these studies demonstrate that COTS predation enhances the likelihood of BrB infection, they do not prove that the starfish itself is a vector of the ciliates. Here, BrB was correlated with feeding scars in only one spatiotemporal analysis out of five. However, the low prevalence of this disease may have hindered the ability to detect a potential correlation between feeding scars and BrB. Indeed, during the 9-month period when the experimental quadrats were surveyed, BrB was observed to appear on colonies that had been preyed upon by both *Drupella* snails and COTS (personal observations). When considered as a whole, these findings suggest that BrB is unlikely to infect healthy colonies (hence the lack of aggregation) but may opportunistically infect corals previously weakened by predation or environmental stressors. While BrB is uncommon on a healthy reef with low numbers of *Drupella* snails and COTS, considering the rapid progression rates of BrB (up to 4.5cm day^{-1}; Katz et al. 2014), the disease could present a substantial threat to coral populations affected by corallivore outbreaks or on reefs under high environmental stress (see Pollock et al. 2014). Further investigation of BrB distribution patterns at various scales and the influence of environmental factors on prevalence and virulence of the disease are required to elucidate BrB dynamics on reefs.

4.5.4 White syndromes (WS)

The random distribution patterns found for WS in the majority (78%) of spatiotemporal analyses suggest that the disease is not contagious. This finding is consistent with modelling studies that have shown that the abundance of WS cases is correlated with environmental variables, as has been found for bleaching, with such reactions to broad environmental factors expected to result in a random distribution of cases at small spatial scales. Hypothesised causes of WS are varied, ranging from programmed cell death (Ainsworth et al. 2007) to bacterial infection (Sussman et al. 2008; reviewed in Bourne et al. 2015). Evidence for a bacterial causative agent is based on studies showing that WS lesions on colonies of several coral species harboured *Vibrio coralliilyticus* during WS outbreaks in Palau, GBR and the Marshall Islands, coupled with confirmation that inoculation of healthy corals with *V. coralliilyticus* from these lesions reproduced WS-like signs (Sussman et al. 2008). However, Luna et al. (2010) isolated and identified a number of different potentially pathogenic organisms (including *V. coralliilyticus*) from corals exhibiting WS and proposed that the disease may be caused by several different pathogens. These discrepancies arise because the term 'white syndromes' was never intended to describe a single disease but rather, is a collective term for conditions producing tissue loss revealing exposed white skeleton (Willis et al. 2004). Evidence from studies of tabular acroporids suggests that some cases of the disease are host density dependent (Hobbs and Frisch 2010; Roff et al. 2011), but are not transmitted via direct contact between colonies (Roff et al. 2011). However, these results do not distinguish whether the disease is associated with a common microbe that becomes virulent under an environmental trigger or results from potentially uncontrolled programmed cell death (Ainsworth et al. 2007). While evidence of typically random distributions for WS and the lack of association with feeding scars of corallivores (only in 1 out of the 8 spatiotemporal analyses) presented here suggests that the disease is not contagious, it does not allow the causative agent to be identified unequivocally. Further studies are required to resolve whether the causative agent(s) of WS is present in healthy coral holobionts (colonies infected but not diseased) but only becomes virulent (causing the disease) under specific environmental factors.

In conclusion, quantifying the spatial distributions of coral diseases provides insights into disease virulence, contagiousness and transmissibility. Such knowledge is fundamental to understanding and managing coral diseases and enables forecasting of the likelihood of increased disease occurrence, for example, following a *Drupella* outbreak or a rise in seawater temperatures. In the face of projected increases in disease prevalence with rising seawater temperatures (Maynard et al. 2015), greater intensity cyclones causing more severe damage on coral reefs, and potential outbreaks of *Drupella* snails known to be attracted to injured corals and to be more abundant on damaged reefs (Morton et al. 2002), understanding which diseases are contagious and which are driven by injury or environmental stress is critical to managing infectious diseases to prevent them from becoming key drivers of coral population loss in the future. This study investigates spatial distributions of multiple coral diseases *in situ* while accounting for the distribution patterns of the coral hosts themselves, providing new and essential information on BBD, BrB, SEB and WS etiology and transmission mechanisms. The use of spatial analysis as a tool for coral disease research should be extended and added to other analyses of disease pathogenesis, virulence and transmissibility. Further research should focus on how environmental factors affect disease incidence and transmission patterns.

CHAPTER 5 – ENVIRONMENTAL FACTORS AFFECTING PREVALENCE AND VIRULENCE OF FIVE CORAL DISEASES ON THE GREAT BARRIER REEF

5.1 Abstract

Changes in environmental conditions influence the dynamics of diseases, and mediate the potential for disease to cause population declines and threaten biodiversity. This study quantifies the impacts of various environmental factors on the abundance (number of infected branches) and progression rates of five common coral diseases under natural field conditions: black band disease (BBD), brown band disease (BrB), white syndromes (WS), skeletal eroding band (SEB) and atramentous necrosis (AtN). Results demonstrate that progression rates of diseases vary significantly with seasonal changes in environmental variables. Notably, total dissolved nutrients (TDN) and seawater temperature were the most important factors affecting progression of all five diseases, with progression rates generally enhanced at high temperatures and high TDN. Different environmental variables, however influenced the dynamics of the different diseases and non-linear, threshold relationships were observed. For example, there was a strong increase of BrB progression rate above a threshold of 6 μmol L^{-1} in TDN. In contrast, there were no strong effects of environmental factors on the overall abundance of any of the five different coral diseases. Nevertheless, the increased rates of disease progression at increased seawater temperature and TDN suggest that declining water quality and ocean warming have the potential to exacerbate disease-related coral tissue loss. Reducing land-based pollution, terrestrial runoff and seafloor dredging would moderate the impact of environmental variables on coral diseases and may therefore be a powerful tool for lessening human impacts on coral reefs.

5.2 Introduction

Coral reefs are among the most vulnerable ecosystems to global climate change, and changes in environmental conditions over the last few decades have contributed to significant degradation of reef ecosystems worldwide (Pandolfi et al. 2003; Bellwood et al. 2004; Hughes et al. 2005; Knowlton and Jackson 2008). Importantly, sustained increases in seawater temperature and ocean acidification have surpassed direct human-induced disturbances (e.g., overfishing, eutrophication and land-based pollution) as the greatest threats to the persistence of coral-dominated reef habitats (Harvell et al. 2007; Maynard et al. 2015). Climate-induced changes in environmental conditions can contribute to declines in the abundance of reef-building corals, both by causing direct increases in rates of coral mortality (e.g., as a consequence of severe coral bleaching), and by constraining key demographic rates, such as tissue growth and fecundity (Jokiel and Coles 1990; Koop et al. 2001; Pandolfi et al. 2011). Environmental change can also affect coral health by contributing to increased prevalence of coral diseases (Harvell et al. 2007; Maynard et al. 2015). For instance, several coral diseases (including, black band disease, Caribbean necrotic patch, *Porites* tissue loss, white pox, white plague I, II and Indo-Pacific white syndromes) show increased prevalence with increasing temperature (Antonius 1985; Bruckner and Bruckner 1997; Harvell et al. 2001; Patterson et al. 2002; Remily 2004; Willis et al. 2004; Bruno et al. 2007; Williams et al. 2010; Maynard et al. 2011).

Diseases have the potential to cause population declines, threaten biodiversity and, through high mortality of susceptible species, result in a change in community structure. For example, the fungus *Batrachochytrium dendrobatidis*, which results in the disease chytridiomycosis in amphibians, has caused the decline or extinction of 200 species of frogs, the most spectacular loss of vertebrate biodiversity due to disease in recorded history (Skerratt et al. 2007). For corals, the prevalence and intensity of coral diseases have increased rapidly (Harvell et al. 2002, Precht et al. 2016) since their occurrence was first reported by Squires in 1965 (Sutherland et al. 2004). Coral diseases are also contributing significantly to ongoing coral loss; in the Caribbean *Acropora palmata* and *A. cervicornis* decreased in abundance by up to 70% due to diseases in

the 80's and 90's (Goreau et al. 1998; Richardson et al. 1998; Williams and Miller 2005). Similarly, an outbreak of disease also occurred in the summer of 2001/2002 on Magnetic Island (Great Barrier Reef), where atramentous necrosis (AtN) infected up to 80% of the *Montipora aequituberculata* population (Jones et al. 2004). The fact that some coral species are more susceptible to coral diseases means that local outbreaks can alter the species composition of coral communities and cause declines in local species richness.

Covariation among multiple environmental variables can confound identification of the primary drivers of disease dynamics. While some coral diseases are more apparent during warmer months (Harvell et al. 2002; Willis et al. 2004; Bruno et al. 2007; Sato et al. 2009; Haapkyla et al. 2010; Heron et al. 2010), many factors, such as rainfall, light levels, water clarity, run-off, ocean circulation and nutrients, also vary seasonally. For example, atramentous necrosis (AtN) was first described to cause extensive mortality to corals around Magnetic Island, and to co-occur with a bleaching event driven by high seawater temperatures (Jones et al. 2004). However, seawater temperature was only weakly correlated with AtN prevalence and highly correlated with low salinity, which suggests that heavy rainfall during tropical summers, and not increased temperature, influences AtN dynamics (Haapkyla et al. 2011, 2013). Most factors are also expected to work in synergy to create disease onset, and are likely to influence different diseases in dissimilar manners. Among diseases recorded on Hawaiian reefs, both *Porites* growth anomaly (PorGA) and *Porites* tissue loss (PorTL) were associated with low water turbidity, however depth was the other covariate influencing PorGA prevalence while water temperature was the important factor predicting PorTL prevalence (Williams et al. 2010). Covariation among most environmental variables makes it difficult to evaluate the relative importance of each factor as a driver of coral disease dynamics. To date, most studies assessing multiple diseases have been conducted using annual sampling of permanent transects at specific months of the year (not allowing for the investigation of seasonality or spatial variation in diseases), or at different locations across a specific environmental gradient (not allowing for the assessment of temporal variation in disease progression rate). Moreover, holistic studies

evaluating both progression and transmission rates of disease over time and space have, to date, been conducted for single diseases in isolation. Studies over long temporal scales, with frequent monitoring of environmental conditions and quantification of both disease prevalence and disease progression rates, are needed to resolve the drivers of coral disease dynamics on reefs.

The aim of this study was to quantify the impacts of a range of environmental factors on the prevalence and progression rates of coral diseases under natural field conditions. I focused on five diseases that are common on the Great Barrier Reef (Willis et al. 2004): black band disease (BBD), brown band disease (BrB), white syndromes (WS), skeletal eroding band (SEB) and atramentous necrosis (AtN). Black band disease is by far the most well-known and studied disease, with extensive work done on its aetiology and pathogenesis (reviewed by Sato et al. 2016). BBD was the first coral disease to be described (Garret and Ducklow 1975; Antonius 1976) and has been reported to infect at least 42 Caribbean and 57 Indo-Pacific coral species (Sutherland et al. 2004; Kaczmarsky 2006; Page and Willis 2006). BrB was first described by Willis et al. (2004) and is characterized by a brown mat of ciliates directly feeding on coral tissue preceded by healthy tissue and followed by exposed skeleton (Willis et al. 2004; Bourne et al. 2008). Very little is known about BrB ecology and dynamics *in situ*. SEB was first described on Indo-Pacific reefs in 1999 (Antonius 1999) and is caused by ciliates that kill coral tissue and also damage the skeleton (Antonius and Lipscomb 2001). It is present on >90% of reefs on the GBR and affects ~2% of all scleractinians and hydrocorals (Antonius and Lipscomb 2001; Willis et al. 2004; Page and Willis 2008). AtN has, to date, only been recorded on the GBR (Jones et al. 2004) and around Borneo (Miller et al. 2015) and is recognizable by a black lesion covered in a thin light coloured film, giving the lesion a grey appearance (Jones et al. 2004). Lastly, WS is a term first used in 2004 to describe all conditions producing white signs on the GBR (Willis et al. 2004). Since then, multiple sub-categories have been created to further describe diseases affecting various coral species in different parts of the Indo-Pacific region (see Chapter 1). Here, as WS was only observed on corals in the genus *Acropora*, this disease is referred to as *Acropora* white syndrome. In order to infer the primary drivers of coral disease

dynamics, coral colonies were mapped at replicate sites around Lizard Island (northern Great Barrier Reef) and disease abundance and progression were monitored over a two-year period. At the same time, a suite of environmental variables were recorded: seawater temperature, light level, total dissolved nutrients (TDN), dissolved inorganic nutrients (DIN), particulate phosphorus (PP), particulate carbon (PC), particulate nitrogen (PN), total suspended solids (TSS), and chlorophyll *a* (Chl *a*). Overall, 519 disease cases across 20 weeks were recorded (40 sampling days; total n = 2814 observations of progression rate) which provides appropriate resolution to partition the effects of different environmental variables on disease dynamics.

5.3 Methods

5.3.1 Study sites

This study was conducted over 18 months comprising four separate observation periods (20 weeks total); from the 24th of April to 3rd of June 2013 (7 weeks), the 28th of October to 13th of November 2013 (3 weeks), the 16th of January to 6th of March 2014 (7 weeks) and the 23rd of July to 11th of August 2014 (3 weeks) as per Chapter 4. Study sites were established on reefs around Lizard Island (14˚40'08''S 145˚27'34''E). In March 2013, I completed extensive surveys of 12 reefs around the island, to locate sites with high prevalence of coral diseases. Disease prevalence was highest at three sites on Trawler reef (northern lagoon, depth 2 – 5 m) and two sites on Horseshoe Reef (southwestern lagoon, depth 2 – 4 m; Figure 5.1).

5.3.2 Reef mapping and disease progression

To study the dynamics of coral diseases in the reef community, five 10 x 10 m quadrats (100 m^2) were established; 3 quadrats on Trawler reef and 2 quadrats on Horseshoe Reef. The substratum of all quadrats was mapped using photography of the reef taken from directly above using a Canon G11 camera. Pictures (each representing 1 x 1 m^2 of reef substrate) were meshed together in Adobe Illustrator and maps were created by outlining every component of the substratum: coral colony (hard and soft), rock, sand and others such as giant clams. All corals present in each quadrat were surveyed by SCUBA or snorkel diving every 3 days during each

observation period (weather permitting) and all infected coral branches were tagged using cable ties positioned on the bare skeleton below the disease lesion, given a unique identifier and their spatial position recorded. Disease abundance was recorded as the number of infected branches, as distinct from disease prevalence which records the proportion of colonies that are infected.

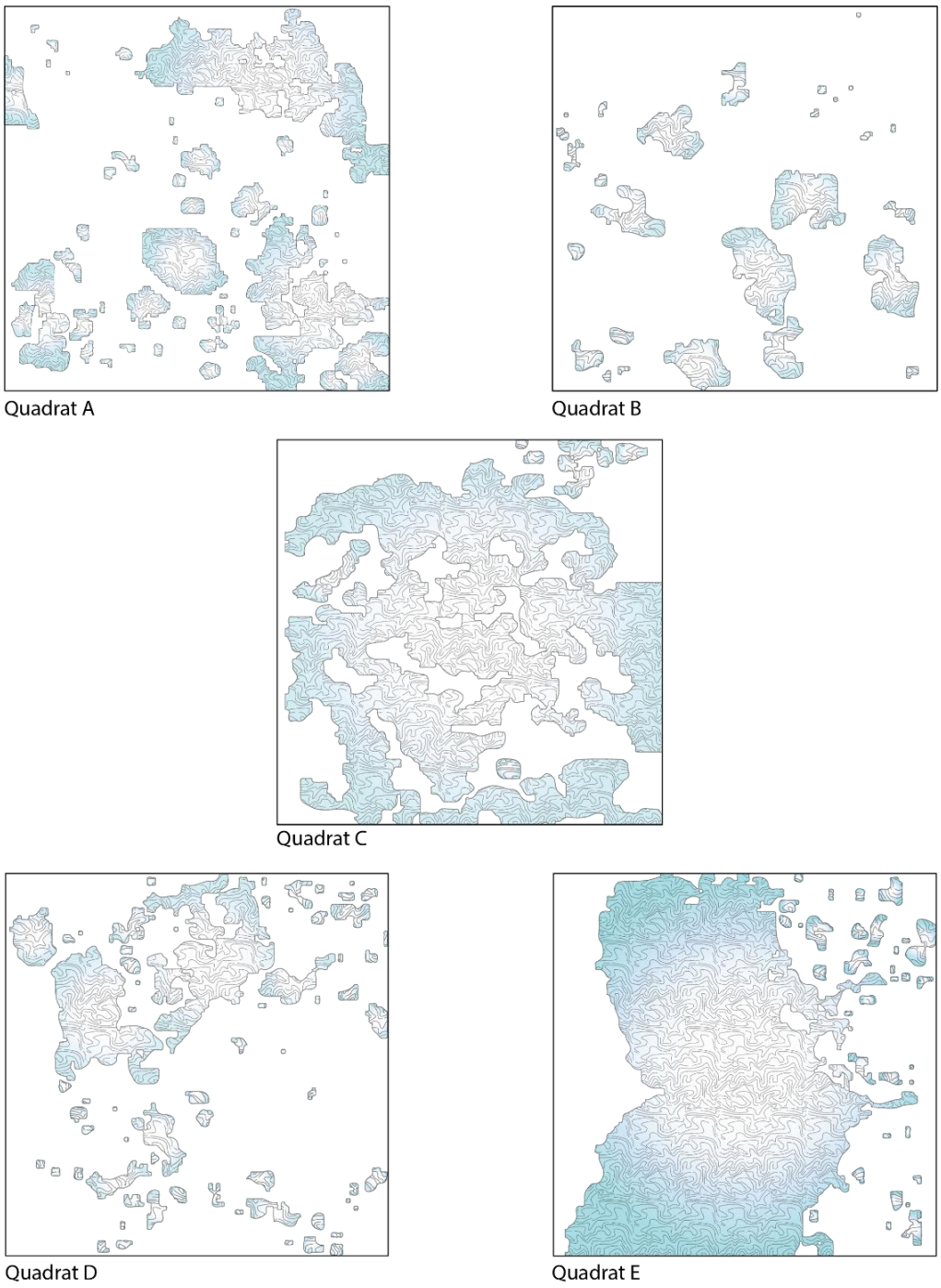


Figure 5.1: Illustration of the five quadrats used in spatial analysis of coral diseases. The blue and white shading representing represent live coral cover. All other substrate types (rock, sand, soft coral and others such as giant clams.) are not represented here.

Disease progression along infected branches (referred to as progression hereafter) was monitored every three days by taking pictures of tagged diseased branches including a ruler as a scale. Images on successive days were taken from the same angle and distance from the band to minimise measurement error. Progression rate was estimated using the program ImageJ (1.44o, Java 1.6.0_29, public domain); the linear distance the disease band moved along coral branches over time was calculated by comparing the distance between the tag (cable tie) and the margin of healthy tissue in subsequent images. Initially, one lesion per branch was monitored, but when lesions progressed past bifurcation points on branches, newly diseased branches were considered separately from the original branch to avoid overestimating progression rates. As a consequence, the number of diseased branches per colony varied over time. All data analyses use the mean (over branches) for each colony on each day to avoid pseudo-replication.

5.3.3 Environmental variables

Measurements of environmental variables were taken alongside surveys of disease abundance and progression rate throughout all four field trips. Two HOBO light and temperature loggers (onset HOBO Pendant®) were deployed, one on Trawler Reef and one on Horseshoe Reef, in close vicinity and at the same depth as the study sites. Light (LUX) and temperature (˚C) were logged every hour. Every 2-3 days, the loggers were brushed manually to remove any sedimentation and to avoid settlement of algae and CCA. Averages of light readings taken between sunrise and sunset (0600 to 1900 hrs) were used for daily light values, whereas daily temperature data were calculated as the average over each 24 h period. Given the relatively small size and consistent depth of colonies within the quadrats, environmental variables measured were considered to be consistent at the scale of individual sites. Salinity data were recorded by GBROOS loggers located at several locations in the northern and southern part of the Lizard Island lagoon, where salinity was logged every five min at a depth of 10 m. Data were acquired from the GBROOS website (http://data.aims.gov.au/gbroos/ on the 18th of November 2016), however a malfunction of the data recorders meant that salinity data were missing for more than half of the sampling days and were therefore excluded from the analysis.

To measure specific water quality parameters, water samples were taken every 3 days (coinciding with the days when progression rate measurements were made) by collecting water directly from the sites while snorkelling. Samples were always taken at the same depth adjacent to the coral colonies, between 2 and 5 m depending on the tide and site. Each water sample was collected in a clean plastic container (2 L), with one sample per site per sampling day. Upon return to the boat, water containers were placed on ice inside an insulated box and returned to the research station as quickly as possible. Bulk water samples were divided into separate vials for different analyses, with two duplicate samples per day per site and per analysis (2 sites x 7 water quality variables x 2 replicate samples = 28 samples per collection day). The water quality variables measured included: chlorophyll *a* (Chl *a*), total suspended solids (TSS), dissolved inorganic nutrients (DIN), total dissolved nutrients (TDN), particulate nitrogen (PN), phosphorus (PP) and carbon (PC). Sampling and processing followed protocols in the manual "Sampling seawater for particulate and dissolved nutrients" (version 01-2013) issued by the Australian Institute of Marine Science (AIMS). Samples were then sent to the AIMS Analytical Services Laboratory to measure TDN and DIN. I conducted analyses of Chl *a*, TSS and particulates (PN, PP, PC) according to standard procedures outlined in the AIMS Analytical Laboratory manuals (Great Barrier Reef Marine Park Authority 2016; find at elibrary.gbrmpa.gov.au).

5.3.4 Statistical analysis

A principal components analysis (PCA) was first used to explore associations among the different environmental variables, and to ordinate the quadrats across the multiple field trips based on measured environmental conditions. These analyses, conducted using the 'vegan' package in R, illustrate spatial and temporal variation in environmental conditions at the study site and identify the main axes of environmental variation. Based on this PCA, redundant environmental variables were excluded from subsequent analyses (see below).

For the analysis of disease abundance, individual observations of disease cases were pooled to provide a count of the number of infected branches present in each quadrat during each field trip. One-way ANOVA was used to assess whether disease abundance changed over time, and to test for seasonal differences in abundance. Analyses first considered overall disease abundance (the total number of diseases, all cases combined) and subsequently were analysed separately by disease type. For these analyses, 'quadrats' were treated as replicates and 'trip' (which summarises different seasons) as a fixed factor. I also used 'disease type' as a factor and tested for interactions between disease type and trip using two-way ANOVA. To standardize for sampling intensity during each field trip, the total number of infected branches observed in each quadrat on each trip was divided by the number of observation days in that trip. Analysis of variance was also used to assess overall seasonal variation in disease progression rates (as per the disease abundance analysis) on pooled and averaged data (mean progression rate per quadrate, per disease and per date). These ANOVA tests were conducted using the 'stats' package in the R statistical platform.

Boosted Regression Trees (BRTs) were used to identify the specific environmental variables associated with variation in disease progression. In these analyses, the entire dataset was used, however progression rate was averaged for each colony, since some colonies had multiple infected branches. BRTs can incorporate different types of predictor variables (continuous and categorical) and can quantify complex nonlinear relationships. BRTs combine two different statistical methods: regression trees (models that relate a response variable to multiple different predictors by repeated binary splits of the dataset) and boosting (an adaptive method for combining many simple models to improve prediction accuracy; see Elith et al. 2008). BRT analyses were used because these methods are non-parametric and allow detection of non-linear associations (De'Ath 2007; Williams et al. 2010). Other approaches, like multiple regression or multiple correlation, assume monotonic relationships between variables. Although BRTs are robust to confounding variables, a recurring assumption of the test is the independence of the data. While progression rate per colony was recorded every three days, no infected colony

remained from one trip to the next, reducing autocorrelation in the dataset. In addition, an Intraclass Correlation Coefficient (ICC) was calculated to check for autocorrelation in the dataset, which produced a low ICC of 0.488, a "within colony" variance of 0.112 and an "among colony" variance of 0.106. The low ICC value and the similar variance within and among colonies support the independence of the data points, making the use of the BRTs possible. BRT analyses were first run using all diseases, before running separate analysis for each disease type. All BRTs were computed using the 'dismo' and 'gbm' packages, specifically the gbm.step function in R. For each analysis, at least five trees were tested, with tree complexity, learning rate and bag fraction varying between replicate trees. For both AtN and SEB, the variance in the dataset was low and, in order for the BRTs to be meaningful, the learning rate of the model had to be reduced, thus increasing the number of trees. Trees were then selected by using the self-statistic function, which provide basic information of the model, such as deviance and mean. The best model was selected by dividing the difference of the mean and deviance by the mean, and the tree with the highest value was considered to be most accurate. All analyses (ANOVA, PCA, BRTs) were implemented in RStudio (R version 3.0.2, see Appendix D for R scripts).

5.4 Results

In all 5 quadrats, staghorn species of *Acropora* were the most abundant corals, and were the most susceptible to infections. Over the course of the 18-month survey period, five major diseases were observed affecting corals on Trawler and Horseshoe reefs: black band disease (BBD), brown band disease (BrB), skeletal eroding band (SEB), atramentous necrosis (AtN) and white syndromes (WS). Besides these, other cyanobacterial diseases and other unknown syndromes were noted, but since their aetiologies were unclear, they were not considered further. In total, 544 branches showing known signs of compromised health were tagged and surveyed over all quadrats and field trips. Of those, 137 branches showed signs of WS, 130 were affected by BBD, 118 by SEB, 81 by branches BrB and 78 by AtN. Over 95% of disease cases were observed on staghorn *Acropora*, the coral which was the most abundant at all sites.

Sample size differs in this chapter compared with Chapter 4 because x and y coordinates could not be provided with certainty for all infected branches used in the last chapter.

5.4.1 Variation in environmental conditions

Even though study sites were separated by 2.5 km, environmental conditions were relatively consistent among sites, but varied greatly through the course of the study (Figure 5.2). From the PCA analysis, the July to August sampling times clustered together, and were most distinct from the January to March samples (Figure 5.2). These results indicate that, as expected, environmental factors varied seasonally, but that for a specific day, environmental conditions at the Trawler and Horseshoe sites were similar.

Several environmental variables were highly correlated with each other (Figure 5.2), specifically concentrations of particulates (PP, PC, PN) and Chl *a*, with all vectors pointing in the same direction, which indicates that these variables have a similar role in separating sampling points. Since PN and PC are associated with each other, only PC was retained. Both Chl *a* and PP were retained since they are not directly correlated with PC. Since the DIN vector had a similar direction as temperature, DIN was removed from the BRT analyses. As a result, the following variables were retained for the BRTs: Temperature, Light, TDN, Chl *a*, PC, PP and TSS (Figure 5.2). TDP, while standing alone on the PC axes, explained less than 7% of the variance of any disease in preliminary BRTs, and was removed from the final analysis.

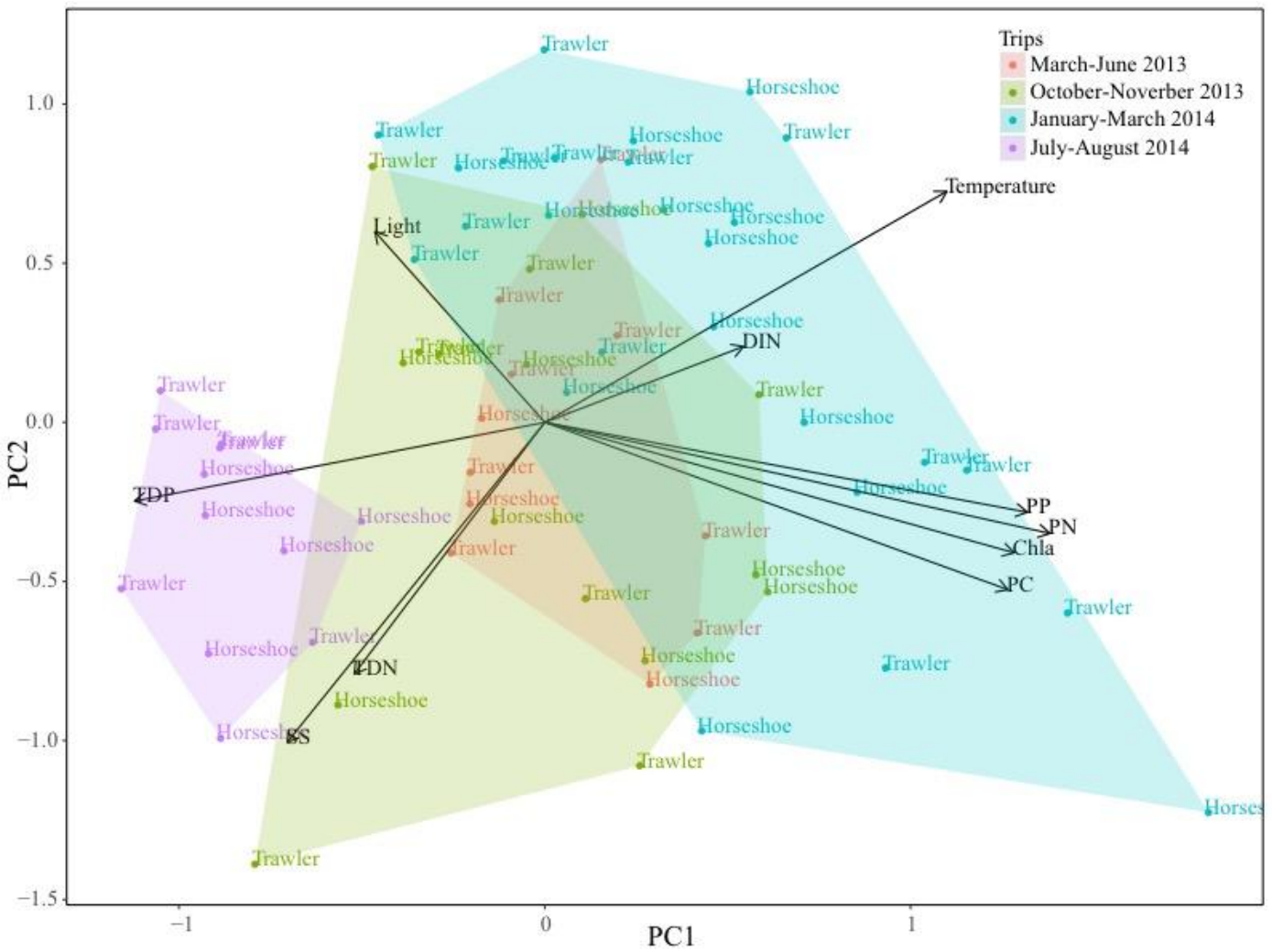


Figure 5.2: Principal components analysis (PCA) illustrating associations among the different environmental variables. Each vector (in black) represents an environmental variable (see methods for meaning of abbreviations). Each point illustrates a sampling day (quadrats grouped by sampling site: Horseshoe or Trawler) and are ordinated across the multiple field trips (colour coded) based on environmental conditions. The acronyms are as follows: total dissolved nutrients (TDN), dissolved inorganic nutrients (DIN), particulate phosphorus (PP), particulate carbon (PC), particulate nitrogen (PN), total suspended solids (TSS), and chlorophyll a (Chl a)

5.4.2 Disease abundance

The overall abundance of all coral diseases combined was generally consistent among field trips, with a mean of 3.4 (± 4.7) infected branches per quadrat and per trip (ANOVA, 'trip' effect, $F_{3,96} = 0.867$, df = 3, p = 0.461), with no evidence of seasonal variation in disease abundance. Disease abundance did vary with disease type (ANOVA, 'disease type' effect, $F_{4,95} = 2.92$, df = 4, p = 0.025; Figure 5.3), but there was no significant interaction between disease type and field trip.

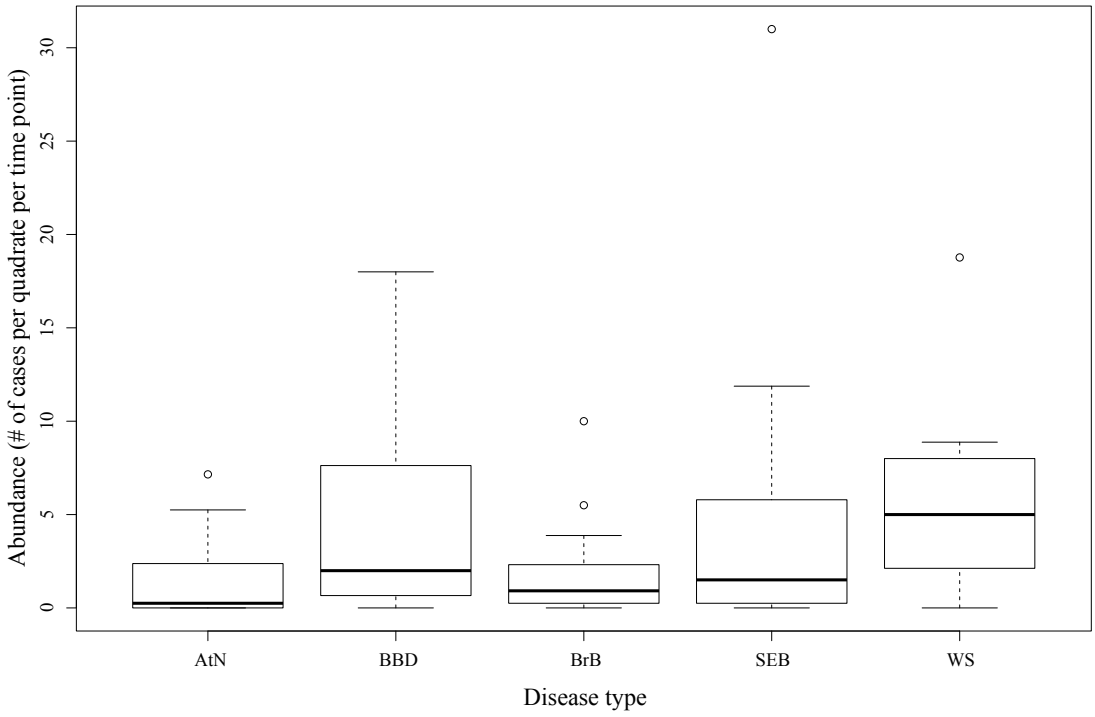


Figure 5.3: Mean disease abundance per disease type across all trips and quadrats. To standardize for sampling intensity during each field trip, the total number of infected branches observed in each quadrat in each trip was divided by the number of observation days for that trip. The diseases acronyms are as follows: atramentous necrosis (AtN), black band disease (BBD), brown band disease (BrB), skeletal eroding band (SEB) and white syndrome (WS)

When investigating the abundance of specific diseases individually, the abundance of BBD cases was highest in March-June 2013 (mean of 8.7 infected branches per quadrat per day, or 0.087 branches m^{-2}) and lowest in July and August of 2014 (mean 0.026 branches m^{-2}). The number of BrB cases was consistent throughout the year, with a mean between 0.012 and 0.032

branches m^{-2}. WS was most abundant in March-June of 2013 (mean 0.063 branches m^{-2}) and least abundant in October-November 2013 (mean 0.047 branches m^{-2}). SEB abundance was greatest in July-August 2014, with a mean of 0.064 branches m^{-2}, and lowest in March-June 2013 (mean 0.033 branches m^{-2}). This difference was due to a great increase in the number of SEB cases in July-August in quadrat E, where the mean SEB count was 31 branches (i.e. 0.31 branches m^{-2}). Finally, the abundance of AtN was very low at all sampling times, ranging between 0.001 (Jul-Aug 2014) and 0.022 branch per m^{-2} (Mar-Jun 2013). However, none of these differences in the mean of individual diseases between field trips were significant due to high variation within sample periods, between quadrats (ANOVA, all $p > 0.05$).

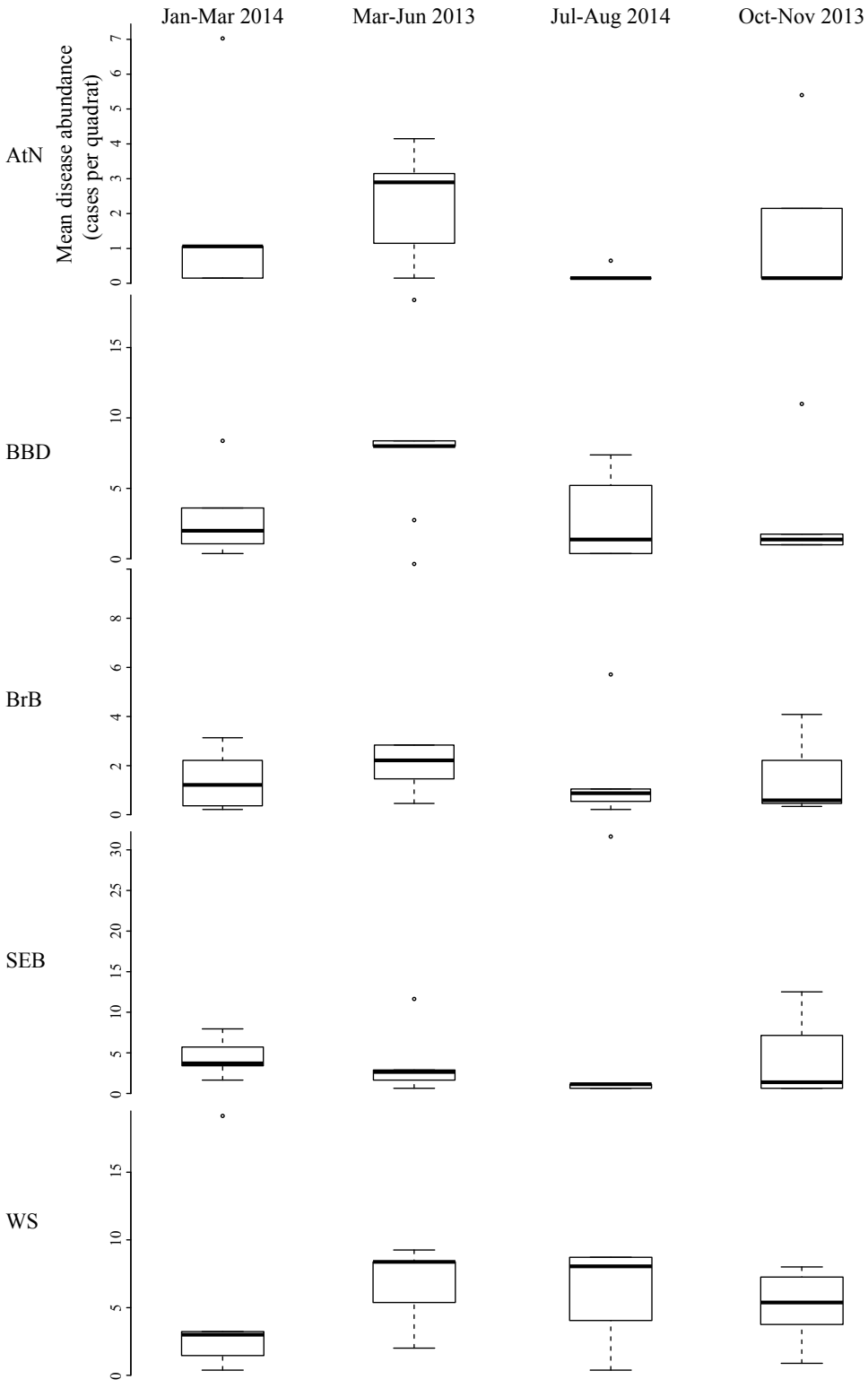


Figure 5.4: Mean abundance of infected branches per field trip for each disease. To standardize for sampling intensity in each field trip, total number of infected branches observed in each quadrat in each trip was divided by the number of observation days for that trip. Means presented here are thus mean abundance per quadrat (100 m^2) and not per m^2. Note the different y-axis scales for each disease: atramentous necrosis (AtN), black band (BBD) and brown band disease (BrB), skeletal eroding band (SEB) and white syndrome (WS). Thick lines

inside boxes represent the median, while the lower and upper lines of boxes represent quartiles 1 and 3. The box itself is the inter-quartile range. The whiskers show 1.5 times the inter-quartile range and individual points on the graph show data outside the range of the whiskers.

5.4.3 Disease Progression

5.4.3.1 Relationships between environmental conditions and disease progression overall

Progression rates were highly variable between the different diseases (Table 5.1, ANOVA, Disease, df = 4, F = 76.12, $p < 0.001$), and also highly variable over time (ANOVA, Trip, df = 3, F = 11.12, $p < 0.001$), and among quadrats (ANOVA, Quadrat, df = 4, F = 13.31, $p < 0.001$). All interaction terms between disease type, time period and quadrat were also significant (Disease:Trip, $p < 0.001$; Disease:Quadrat, $p = 0.01$; Trip:Quadrat, $p < 0.001$; Disease:Trip:Quadrat, $p < 0.001$) indicating the different dynamics of each disease. When considering the entirety of the dataset (all 5 diseases pooled across all sites and time points), total dissolved nutrients (TDN) was the most influential factor (explained 27% of the variance in progression rates), followed by chlorophyll *a* (Chl *a*, 18%), light intensity (15%) and water temperature (15%). TDN displayed a strong threshold type of response, wherein disease progression rate increased strongly above 6 μmol L^{-1}. In contrast, Chl *a* was negatively correlated with progression rate but only at values less than 0.2 μg L^{-1}. Light intensity showed a clear positive correlation with progression rate, with a steady, approximately linear, increase of disease progression rate with increasing light intensity. Finally, temperature did not appear to be associated with increased progression rate of diseases below a temperature threshold of approximately 29˚C. Above 29˚C, however disease progression rate increased quickly as temperature rose. The other variables included in the analysis, PC, TSS and PP, explained less than 7% of the variance in progression rate.

5.4.3.2 Relationships between environmental conditions and progression of different diseases

BBD progression rate varied significantly among observation periods, and the nature of this variation differed among quadrats (ANOVA, Trip, df = 3, F = 9.99, $p < 0.001$; Quadrat, df = 4, F = 6.52, $p < 0.001$;Trip:Quadrat, df = 9, F = 3.79, $p < 0.001$). Similar patterns were observed

for BrB; progression rate varied significantly among observation periods but the temporal variation was different among quadrats (Trip, df = 3, F = 8.87, p < 0.001; Trip:Quadrat significant, df = 10, F = 2.54, p = 0.01). A different pattern was observed for WS progression rate, which remained constant through time in some quadrats but not in others (Quadrat, df = 4, F = 6.67, p <0.01; Trip:Quadrat, df = 10, F = 4.03, p < 0.001). Both AtN and SEB showed approximately constant progression rates through both time and space (all factors analysed by ANOVA returned p > 0.05). Boosted Regression Trees (BRTs) revealed that the different diseases respond differently to environmental variables (Table 5.2, see Appendix E for BRTs graphics). Results are presented below and separated by environmental stressor.

Table 5.1: Minimum, median, mean and maximum disease progression rates (cm day^{-1}) for all diseases combined, and for each disease individually: atramentous necrosis (AtN), black band (BBD) and brown band disease (BrB), skeletal eroding band (SEB) and white syndrome (WS).

Progression (cm day^{-1})	All Diseases	AtN	BBD	BrB	SEB	WS
Min	0.0001	0.0001	0.0001	0.002	0.0001	0.0001
Median	0.148	0.098	0.429	0.575	0.084	0.101
Mean	**0.337**	**0.154**	**0.497**	**0.840**	**0.170**	**0.289**
Max	4.095	1.098	3.193	4.095	1.720	3.782

Total dissolved nutrients

Variation in TDN was consistently associated with variation in progression rates for all five coral diseases analysed here. Progression of all diseases except for SEB was positively associated with an increase in TDN concentrations. BrB progression was the most strongly associated with TDN (this variable explained 45% of the variance in BrB progression) and a clear threshold was observed at 6 μmol L^{-1} above which BrB progression rate increased rapidly.WS progression was also strongly associated with an increase of TDN (22.1%) and exhibited a similar threshold at 6 μmol L^{-1}. Both AtN (13.3%) and BBD (9.2%) showed a slight and approximately linear increase in progression with increasing TDN values, while SEB progression rate declined for values of TDN below 5 μmol L^{-1}.

Table 5.2: Direction and magnitude of effects of different environmental variables on coral disease progression rate. Bolded values indicate the factor(s) having the greatest impact on progression rates of each disease: atramentous necrosis (AtN), black band disease (BBD), brown band disease (BrB), skeletal eroding band (SEB) and white syndrome (WS).

Disease	**TDN**	**Temperature**	**Light**	**PC**	**PP**	**Chl *a***	**SS**
Combined diseases	**27.7%** Increasing strongly after 6 µmol L^{-1}	15.7% Increasing strongly after 29°C	15.7% Steady increase above 1000 LUX	6.2% No clear correlation	7% No clear correlation	18.3% Decrease under 0.2 µg L^{-1}	9.4% No clear correlation
BBD	9.2% Slight linear increase	**46.8%** Increasing strongly after 27°C	11.9% Linear increase	12.3% Drop between 0 and 60 µg L^{-1}	8.4% No clear correlation	6% No clear correlation	5.4% No clear correlation
BrB	**45.5%** Increasing strongly after 6 µmol L^{-1}	8.1% No clear correlation	8.2% No clear correlation	7.4% No clear correlation	7.8% No clear correlation	14.2% Decrease under 0.2 µg L^{-1}	8.7% No clear correlation
WS	**22.1%** Increasing after 6 µmol L^{-1}	10.3% No clear correlation	14.9% Slight linear increase	10.3% Slight increase after 140 µg L^{-1}	**20.3%** Decrease strongly under 0.04 µM	12.6% Decrease under 0.2 µg L^{-1}	9.5% No clear correlation
AtN	13.3% Slight linear increase	14% Slight decrease between 27 and 29°C	13.7% Increase between 15000 and 25000 LUX	**32.6%** Decrease strongly under 100 µg L^{-1}	8.4% Slight increase between 0.06 and 0.08 µM	8.3% Slight increase between 0.2 and 0.3 µg L^{-1}	9.5% No clear correlation
SEB	11.3% Decrease under 5 µmol L^{-1}	18.4% Increasing strongly after 29°C	19.6% Increasing strongly after 15000 LUX	8.8% No clear correlation	17.3% Increase strongly after 0.09 µM	15.9% Steady increase between 0.2 and 0.4 µg L^{-1}	8.7% No clear correlation

Temperature

Progression rate was positively associated with increasing temperature for two of the five coral diseases, BBD and SEB. Temperature explained 46% of the variance in BBD progression rate, which increased as temperature increased above a clear temperature threshold at 27ºC. A similar threshold-type relationship was observed for SEB (18% of variance explained by temperature), where progression rate increased rapidly with increasing temperature above 29ºC. In contrast, temperature had no clear association with progression rates of BrB, WS or AtN, explaining only ~10-14% of variance in progression for these diseases.

Light

With the exception of BrB, progression rates of all diseases generally increased with increasing light intensity. The shape of the relationship between progression and light intensity differed among diseases, ranging from an approximately linear association between light intensity and progression for BBD and WS (which explained 12% and 15% of the variance in progression for these diseases, respectively), to a threshold–type association between light intensity and SEB progression, where progression rate increased rapidly above a threshold of 15000 LUX.

Particulate carbon

PC primarily affected progression rates of AtN, explaining 32.6% of the variance in AtN progression rates, which strongly decreased when PC levels declined below 100 μg L^{-1}. BBD progression rate (12.3%) likewise declined when PC was between 0 and 60 μg L^{-1}, while WS progression rates (10.3%) slightly increased at levels above 140 μg L^{-1}. Progression rates of BrB and SEB did not show any association with PC.

Particulate phosphorus

PP was one of the main factors affecting WS (20.3%), with disease progression rates decreasing strongly when PP concentrations were less than 0.04 μM. The only other disease affected by this variable was SEB (17.3%), with progression rates increasing strongly above 0.09 μM.

Progression rates of all other diseases were not clearly associated with PP.

Chlorophyll a

Both BrB (14.2%) and WS (12.6%) progression rates were negatively associated with Chl *a* content of seawater below a threshold of 0.2 µg L^{-1}, which is the average value for "clear" offshore waters (De'Ath and Fabricius 2010). In contrast, progression rates of SEB (15.9%) and AtN (8.3%) were approximately linearly associated with increasing Chl *a* between the values of 0.2 and 0.3 µg L^{-1}, but were otherwise not affected by changing Chl *a* concentrations. BBD was not affected by Chl *a* in any significant manner.

Suspended solids

This variable never explained more than 9.5% of the variance in progression rates of the five diseases and was never associated with any disease type in any significant manner.

5.5 Discussion

This study demonstrates that progression rates for each of the five coral diseases investigated (BBD, BrB, SEB, AtN and WS) vary temporally with seasonal changes in environmental conditions. In contrast, I found no evidence that seasonal changes in environmental conditions affect the abundance of these diseases, with low numbers of each disease consistently present at all study sites, in all seasons and both years. Temporal variation in disease progression was best explained by seasonal differences in total dissolved nutrients (TDN) and water temperature, with a general enhancement of progression rates at high temperatures and high TDN. This is consistent with previous studies showing that declining water quality (Koop et al. 2001) and ocean warming (Maynard et al. 2016) have the potential to greatly increase coral tissue loss caused by coral diseases, thereby contributing to the sustained coral loss that has been recorded at many locations around the world (e.g., Bellwood et al. 2004, Gardner et al. 2014; Machendiranathan et al. 2016; Precht et al. 2016).

Increasing ocean temperature is the most commonly investigated indirect impact of human activities on coral reefs. In this study, increased water temperatures explained over 15% of the observed variance in progression rate across all diseases combined. Furthermore, temperature threshold dynamics were observed with a strong increase in disease progression above 29°C (for all combined diseases) rather than a linear association between temperature and progression rate. Such threshold dynamics were also apparent for specific diseases: BBD progression was strongly associated with temperature (46% of variance explained by temperature) with progression increasing rapidly at temperatures above 27˚C. Previous studies have indicated a synergistic effect of light and temperature on BBD dynamics (Antonius 1981; Edmunds 1991; Kuta and Richardson 1996, 2002; Borger 2005; Page and Willis 2006; Boyett et al. 2007; Sato et al. 2009, 2011). Two previous laboratory studies concluded that light was the primary driver of BBD progression, which increased significantly in high light treatments, but did not differ between temperature treatments (28, 29 and 30.5˚C) when light was consistently high (Boyett et al. 2007; Sato et al. 2011). However, both studies used temperature treatments above the 27˚C threshold found in the present study (27-32˚C: Boyett et al. 2007; 28-30.5˚C: Sato et al. 2011). As seawater temperatures at Lizard Island typically vary between 24 to 30˚C, the contrasting results of these studies highlight the need to quantify disease dynamics across the full range of environmental conditions.

Progression rates of SEB and AtN were mostly influenced by both water temperature (18.4% SEB, 14% AtN) and light levels (19.6% SEB, 13.7% AtN), whereas abundance of these diseases was generally consistent over time. Although SEB was the first disease to be described from the Indo-Pacific region (Antonius 1999), with a known putative agent (Antonius 1999, Antonius and Lipscomb 2001), and some knowledge of its aetiology (Page and Willis 2008), very little is currently known about the environmental drivers of this disease. Consistent with my results, the only previous study of SEB ecology found consistency in SEB prevalence between winter and summer months (Haapkylä et al. 2010). In contrast, AtN was first described by Jones and colleagues (2004), who reported higher prevalence of AtN in summer, suggesting

that AtN was influenced by seawater temperature. A subsequent study found that AtN prevalence was linked with a decrease in salinity resulting from increased rainfall during the warmer and wetter months of the austral summer (Haapkylä et al. 2013). This latter study also found an effect of temperature, although only above 31°C, when AtN prevalence suddenly increased (Haapkylä et al. 2013). In the present study, AtN abundance was not associated with seasonal changes in water temperature. These contrasting results are likely related to differences in local seawater temperatures; the highest temperature recorded at the study location between 2013 and 2014 was 30°C, suggesting that effects of temperature on AtN ecology occur during abnormally high ocean temperatures. To the best of my knowledge there are no previous studies relating disease progression rates of SEB and AtN to environmental conditions. In contrast to SEB, AtN progression rate was negatively associated with water temperature between 27 and 29°C, but positively associated with light levels between 15000 and 25000 LUX. While a few diseases have been positively associated with light levels -potentially due to the increased pathogen virulence and/or the compromised host resistance (Sato et al. 2011)- the decrease in AtN progression rate between 27 and 29°C is difficult to explain. Previous studies have reported a higher AtN prevalence in summer months (Jones et al. 2004; Haapkyla et al. 2011), however further reports suggest that water temperature itself has little effect on the disease prevalence (Anthony et al. 2008; Haapkyla et al. 2013). No studies have yet experimentally investigated the link between AtN progression rate and water temperature and further research is needed to clarify this relationship. This study, therefore, provides the first evidence of an association between progression rates of these two diseases with water temperature and light levels in the natural environment.

Neither WS nor BrB progression rates were significantly influenced by temperature, highlighting the need to examine a number of environmental variables when investigating disease dynamics. Previous studies investigating BrB progression and prevalence have focused on the effect of light and temperature (Nash 2003; Boyett 2006; Page et al. 2009; Haapkyla et al. 2010), but have found contradictory results. Generally, BrB is more prevalent in summer

(Nash 2003, Boyett 2006) and a correlation between BrB prevalence and bleaching has been suggested (Page et al. 2009). However, other studies recorded higher prevalence of BrB in winter months at Heron Island (southern GBR, Haapkylä et al. 2010), or did not detect any difference in progression rates of BrB across different temperature treatments (Boyett 2006). These results suggest that temperature-mediated effects on BrB prevalence and progression rate may be attributed to other local environmental factors, and/or driven mainly by host susceptibility rather than pathogen virulence. Other changes in host physiology may be required before BrB becomes infectious.

The lack of correlation between WS progression and temperature observed in this study is surprising because several other studies have linked WS prevalence to high temperatures (Willis et al. 2004; Bruno et al. 2007; Heron et al. 2010; Maynard et al. 2011; Ban et al. 2013). Most studies however, have linked warm temperature anomalies to WS prevalence; some suggesting that bleaching preceded the onset of the disease (Bruno et al. 2007; Heron et al. 2010; Maynard et al. 2011). In the present study, summer seawater temperatures remained below 30°C and no visible signs of thermal stress were observed during the 20-week monitoring period. As noted for AtN above, comparison between this study and the literature indicates that a higher temperature threshold (>30˚C, outside the normal range experienced at the study location) must be breached before WS progression and/or prevalence increases with increasing temperature. I note, however that since the term 'white syndrome' was coined to describe all conditions producing white signs (Willis et al. 2004), different 'types' of WS may have different aetiologies, pathogenesis and ecology. My results are, thus, only applicable for white syndromes affecting branching species of *Acropora* at Lizard Island sites.

This study shows that variation in seawater temperature can alter coral disease dynamics, however total dissolved nutrient concentration was the main driver of coral disease progression rates when all five diseases were considered together (27.7%), with disease progression increasing when TDN levels were above a threshold of 6 μmol L^{-1}. Among the coral diseases

investigated here, BrB progression was the most strongly associated with TDN (45.5%) followed by progression of branching *Acropora* WS (22.1%). The results of my study are conservative as to the effects of nutrient addition on coral disease in the natural environment because I observed a strong association between TDN and disease progression even though loads of suspended sediments and nutrients were generally low in my study (max Chl *a* = 0.65 µg L^{-1}; max DIN = 4.06 µmol L^{-1}; max TSS = 2.75 mg L^{-1}). Other studies on the GBR have reported up to 22 µg L^{-1} in Chl *a*, over 25 µmol L^{-1}in DIN and 334 mg L^{-1} in TSS (Joo et al. 2012; Devlin et al. 2015). Even though these latter measurements are extremes taken in river catchments, these findings indicate that the range of suspended sediments and nutrients observed in the vicinity of the GBR has the potential to greatly influence disease-related coral tissue loss on inshore and mid-shelf reefs.

In contrast to the strong effect of TDN on progression rates, BrB and WS abundances were not significantly associated with nutrient levels, likely because these diseases were consistently present in low numbers. To my knowledge, no study to date has experimentally assessed the impact of increased nutrient levels on abundance and progression rates of BrB and *Acropora* WS. However, previous studies have linked the prevalence of BrB and WS to poor water quality due to river discharge or heavy loads of sediments associated with dredging activities, with these metrics often associated with increased nutrient input (Williams et al. 2010; Pollock et al. 2014; Sheridan et al. 2014). While further research is needed to elucidate the mechanisms underlying the association between TDN and increased disease progression, previous studies indicate that high nutrient loads can destabilise the nutritional symbiosis between corals and *Symbiodinium* spp. (e.g., Dubinsky & Berman-Frank 2001). This might, in turn, act as an additional stressor that lowers coral disease resistance. Further studies should test the effects of nutrient addition on coral physiology, *versus* the potential effects on nutrients on rates of ciliate proliferation, using nutrient addition treatments based on the threshold TDN concentration of 6 µmol L^{-1} that the present study has identified.

Increases in particulate phosphorus promoted SEB progression rate and explained 17% of the variance in progression rates for this disease. While very little is known about SEB ecology and dynamics, one study observed the disease to be more prevalent around offshore tourism platforms compared with nearby reefs without platforms (Lamb and Willis 2011). The results of the present study support the nutrient hypothesis proposed by Lamb and Willis (2011), wherein disease prevalence was thought to be associated with nutrient input through guano deposited by seabirds that roost on offshore pontoons (Lamb and Willis 2011). Progression rate of SEB was also shown here to increase slightly with increasing Chl *a* concentrations between 0.2 and 0.4 ug L^{-1} (15.9%), a proxy for poor water quality (see Boyer et al. 2009). A previous study of water quality across a longitudinal gradient on the GBR revealed that Chl *a* concentrations were more than two-fold higher in coastal waters (mean = 0.46 ug L^{-1}) than in clear offshore water (<0.2 ug L^{-1}; De'Ath and Fabricius 2010), a range consistent with the values observed at my study sites. These results suggest that SEB progression rate is likely to be greater at sites directly impacted by coastal development and terrestrial runoff (e.g., discharge of polluted water) and to decrease in mid and offshore reefs. Likewise, AtN progression rate was positively correlated with TDN (13.3%), PP (8.4%) and Chl *a* (8.3%), while being strongly negatively correlated with particulate carbon concentrations (32.6%), again, suggesting that AtN is promoted by poor water quality. AtN outbreaks have been recorded following heavy rainfalls and subsequent decrease in salinity (Haapkylä et al. 2011), although the same study also found a positive correlation between AtN prevalence and particulate organic carbon. Due to a failure of salinity loggers, unfortunately, it was impossible to investigate the impact of salinity on AtN progression rates here.

Implications for disease dynamics in a changing world

The increased progression rates of BBD and SEB with temperature found here is of concern as these diseases, while typically in low prevalence, are omnipresent on GBR reefs (BBD present in >70% of surveyed reefs, SEB in >90%; Page and Willis 2006, 2008). Furthermore, both diseases affect a wide range of coral species (BBD: >50 scleractinian species, SEB: >80

scleractinian species; Page and Willis 2006, 2008), and any increases in progression rates and associated coral tissue loss will have significant consequences. Coral fitness (e.g. fecundity, growth rate) is linked to tissue surface area (Hall and Hughes 1996), so loss of coral tissue, even in the absence of total colony mortality, can decrease coral reproductive output and associated population replenishment. Increases in the number of coral diseases recognised in the literature (Green and Bruckner 2000, Sutherland et al. 2004, Sokolow 2009, Chapter 1), in the number of infected colonies present on reefs (e.g. Willis et al. 2004; Page and Willis 2008; Bourne et al. 2016; Precht et al. 2016), and in rates of disease-related tissue loss, all of which have been associated with environmental change, indicate that diseases may have increasingly negative impacts on coral populations and communities (Maynard et al. 2015).

Water quality variables measured here (TDN, PC, PP and Chl *a*) were associated with progression rates of all five diseases surveyed during the 18 month sampling period, with total dissolved nutrients explaining a large proportion of the variation in disease progression. Human activities have driven changes in nutrient cycles (particularly those of nitrogen and phosphorus), primarily via fossil fuel combustion, but also via fertilizer production and application (Johnson et al. 2010). As a result, nutrient loads in rivers have increased greatly, which has further modified nutrient cycling in freshwater and marine ecosystems (Johnson et al. 2010). My finding that disease-related tissue loss is strongly associated with seasonal variation in nutrient loads is consistent with other studies that have correlated outbreaks of coral diseases with increases in nutrient runoff (Kim and Harvell 2002; Sutherland et al. 2004). For instance, addition of fertilizer directly on infected corals increased the severity of black band, aspergillosis and yellow band disease (Bruno et al. 2003; Voss and Richardson 2006). Even the relatively small seasonal changes in nutrients that occur on reefs that are subjected to only minor human impacts were strongly correlated with disease progression, highlighting the likely role of increased nutrients and particulates in enhancing rate of tissue loss.

Conclusion

This study further highlights the importance of managing anthropogenic impacts, primarily temperature change and nutrient addition, for the conservation of coral-dominated habitats. In the present study, the detailed environmental data recorded at the study site, the long temporal duration, the spatial replication, and the simultaneous quantification of multiple diseases allowed for detailed comparisons between environmental variables and both the abundance and progression of diseases. Consistent with the literature, seasonal increase in seawater temperature was found to be an important driver of coral disease progression rate. In parallel, total dissolved nutrients were also related to increased disease progression for all but one naturally occurring disease. This suggests further that local management of terrestrial and coastal activities can be a powerful tool for lessening human impacts on coral reefs.

CHAPTER 6 – DISCUSSION AND CONCLUSIONS

6.1 New knowledge derived from this thesis

The study of coral disease ecology recognises the intrinsic connections between disease dynamics and environmental conditions, especially temperature. An infectious disease arises through interactions between a pathogen and a host, wherein pathogen virulence and host susceptibility are both influenced by the environment (Sokolow et al. 2009a,b). The role of the environment as a driver of disease dynamics is, therefore, multi-layered and extremely complex. The breadth of studies investigating coral disease ecology, aetiology and pathogenesis has expanded over the last 30 years, and a clear trend of increasing impacts of human activities on coral health has emerged. Such studies have enabled the development of models that forecast the future of our oceans (e.g. Maynard et al. 2015). My thesis sought to elucidate the multi-layered factors that influence disease dynamics by directly linking disease progression and abundance to environmental conditions (Chapter 5), evaluating whether corallivory meditates rates of disease progression (Chapter 2), assessing the role of vectors in disease transmission (Chapter 3), and resolving contagiousness through quantifiable differences in spatial distributions of disease (Chapter 4).

Understanding the complexities of coral pathogen-host-environment interactions requires the use of a diverse array of tools, and also requires long-term observations of where diseases occur on the reef, and how quickly diseases result in coral tissue loss. By spending 18 months in the field, I was able to closely monitor the dynamics of all naturally occurring diseases at multiple study sites. By tagging and monitoring all infected branches within large areas of the reef, I was able to determine when and where new disease cases arose. This provided new insights into whether diseases are contagious, and how they are transmitted between colonies. Similarly, by monitoring diseased corals over time, I was able to estimate mean progression rates for each individual disease and to understand how environmental conditions influenced disease progression. Finally, using manipulative field experiments and controlled aquarium

experiments, I was able to assess the effect of corallivorous fish and invertebrates on coral disease virulence and transmission patterns.

My thesis reveals that:

- *Corallivory by butterflyfishes does not slow rates of BBD disease progression* – While a diverse community of corallivorous fishes have been observed to selectively target coral disease lesions as a source of nutrition, this behaviour had little to no capacity to constrain the progression of the disease, neither under controlled laboratory conditions nor in a natural field setting. While there was high variability in rates of disease progression among colonies, and over time, this variation was mainly associated with characteristics of the coral host (e.g. genotypic differences in disease susceptibility or health), the pathogens (e.g. successional stage of the microbial community, as indicated by the width of the disease band), and the environment (e.g. seasonal variation in light or temperature, as measured in the field) (Chapter 2). The precedence of inter-colony variability in explaining progression rate variability highlights that some colonies are naturally more resistant to diseases and avoid substantial tissue mortality. This is the first time that the effect of corallivory on coral disease progression rate has been quantified, and results demonstrate that it is unlikely that direct removal of pathogens from lesions by fish significantly slows the rate of tissue loss due to BBD infections.

- *Drupella snails, but not butterflyfish, can be vectors of BrB but not BBD* – *Drupella* snails transmit brown band disease (BrB), both immediately after feeding and for at least 24 h after feeding on disease lesions. Survival of BrB ciliates within the snail for 24 hours, and potentially longer, would facilitate disease transmission, both within and between coral colonies *in situ*. In contrast to BrB, I found that *Drupella* snails did not transmit BBD, most likely because of the complexity of the BBD pathogenic community. *Chaetodon plebeius* did not transmit either disease. Interestingly, BBD spatial distribution on the reef was associated with *Drupella* snails and COTS feedings scars on 46% of sampling occasions,

whereas BrB was associated with scars on only one sampling occasion. The results of my thesis indicate, therefore, that although *Drupella* can be a vector for BrB (Chapter 3), transmission might be rare in the natural environment, when BrB prevalence is low and *Drupella* are present at low abundance. Overall, the results of Chapter 4 are consistent with the hypothesis raised in Chapter 3 that organisms creating larger feeding scars (e.g. *Drupella*, COTS) than those created by butterflyfish are more like to contribute to disease transmission.

- *BBD and SEB behaved as contagious diseases, spreading from one colony to other nearby colonies, whereas WS was randomly distributed and thus is likely to be triggered by environmental conditions. In contrast, spatial patterns in occurrences of BrB were variable, suggesting that multiple interacting factors culminate in BrB disease signs*. Quantifying spatial distributions of coral disease occurrence enables assessments of disease contagiousness and transmission mechanisms, both of which are fundamental for understanding and managing diseases in the natural environment. My thesis is the first to test for spatial aggregation of four coral diseases while specifically accounting for the distribution of potential host corals, resulting in robust inferences about BBD, BrB, SEB and WS transmissibility and contagiousness. Lastly, by investigating the relationship between feeding scars of invertebrate corallivores and disease distribution, inferences could be made about the importance of physical weakening of the coral host for disease onset. BBD and SEB retained their contagiousness independent of physical injury, and WS was never found to be associated with feeding scars. BrB remained elusive, clustering on very rare occasions and being associated with feeding scars only once, suggesting that either a physical or a physiological weakening of the host (i.e. due to variations in environmental factors) might be required for the onset of BrB.

- *Increased ocean temperature and increased nutrient levels are associated with increased rates of coral tissue loss from disease.* In combination, detailed environmental data

recorded at the study sites, the long temporal scale, and the simultaneous quantification of multiple diseases allowed for rigorous assessment of the relationships between environmental variables and disease prevalence and progression. Consistent with previous literature, seasonal increase in seawater temperature was found to be a major driver of coral disease progression rates. This was especially true for BBD, with this variable alone explaining 47% of variation in disease progression rate. This is consistent with results found in Chapter 1, where BBD progression rate was highly correlated with seawater temperature and light intensity. In parallel, however total dissolved nutrients played an even greater role in increasing the progression of all but one naturally occurring disease. In particular, TDN greatly influenced BrB progression rate (46%), providing new insights into environmental drivers of this disease and increasing understanding of disease dynamics *in situ*.

6.2 Caveats and limitations

As for any ecological study, the present thesis is subject to a series of caveats and limitations due to the variability of the natural environment. High variation in disease progression rates and abundance recorded in this study meant that even after 20 weeks of *in situ* monitoring, some trends were unclear or not statistically significant. Furthermore, the low prevalence of most coral diseases at my study sites reduced the number of replicates available for experimental investigation and thus reduced statistical power or made some analyses impossible. For example, out of the 20 potential replicates for disease clustering analysis (5 quadrats x 4 field trips), only 5 replicates had sufficient BrB infected branches for the Ripley's K function to be applied to disease distribution data. The same was true when investigating the relationship between disease distribution and the presence of feeding scars. The low abundance of diseases also prevented use of Boosted Regression Trees to analyse disease abundance data or to detect any significant variation in abundance with seasonal changes in environmental factors. Extending the spatial or temporal scales of data collection would have allowed for a higher statistical power, unfortunately this was unrealistic due to time and monetary constraints.

Disease aggregation results presented here were necessarily restricted to spatial scales of between 0 and 2.5m, due to limits on what was considered to be a manageable quadrat size and modifications of statistical windows to account for coral cover. While this spatial scale allowed for the investigation of contagiousness of diseases, examination of disease distribution patterns at larger scales would give a more holistic understanding of coral disease behaviour *in situ*. My focus on a more precise investigation of disease clustering (while simultaneously accounting for host distribution), enabled by the comparatively small spatial scale of my quadrats, was at the expense of a larger spatial scale of disease observations. My design represents a trade-off between depth and breadth of research, a conundrum that is always problematic in ecological research.

Finally, the low prevalence of even the most common diseases made finding over 15 coral colonies of the same species infected with the same disease at the same time and on the same reef impossible, even after monitoring the reef over a two-year period, thereby constraining the sample size for the caging experiment in the field (Chapter 2). More importantly, all laboratory experiments focused on one coral species, one fish and one snail species. Results concerning the effect of corallivory on disease progression and transmission rate may vary with different corallivore or host species, although the importance of the size of feeding scars in disease transmission is expected to be maintained across corallivores and host species.

6.3 Factors influencing the dynamics of common coral diseases on the Great Barrier Reef

Table 6.1 Environmental factors influencing (A) disease prevalence/abundance and (B) disease progression rates based on results from this study and in the literature. 0 = no effect, + = increase, - = decrease, nd = no data, ? = potential effect suggested but not measured. Disease acronyms: atramentous necrosis (AtN), black band disease (BBD), brown band disease (BrB), skeletal eroding band (SEB) and white syndrome (WS)

Disease	**(A) Prevalence**			
	Factor	**My thesis**	**Literature**	**Source**
BBD	Temperature	0	+	Antonius 1985; Kuta and Richardson 2002 ; Boyett et al. 2007; Sato et al. 2009; Zvuloni et al. 2009 ; Kuehl et al. 2011
	Light level	0	+	Antonius 1985; Boyett et al. 2007; Sato et al. 2009; Kuehl et al. 2011
	Nutrients	0	+	Antonius 1981; Kuta and Richardson 2002
BrB	Temperature	0	+	Boyett et al. 2006; Page et al. 2009
	Nutrients	0	nd	
SEB	Temperature	0	0	Haapkylä et al. 2010
	Nutrients	0	?	Lamb and Willis 2011
WS	Temperature	0	+	Willis et al. 2004; Bruno et al. 2007; Heron et al. 2010 ; Maynard et al. 2011; Ban et al. 2013
	Nutrients	0	?	Lamb and Willis 2011; Pollock et al. 2014
AtN	Temperature	0	+	Jones et al. 2004
	Nutrients	0	+	Haapkyla et al. 2011
	Salinity	N/A	+	Haapkyla et al. 2011
Disease	**(B) Progression**			
	Factor	**My thesis**	**Literature**	**Source**
BBD	Temperature	+	+	Boyett et al. 2007; Muller and van Woesik 2011; Sato et al. 2011
	Light level	+	+	Antonius 1985; Boyett et al. 2007; Muller and van Woesik 2011; Sato et al. 2011
	Nutrients	0	+	Voss and Richardson 2006
BrB	Temperature	0	0	Boyett et al. 2006
	Nutrients	+ (TDN)	nd	
SEB	Temperature	+	nd	
	Light level	+	nd	
	Nutrients	+ (TDN)	nd	
WS	Temperature	0	+	Dalton et al. 2010
	Light level	+	nd	
	Nutrients	+ (TDN)	+	Redding et al. 2013
AtN	Temperature	-	nd	
	Light level	+	nd	
	Nutrients	- (PC)	nd	

6.4 Synthesis of thesis findings by disease type

6.4.1 Black band disease

Black band disease was one of the most prevalent diseases on the reefs surveyed around Lizard Island between March 2013 and September 2014, with a total of 122 infected colonies across all quadrats and field trips. The disease was most abundant between March and June 2013 (mean = 8.7 cases per 100m^2), and least abundant between July and August of 2014 (mean = 2.6 cases per 100m^2). BBD abundance around Lizard Island has been reported to reach much greater values in previous studies, with close to 40 cases per 100 m^2 in summer and 4.5 cases per 100 m^2 in winter (data from Willis et al. 2004, reported as: 95 cases in summer and 11 cases in winter, over a surveyed area of 240 m^2). Furthermore, mean progression rate of BBD across all study periods was among the most rapid of the diseases studied here, at ~0.5 cm per day. The constant presence of BBD on all reefs surveyed, and the speed at which the disease consumes coral tissue, are likely to make it a significant driver of coral mortality on the GBR.

Ten fish species were observed feeding on BBD lesions, taking a mean of 0.46 bites min^{-1} on the disease band in natural conditions, and *Chaetodon plebeius* was responsible for more than half (52.7%) of the total predation. However, selective feeding on BBD lesions and direct removal of pathogens by corallivorous fish did not influence progression rates of BBD. Indeed, variability in BBD progression among predation treatments in the field (cage and uncaged) was negligible (<0.1%) and the greatest variability in disease progression was primarily due to inter-colony variation (~24%), variance over time (i.e., days of experimental exposure; ~38%), and changing band width over time for each colony (~38%).

Analyses of spatiotemporal distributions of BBD within the 10 x 10 m quadrats revealed that the disease was clustered in almost 80% of cases (Chapter 4). All clusters were observed between 0 and 1 m, but sometimes up to 2.5 m, proving that BBD is a contagious disease spreading from a diseased colony to the next. These patterns are consistent with the high contagiousness of BBD observed during aquaria experiments, where 92% of contact nubbins

became infected (Chapter 3), a result consistent with previous studies reporting 100% infection of nubbins in direct contact with infected corals (Boyett 2006; Antonius 1985). BBD has also previously been observed easily transmitting to colonies in close contact in the field and even bridging gaps of 2-3mm (Antonius 1985). In the present thesis, BBD was associated with feeding scars of *Drupella* snails and COTS 46% of the time, suggesting that injuries caused by predation may contribute to the likelihood of infection. However, neither *Chaetodon plebeius* nor *Drupella* snails were able to directly transmit the disease in laboratory experiments. However, physical injury has been shown to influence the vulnerability of corals to BBD, with all mechanically injured coral fragments contracting the disease and uninjured corals remaining healthy after exposure to BBD (Aeby and Santavy 2006). While corallivores might be unable to transport BBD pathogens directly, injuries created by corallivore feeding behaviour might increase host susceptibility if the pathogenic consortium is present locally.

Lastly, progression rate of BBD was predominantly influenced by temperature, with this variable alone explaining close to half of the observed variation in BBD progression among sampling occasions (47%) and progression rates increasing dramatically after a threshold at 27°C. Other variables, such as light and total dissolved nutrients, also had an effect on BBD progression, but to a lesser extent. While abundance around Lizard Island over the 20 weeks of monitoring was too low (average density of BBD diseased branches was 0.042 branches m^{-2}) for meaningful statistical analysis of the relationship between abundance and environmental conditions, numerous other studies have demonstrated a general increase in BBD prevalence with warmer seawater temperature (e.g. Antonius 1985; Kuta and Richardson 2002; Boyett et al. 2007; Sato et al. 2009; Zvuloni et al. 2009; Kuehl et al. 2011). Given that BBD is contagious (Chapter 4), results from my thesis research, combined with the literature, suggest that the observed increase in BBD prevalence at high temperatures is likely associated with increased progression rates observed in this thesis (Chapter 2, Chapter 5). Given that the range of temperatures over which increased progression was observed in this study was below temperatures that are generally considered to be stressful for corals (Ainsworth et al. 2016),

increased progression and abundance are more likely due to increased virulence of the BBD microbial mat. Whatever the mechanism, these results lead to a prediction that BBD will cause increased rates of coral tissue loss under future climate-induced changes in seawater temperatures.

6.4.2 Brown Band Disease

Abundance of BrB was very low during the two years of the field experiments, with only 81 branches affected by the disease in the 5 quadrats monitored (average density of BrB diseased branches was 0.018 branches m^{-2}). Nevertheless, the progression rate of BrB (average of ~0.85 cm day^{-1}, maximum of 4 cm day^{-1}) was the highest observed among the set of diseases analysed here, and was almost double that of BBD. Other studies have reported values for BrB prevalence of 0 to 10% on reefs around Lizard Island (Chong-Seng et al. 2011). Consequently, the high progression rate of BrB, and the potentially localised high prevalence of this disease, makes it an important driver of diseased-induced coral loss on Indo-Pacific reefs.

Spatial distributions of BrB cases were not consistent among sites or over time, with BrB cases being aggregated in 40% of datasets but otherwise random, suggesting that the disease is generally not contagious. Moreover, the disease was found to cluster around feeding scars only once, and in the analysis of the quadrat at that specific time period, the disease itself was also found to cluster within the space. It is possible, therefore, that the aggregated disease distribution found in that one analysis was more a reflection of the distribution of feeding scars, which are inherently clustered, than a reflection of the contagiousness of the disease. The lack of correlation between BrB spatial distribution and *Drupella* feeding scar was surprising, considering that *Drupella* snails were found to transmit BrB in laboratory conditions, both directly after snail exposure to BrB and 12 and 24h after exposure. Furthermore, I directly observed *Drupella* and COTS feeding scars becoming the origin of BrB infection at the study sites (although only on rare occasions). It is possible that the disease is contagious and transmitted both by direct contact and by vectors, but the low prevalence of BrB meant that a

source of BrB ciliates was only present on certain occasions. To support this hypothesis that BrB is contagious, I observed high rates of contact-transmission in the laboratory experiment (BrB was transmitted to 90% of healthy nubbin) and non-contact transmission in the field (healthy nubbins 20 cm away from the focal diseased coral became infected, Chapter 3). In a previous study at Lizard Island in 2003/2004, BrB transmitted to 50% of healthy corals tied to infected nubbins (Boyett 2006). Further studies are required to understand BrB contagiousness and transmission mechanisms on the reef.

Lastly, BrB progression rate was most strongly associated with total dissolved nitrogen, with this variable alone explaining ~46% of the variation in BrB progression. Furthermore, a clear threshold was apparent, with BrB progresson increasing when TDN > 6 μmol L^{-1}, a level which is well within the values observed on tropical coral reefs (Tanaka et al. 2011). Other studies on turbid inshore reefs have reported much higher concentrations of nutrients, reaching 827μg L^{-1} in total nitrogen (Joo et al. 2012) and >25 μmol L^{-1} of dissolved inorganic nitrogen (Devlin et al. 2015). While the contagiousness of BrB is still unclear, it is possible that the coral host can resist BrB infection, except when weakened by physical injury or when the disease is facilitated by a vector. Concentrations of TDN at the study locations (up to 9 μmol L^{-1}-) are unlikely to significantly compromise the condition of the coral host host, since most reefs experience similar values regularly (Tanaka et al. 2011). It is possible however, that BrB ciliates might become more virulent when TDB >6 μmol L^{-1}, which would imply that ciliates are able to infect weakened hosts, and also healthy corals when nutrients levels promote virulence. Further studies are needed to elucidate BrB dynamics, however the present results suggest that disease spread and severity will be increased by direct human impacts on the reef (e.g., sediment input, physical breakage), as well as by biotic factors, such as *Drupella* snails or COTS outbreaks.

6.4.3 White Syndromes

White syndromes (WS), specifically branching *Acropora* white syndrome, were the most prevalent disease type around Lizard Island during this study, with a total of 137 branches

affected across all monitoring periods (average density of 0.054 infected branches m^{-2}). WS were most abundant in March-June of 2013 (mean 0.063 branches m^{-2}) and lest abundant in October-November 2013 (mean 0.047 branches m^{-2}), but WS abundance did not differ significantly among observation periods. Other studies demonstrate that WS abundance can reach 0.2 cases m^{-2} during 'outbreaks' (reported as 304 cases per 1500m^2 area; Willis et al. 2004) but is otherwise between 0.001 and 0.03 cases m^{-2} (reported as 1.7 and 47.7 cases 1500m^2; Willis et al. 2004). The mean progression rate of WS was 0.29 cm day^{-1}, which remained approximately constant through time but was more rapid at some sites than others (maximum of 3.8 cm day^{-1}). Consistent with these results, rates of linear progression of WS on tabular corals infected with *Acropora* WS were previously found to be highly variable, ranging from 0 to 1.9 cm day^{-1}, resulting in tissue loss of up to ~164cm^{-2} day^{-1} (Roff et al. 2011). Very few studies have reported progression rates of *Acropora* WS in natural conditions (mean rate of 2.28 cm day^{-1} in aquaria; Pollock et al. 2013), nonetheless, the rates reported here (between 0 and 3.8 cm day^{-1}) are consistent with previous reports.

Spatial and temporal distributions of WS cases were found to be random nearly 80% of the time, suggesting that the disease is not contagious. Furthermore, WS distribution was only associated with feeding scars of corallivores in one instance (12.5%), highlighting that occurrence of the disease is independent of physical weakening of the coral host through predation. There is an ongoing debate in the scientific literature about the cause of the different types of white syndromes. It has been suggested that WS can be caused by either a pathogenic agent (most likely a *Vibrio* bacterium; Sussman et al. 2008; Luna et al. 2010) or simply a "shut-down reaction" (i.e., programmed death of coral cells; Ainsworth et al. 2007). Results presented here are consistent with either interpretation of the causative agent. It is possible that the causative agent(s) of WS is present in the healthy coral holobiont (colony infected but not diseased) but only becomes infectious (causing the disease) under specific environmental factors. Under such a scenario, the disease does not spread from colony to colony, resulting in a random spatial distribution of disease cases. Alternatively, WS could be the consequence of

programed cell death triggered by an environmental variable, resulting in the same random distribution. In the latter scenario, WS is not an infectious disease (i.e., does not result from a pathogenic agent) but a physiological reaction of the coral holobiont, comparable to coral bleaching. Lastly, the random spatial distribution of the disease, and the lack of association with corallivore feeding scars suggest that the disease is unlikely to be transmitted by corallivorous vectors.

In either scenario (i.e., infected but not diseased corals (pathogen present but no disease signs) vs programmed cell death), specific environmental factors are required to trigger disease onset. Most of the literature thus far has focused on the relationship between WS abundance and the occurrence of thermal anomalies (Bruno et al. 2007; Heron et al. 2010; Maynard et al. 2011; Ban et al. 2013). There is now a general consensus in the literature that abnormally high ocean temperatures increase WS prevalence in the Indo-Pacific, yet little is known about the dynamics of WS under average summer conditions. In the present study, the maximum seawater temperature was 30°C, which does not constitute a thermal anomaly. In these conditions, TDN is the variable most likely to influence WS progression rates (22%). Furthermore, disease progression increased strongly after a threshold of 6 μmol L^{-1}, suggesting that direct human disturbance of seawater nutrient levels (e.g. fertilizer runoff in rivers, dredging, untreated sewage) is likely to influence WS severity in *Acropora* species. It also implies, however that management actions that improve water quality in coastal areas have the potential to lessen the impact of WS on coral populations.

6.4.4 Other diseases

SEB and AtN infected 118 and 78 branches, respectively, during the 18-month period when the 5 quadrats were monitored. SEB abundance was greatest in July-August 2014, with a mean of 0.064 infected branches m^{-2}, and lowest in March-June 2013 (mean = 0.033 infected branches m^{-2}). This difference was due to a spike in SEB abundance in July-August in quadrat E, where the mean SEB count was 31 infected branches (0.31 branches m^{-2}). This occasional high

abundance of SEB has been observed on Lizard Island before, with a mean of 1.4 cases of SEB per m^2 in the summer of 2003 (reported as 342 cases per 240 m^{-2}; Willis et al. 2004). However, while SEB affects a wide range of coral species (82 species) and was present on most reefs (>90% of reefs surveyed), its prevalence is typically low, infecting only 2% of corals surveyed (Willis et al. 2004; Page and Willis 2008). The abundance of AtN was very low at all sampling times, ranging between 0.001 (Jul-Aug 2014) and 0.022 branch per m^2 (Mar-Jun 2013). However, this disease has only rarely been reported on the GBR. The first account of the disease reported outbreak levels, with the disease affecting 80% of the *Montipora aequituberculata* population around Magnetic Island (Jones et al. 2004). Later studies also revealed very high abundance of AtN in summer (1.76 case m^{-1}) and lower abundance in winter (0.44 case m^{-1}) within the population of *M. aequituberculata* (Haapkylä et al. 2011). However, a recent study conducted around Borneo revealed low abundance of AtN (mean of 0.06 case m^{-2}; Miller et al. 2015), comparable to the values reported here. In the present study, both AtN and SEB exhibited very similar mean progression rates (AtN: 0.15 cm day^{-1}; SEB: 0.17 cm day^{-1}) and both retained constant progression rates through both time and space. However, SEB had a higher maximum progression rate (1.7 cm day^{-1}) than AtN (1.1 cm day^{-1}). The mean linear progression rate of SEB is typically low, with reported values between 0 to 0.3 cm day^{-1} (Page and Willis 2008).

Progression rates of both SEB and AtN varied only slightly across survey periods and seasons, which decreased the potential for statistical analysis to determine what environmental factors most impact their diseases dynamics. AtN was only significantly influenced by levels of particulate carbon, a variable that explained over 30% of the variation in AtN progression rate. This correlation was negative however, with fastest AtN progression under low levels of PC. AtN outbreaks have been recorded following heavy rainfalls and subsequent decreases in salinity (Haapkylä et al. 2011), although the same study also found a positive correlation between AtN prevalence and particulate organic carbon. Due to a failure of salinity loggers, it

was not possible to investigate how AtN progression rates respond to salinity here, limiting conclusions about AtN ecology and dynamics.

SEB progression rate was mostly influenced by seawater temperatures (18.4%) and light levels (19.6%). A threshold response was observed for both variables, with progression rates increasing rapidly above light levels of ~15000 LUX and temperatures of 29°C. This study provides the first evidence of an effect of both water temperature and light levels on SEB progression rate, as all previous studies on SEB have focused on disease prevalence rather than progression. The only study reporting rate of SEB progression (Page and Willis 2008) did not investigate seasonal patterns in progression rate. Lastly, SEB distribution patterns were highly aggregated, which is consistent with contagiousness suggested by other studies (Winkler et al. 2004, Page and Willis 2008, Lamb et al. 2014, 2015). Notably, my results suggest that the ciliates causing SEB (*Halofolliculina corallasia*) can travel up to 2.5 m to infect new coral colonies. SEB distribution was not associated with corallivore feeding scars, suggesting that injury alone is not sufficient for SEB onset. These results are consistent with previous studies that have shown that *H. corallasia* ciliates readily colonise areas of exposed coral skeleton but fail to form the virulent band characteristic of SEB (Winkler et al. 2004; Page and Willis 2008). Only when additional stressors are present are injured corals more prone to SEB infection (Lamb et al. 2014). The specific light and temperature levels mentioned above could be the stressors necessary for SEB onset and virulence, but further studies are required to accurately tease apart the impact of light, temperature and injury, as well as the synergistic effects of the three, on disease prevalence and progression. Nonetheless, increased SEB progression rate with light and temperature predicts an intensification of SEB-related coral mortality with climate-induced changes in seawater temperatures.

6.5 Concluding remarks

In the natural environment, the dynamics of diseases are influenced by multiple biotic and abiotic factors that interact in complex ways (Sutherland et al. 2004; Work et al. 2008; Sokolow

et al. 2009). These factors influence both the host and the pathogen(s) and, ultimately, it is the outcome of the **host-pathogen-environment** interaction that determines whether the host will become diseased. Here, **host** condition was found to greatly influence the dynamic of diseases, with inter-colony variation accounting for a quarter (24%) of the variance in BBD progression rate in the field (Chapter 2). **Environmental** factors, such as seawater temperature, light levels and water quality, also greatly influenced the dynamics of all diseases investigated here, explaining close to 50% of the variance observed in some diseases' progression rate (Chapter 5). However, measured values of seawater temperature and water quality were within the range of expected values for seawater temperatures and nutrients levels on healthy tropical reefs (Tanaka et al. 2011). These results suggest that, under such conditions, the association between disease-related tissue loss and environmental variation results from a triggering of **pathogen**(s) virulence rather than from the weakening of the coral host. Nevertheless, further studies of the mechanisms of pathogenesis are required to test this hypothesis.

Whether diseases drive populations to local extinction, and thereby affect local biodiversity, depends on whether transmission is density- or frequency-dependent (Smith et al. 2009), where the latter requires the survival of the pathogen outside of the host (e.g., in a vector or reservoir). My thesis shows that brown band disease, for example, can be readily transmitted by *Drupella* snails (Chapter 3). This, and the fact that BrB progression rate could reach up to 4 cm day^{-1}, indicates that diseases have the potential to cause extensive coral tissue loss and contribute to coral population decline. After accounting for the heterogeneous spatial distribution of coral hosts in the environment, my results indicate that both of these diseases are contagious (Chapter 4). Moreover, this contagiousness was retained in the absence of physical weakening of the host, meaning that both diseases have pathogens capable of infecting corals without the creation of an entry point by an external stressor. The new statistical analysis employed here avoids the use of less precise estimates of coral spatial distribution, such as weighting analyses of disease prevalence per transect by the number of coral colonies present or using the underlying distribution of coral as the null distribution for statistical analysis. The results provided in the

present thesis help estimate the contagiousness and transmission mechanisms of diseases, and by doing so, provide a better understanding of coral disease potential to cause coral tissue loss and subsequent coral population decline.

Corals, the foundation species of coral reefs, are declining on a global scale. Coral diseases are one factor that has contributed to this decline (e.g., Precht et al. 2016), and there is evidence to indicate that disease-related coral tissue loss will accelerate under future environmental change (Maynard et al. 2015). During the 18 months over which data were collected on Lizard Island, no abnormal thermal stress was recorded. Furthermore, as the island is a mid-shelf reef, all water quality values presented here fall within a range expected for a 'normal' tropical reef. Even so, seawater temperature and total dissolved nutrients were both significantly positively associated with disease progression rates. It is difficult to estimate standard pre-industrial values of water quality on inshore and mid-shelf reefs (but see Wooldrige et al. 2006), but coral hosts are susceptible to disease at what are now considered baseline levels of nutrients. While reducing global warming and the likelihood of thermal anomalies should remain a priority, results presented here suggest that coastal management and local actions that reduce land-based pollution are tools likely to lessen the impact of coral diseases on their host populations. Otherwise, the combined effects of global warming and decreased water quality are likely to continue to intensify coral disease severity and increase subsequent coral mortality.

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APPENDICES

APPENDIX A: R script Chapter 2

Field Experiment

Testing Before and After Caging

Using all data (Before and After caging) and all replicates (unbalanced nb of branches per colony)

Prog = Progression rate (cm/day, continuous variable)
Exp = Experimental Status (before or after, factor with 2 levels)
Band = Band width (cm, continuous variable)
Colony = Replicate colony (1 to 15, factor with 15 levels)

```
library(nlme)
FIELD.lme<-lme(Prog~Exp+Band,random=~1|Colony,data=FIELD.OUT,
               weights=varExp(form=~Band),
               correlation=corARMA(c(0.2,-0.2),p=2,q=0),method='REML')

summary(FIELD.lme)

## Linear mixed-effects model fit by REML
##  Data: FIELD.OUT
##        AIC      BIC    logLik
##   1462.757 1497.972 -723.3785
##
## Random effects:
##  Formula: ~1 | Colony
##         (Intercept)  Residual
## StdDev:   0.2609254 0.7687856
##
## Correlation Structure: ARMA(2,0)
##  Formula: ~1 | Colony
##  Parameter estimate(s):
##      Phi1      Phi2
## 0.1547977 0.0851155
## Variance function:
##  Structure: Exponential of variance covariate
##  Formula: ~Band
##  Parameter estimates:
##      expon
## 0.07210663
## Fixed effects: Prog ~ Exp + Band
##                 Value  Std.Error  DF  t-value p-value
## (Intercept) 0.4549426 0.09967133 589 4.564428  0.0000
## ExpBefore   0.2541127 0.08274092 589 3.071185  0.0022
## Band        0.3330649 0.08906424 589 3.739603  0.0002
```

```
##  Correlation:
##           (Intr) ExpBfr
## ExpBefore -0.428
## Band      -0.356 -0.122
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -1.3388359 -0.6011315 -0.2613833  0.2925709  5.5621354
##
## Number of Observations: 606
## Number of Groups: 15
```

```
anova(FIELD.lme)
```

```
##             numDF denDF  F-value p-value
## (Intercept)     1   589 88.97312  <.0001
## Exp             1   589 12.63282   4e-04
## Band            1   589 13.98463   2e-04
```

Testing Colony effect

Using all data (Before and After caging) and all replicates (unbalanced nb of branches per colony)

```
FIELD1.kw<-kruskal.test(data=FIELD1,Prog~Colony)
FIELD1.kw
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  Prog by Colony
## Kruskal-Wallis chi-squared = 121.73, df = 14, p-value < 2.2e-16
```

Colonies have significantly different mean progression rates

```
FIELD1.kw1<-kruskal.test(data=FIELD1,Band~Colony)
FIELD1.kw1
```

```
##
##  Kruskal-Wallis rank sum test
##
## data:  Band by Colony
## Kruskal-Wallis chi-squared = 146.14, df = 14, p-value < 2.2e-16
```

Colonies have significantly different mean band width

Testing Time effect

Using all data (Before and After caging) and all replicates (unbalanced nb of branches per colony)

```
(FIELD1.kw2<-kruskal.test(data=FIELD1,Prog~Time))
```

```
##
##  Kruskal-Wallis rank sum test
##
```

```
## data:  Prog by Time
## Kruskal-Wallis chi-squared = 76.577, df = 15, p-value = 2.934e-10

(FIELD1.kw2<-kruskal.test(data=FIELD1,Band~Time))

##
##  Kruskal-Wallis rank sum test
##
## data:  Band by Time
## Kruskal-Wallis chi-squared = 42.081, df = 15, p-value = 0.0002184
```

Both band width (cm) and Progression rate (cm/day) significantly vary over time.

Treatment effect

Only using the "After" caging dataset to tease appart the effect of predation removal

Prog = Progression rate (cm/day, continuous variable)
Treatment = Treatment group (cage, cage ctrl and ctrl, factor with 3 levels)
Band = Band width (cm, continuous variable)
Time = Days since the onset of the experiment (1 to 15, factor with 15 levels)

Adding Colony as random effect did not improve the model.

```
library(nlme)
TREAT.gls<-
gls(Prog~Treatment+Band,correlation=corCompSymm(form=~Time),
                data=FIELDT,method ='REML')
summary(TREAT.gls)

## Generalized least squares fit by REML
##   Model: Prog ~ Treatment + Band
##   Data: FIELDT
##        AIC     BIC    logLik
##   533.1272 554.232 -260.5636
##
## Correlation Structure: Compound symmetry
##  Formula: ~Time
##  Parameter estimate(s):
## Rho
##   0
##
## Coefficients:
##                        Value  Std.Error   t-value p-value
## (Intercept)        0.5560764 0.09156096  6.073291  0.0000
## TreatmentCageCtrl -0.0754284 0.11147726 -0.676626  0.4993
## TreatmentControl  -0.2116638 0.10673388 -1.983099  0.0485
## Band               0.3761309 0.10884679  3.455600  0.0006
##
##  Correlation:
##                   (Intr) TrtmCC TrtmnC
## TreatmentCageCtrl -0.629
## TreatmentControl  -0.696  0.601
## Band              -0.400 -0.152 -0.061
##
```

```
## Standardized residuals:
##        Min         Q1        Med         Q3        Max
## -1.5539459 -0.5864784 -0.2249046  0.2522442  5.0503530
##
## Residual standard error: 0.6660656
## Degrees of freedom: 253 total; 249 residual
```

```
anova(TREAT.gls)
```

```
## Denom. DF: 249
##             numDF   F-value p-value
## (Intercept)     1 205.19180  <.0001
## Treatment       2   2.22099  0.1106
## Band            1  11.94117  0.0006
```

Variance component analysis

Variance component analysis using a linear mixed effect model.

```
library(nlme)
model.1<-lme(prog~1,random=~1|time/treat/colony/band)
summary(model.1)
```

```
## Linear mixed-effects model fit by REML
##  Data: NULL
##        AIC      BIC    logLik
##   250.1925 268.7181 -119.0962
##
## Random effects:
##  Formula: ~1 | time
##         (Intercept)
## StdDev:   0.2627296
##
##  Formula: ~1 | treat %in% time
##          (Intercept)
## StdDev: 3.560073e-05
##
##  Formula: ~1 | colony %in% treat %in% time
##         (Intercept)
## StdDev:   0.3320121
##
##  Formula: ~1 | band %in% colony %in% treat %in% time
##         (Intercept)   Residual
## StdDev:   0.3320527 0.01816356
##
## Fixed effects: prog ~ 1
##                 Value  Std.Error  DF  t-value p-value
## (Intercept) 0.6712117 0.08465648 127 7.928651       0
##
## Standardized Within-Group Residuals:
##          Min           Q1          Med           Q3          Max
## -0.099235467 -0.025997736 -0.005709035  0.022796125  0.093936875
##
## Number of Observations: 163
## Number of Groups:
```

```
##                                     time
##                                       12
##                         treat %in% time
##                                       36
##             colony %in% treat %in% time
##                                      163
## band %in% colony %in% treat %in% time
##                                      163

sds.4<-c(0.2627287,3.478705e-05,0.3320149,0.3320556)
vars.4<-sds.4^2
percent.4<-100*vars.4/sum(vars.4)

percent.4

## [1] 2.384156e+01 4.179794e-07 3.807455e+01 3.808389e+01
```

so time explain 23.84% of variance, treat 0%, colony 38.08%, band 38.08%

Light and Temperature effect

Prog = Progression rate (cm/day, continuous variable)
Temp = Water Temperature (C, average of 24h periode, continuous variable)
Light = Light Incidence (PAR, average of 12h day light, continuous variable)

```
ENV.model<-
gls(Prog~Temp*Light,weights=varPower(form=~Light),data=ENV,method='REML')

summary(ENV.model)

## Generalized least squares fit by REML
##   Model: Prog ~ Temp * Light
##   Data: ENV
##        AIC      BIC    logLik
##   27.69389 30.08126 -7.846943
##
## Variance function:
##  Structure: Power of variance covariate
##  Formula: ~Light
##  Parameter estimates:
##   power
## 2.69735
##
## Coefficients:
##                 Value Std.Error   t-value p-value
## (Intercept) 29.976285 13.295880  2.254554  0.0455
## Temp        -1.042340  0.458875 -2.271514  0.0442
## Light       -0.057386  0.022323 -2.570754  0.0260
## Temp:Light   0.002030  0.000770  2.635304  0.0232
##
##  Correlation:
##             (Intr) Temp   Light
## Temp        -1.000
## Light       -0.996  0.995
```

```
## Temp:Light  0.996 -0.995 -1.000
##
## Standardized residuals:
##        Min         Q1        Med         Q3        Max
## -1.5537757 -0.4434347 -0.1994185  0.5576776  1.5225705
##
## Residual standard error: 2.984557e-09
## Degrees of freedom: 15 total; 11 residual
```

```
anova(ENV.model)
```

```
## Denom. DF: 11
##             numDF  F-value p-value
## (Intercept)     1 407.8677  <.0001
## Temp            1  12.7599  0.0044
## Light           1  34.7554  0.0001
## Temp:Light      1   6.9448  0.0232
```

Laboratory Experiment

Testing effect of predation using a linear mixed effects model

This dataset does NOT include progression rate of "no predation" treatments and controls. Only data for the progression rate in the presence of predation.

Prog = Progression rate (cm/day, continuous variable)
Pred = Predation rate (bite/min, contiunous variable)
Band = Band width (cm, continuous variable)
Replicate = Experimental replicates (A to G, factor with 7 levels)

```
library(nlme)
LAB.model<-lme(Prog~Pred+Band,random=~1|Replicate,
               weights=varExp(form=~Pred),
               correlation=corARMA(c(0.2,-0.2),p=2,q=0),
               data=LAB1,method='REML')
```

```
summary(LAB.model)
```

```
## Linear mixed-effects model fit by REML
##  Data: LAB1
##       AIC      BIC   logLik
##   64.3836 88.78244 -24.1918
##
## Random effects:
##  Formula: ~1 | Replicate
##         (Intercept)  Residual
## StdDev:  0.05181387 0.2805578
##
## Correlation Structure: ARMA(2,0)
##  Formula: ~1 | Replicate
##  Parameter estimate(s):
##      Phi1      Phi2
## 0.1802303 0.4597433
```

```
## Variance function:
##  Structure: Exponential of variance covariate
##  Formula: ~Pred
##  Parameter estimates:
##      expon
## 0.07583732
## Fixed effects: Prog ~ Pred + Band
##                   Value  Std.Error  DF   t-value p-value
## (Intercept)  0.25950882 0.06962589 150  3.727189  0.0003
## Pred         0.05997836 0.02133973 150  2.810642  0.0056
## Band        -0.01263642 0.06241163 150 -0.202469  0.8398
##  Correlation:
##      (Intr) Pred
## Pred -0.336
## Band -0.567  0.124
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -1.2571430 -0.8371828 -0.3199478  0.5489105  3.1057456
##
## Number of Observations: 159
## Number of Groups: 7

anova(LAB.model)

##             numDF denDF   F-value p-value
## (Intercept)     1   150 31.583560  <.0001
## Pred            1   150  8.167733  0.0049
## Band            1   150  0.040994  0.8398
```

This time high levels of predation is positively correlated with progression rate.

Progression without Predation

Testing for the correlation between progression and band width in laboratory settings.

```
library(nlme)
LABW.model<-
lme(Prog~Band+Time,random=~1|Tank,weights=varExp(form=~Band),
               data=LAB.WO,method='REML')

anova(LABW.model)

##             numDF denDF   F-value p-value
## (Intercept)     1    44  38.14983  <.0001
## Band            1    44 120.34844  <.0001
## Time           44    44   2.20091  0.0051
```

Overall model

Overall model including mean progression rate and predation rate for both colonies in the field and repicate tanks in the laboratory.

```
OA.lm<-lm(prog~pred+band, data=overall)
anova(OA.lm)

## Analysis of Variance Table
##
## Response: prog
##           Df Sum Sq  Mean Sq F value Pr(>F)
## pred       1 0.0604 0.060376  0.3800 0.5411
## band       1 0.0659 0.065899  0.4147 0.5233
## Residuals 40 6.3559 0.158898
```

APPENDIX B: R-script Chapter 3

Field Experiment

Looking at the effect of predation on black band and brown band disease transmission rate.

In this dataset: "Status" is the response variable, the health status of the nubbins at the end of the experiment, either healthy of infected. The different factors in the dataset: "nubbin" refers to individual replicate branch; "reef" is the reef site, either Horseshoe or Palfrey; "disease" is the disease type of the infected nubbin in the centre of the block, either black band or brown band; "condition" is the condition treatment of the experimental nubbin before the onset of the experiment, either healthy or bleached in fresh water; "caging" refers to whether the nubbin was protected from predation by a cage "infection" is a detailed version of the response variable "Status". It lists the type of infections observed on the experimental nubbins at the end of the experiment. However, it was found to have no effect in preliminary statistical analysis. It was thus left out of the present analysis.

Data

```
infected<-read.csv("/Users/kjnicolet/Desktop/Disease spread
paper/Stats/Field/Excel and csv
files/field_infected.csv",header=T,strip.white = T)
summary(infected)
```

glmer Laplace approximation model with all factors included

```
library(lme4)

FIELD.glmerL1<-
glmer(Status~caging+condition+reef+(1|block),data=infected,family='bin
omial')

summary(FIELD.glmerL1)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: binomial  ( logit )
## Formula: Status ~ caging + condition + reef + (1 | block)
##    Data: infected
##
##      AIC      BIC   logLik deviance df.resid
##    124.2    136.7    -57.1    114.2       86
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.2834 -0.8505  0.4506  0.8126  1.7110
##
## Random effects:
##  Groups Name        Variance Std.Dev.
```

```
##  block  (Intercept) 0.4502   0.6709
## Number of obs: 91, groups:  block, 12
##
## Fixed effects:
##                  Estimate Std. Error z value Pr(>|z|)
## (Intercept)        0.1767     0.5229   0.338   0.7355
## caginguncaged     -0.3084     0.4660  -0.662   0.5081
## conditionhealthy  -0.8303     0.6081  -1.365   0.1721
## reefPalfrey        1.2524     0.4930   2.540   0.0111 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) cgngnc cndtnh
## caginguncgd -0.440
## condtnhlthy -0.557  0.014
## reefPalfrey -0.356 -0.013 -0.079
```

Caging is not significant
Disease not significant in itself but has an interaction with reef
Reef and block are significant
The interaction betweeen caging and condition is not significant

glmer Laplace approximation with only status, condition and reef as factors and block as random factor.

```
library(lme4)

FIELD.glmerL2<-
glmer(Status~condition+reef+(1|block),data=infected,family='binomial')

summary(FIELD.glmerL2)
```

```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: binomial  ( logit )
## Formula: Status ~ condition + reef + (1 | block)
##    Data: infected
##
##      AIC      BIC   logLik deviance df.resid
##    123.4    133.5    -57.7    115.4       89
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -2.2096 -0.7995  0.4526  0.8004  1.9256
##
## Random effects:
##  Groups Name        Variance Std.Dev.
##  block  (Intercept) 0.5827   0.7633
## Number of obs: 93, groups:  block, 12
##
## Fixed effects:
##                    Estimate Std. Error z value Pr(>|z|)
## (Intercept)      -0.0002163  0.4934440   0.000  0.99965
## conditionhealthy -0.8010346  0.6454369  -1.241  0.21458
```

```
## reefPalfrey       1.3348739  0.4878150   2.736  0.00621 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) cndtnh
## condtnhlthy -0.619
## reefPalfrey -0.380 -0.091
```

```
anova(FIELD.glmerL2,test="LRT")
```

```
## Analysis of Variance Table
##           Df Sum Sq Mean Sq F value
## condition  1  0.984   0.984   0.984
## reef       1  8.241   8.241   8.241
```

Only reef site is significant. The bleaching has no effect.

Model testing

Overdispersion

```
overdisp_fun<-function(model)
  {vpars<-function(m)
    {nrow(m)*(nrow(m)+1)/2}
  model.df<-sum(sapply(VarCorr(model),vpars))+length(fixef(model))
  rdf<-nrow(model.frame(model))-model.df
  rp<-residuals(model,type="pearson")
  Pearson.chisq<-sum(rp^2)
  prat<-Pearson.chisq/rdf
  pval<-pchisq(Pearson.chisq,df=rdf,lower.tail=FALSE)
  c(chisq=Pearson.chisq,ratio=prat,rdf=rdf,p=pval)}

overdisp_fun(FIELD.glmerL2)
```

```
##      chisq      ratio        rdf          p
## 80.2652383  0.9018566 89.0000000  0.7346758
```

p values isn't significant so we don't have overdispersion in the data.

R^2 for glmerL2 model

```
totalss<-
var(resid(FIELD.glmerL2,type='pearson')+predict(FIELD.glmerL2,type='li
nk'))
1-var(residuals(FIELD.glmerL2,type='pearson'))/(totalss)
```

```
## [1] 0.5649683
```

By removing caging and disease, we reduced the R^2 a little.

Drupella Experiment

This dataset only includes the BrB data (since BBD was never transmitted by Drupella or Chaetodontids)

Generalized linear model testing the effect of treatments and controls on coral disease infection rate. Status refers to the health of the coral nubbin at the end of the trial (healthy or infected) and Factor has two levels, Drupella (Direct, 12h and 24h pooled together) and the controls (injury and water control combined). Treatment containes all 5 treatments and tests whether infection rate differs between them.

```
DRUP<-read.csv('/Users/kjnicolet/Desktop/Disease spread
paper/Stats/Drupella/Excel and csv files/Drupella BrB.csv',strip.white
= T,header=T)
summary(DRUP)

DRUP.glm<-glm(Status~Treatment+Factor,family=binomial, data=DRUP)
summary(DRUP.glm)

##
## Call:
## glm(formula = Status ~ Treatment + Factor, family = binomial,
##     data = DRUP)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.96954  -0.51678  -0.00008  -0.00008   2.03933
##
## Coefficients: (1 not defined because of singularities)
##                   Estimate Std. Error z value Pr(>|z|)
## (Intercept)     -1.946e+00  1.069e+00  -1.820   0.0687 .
## Treatment24h    -1.908e-15  1.512e+00   0.000   1.0000
## TreatmentDirect  1.435e+00  1.295e+00   1.108   0.2677
## TreatmentInjury -1.762e+01  3.802e+03  -0.005   0.9963
## TreatmentWater  -1.762e+01  3.802e+03  -0.005   0.9963
## FactorDrupella          NA         NA      NA       NA
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 30.142  on 39  degrees of freedom
## Residual deviance: 22.642  on 35  degrees of freedom
## AIC: 32.642
##
## Number of Fisher Scoring iterations: 18

anova(DRUP.glm,test="LRT")

## Analysis of Deviance Table
##
## Model: binomial, link: logit
##
## Response: Status
##
## Terms added sequentially (first to last)
##
##
##           Df Deviance Resid. Df Resid. Dev Pr(>Chi)
```

```
## NULL                         39     30.142
## Treatment  4      7.5        35     22.642   0.1117
## Factor     0      0.0        35     22.642
```

Injection rate does not significantly vary between the 5 treatment groups. Meaning that the Direct treatment group is not significantly different from the 12h, 24h, injury or water control group.

To compare only the Drupella treatments against each other, we tested only a subset of the data:

```
SUB.DRUP<-
subset(DRUP,Factor=="Drupella"&Treatment%in%c("Direct","12h","24h"))
DRUP.glm1<-glm(Status~Treatment,data=SUB.DRUP,family=binomial)

summary(DRUP.glm1)

##
## Call:
## glm(formula = Status ~ Treatment, family = binomial, data =
SUB.DRUP)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -0.9695  -0.5168  -0.5168  -0.5168   2.0393
##
## Coefficients:
##                   Estimate Std. Error z value Pr(>|z|)
## (Intercept)     -1.946e+00  1.069e+00  -1.820   0.0687 .
## Treatment24h     1.635e-15  1.512e+00   0.000   1.0000
## TreatmentDirect  1.435e+00  1.295e+00   1.108   0.2677
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 24.564  on 23  degrees of freedom
## Residual deviance: 22.642  on 21  degrees of freedom
## AIC: 28.642
##
## Number of Fisher Scoring iterations: 4

anova(DRUP.glm1,test="LRT")

## Analysis of Deviance Table
##
## Model: binomial, link: logit
##
## Response: Status
##
## Terms added sequentially (first to last)
##
##
##           Df Deviance Resid. Df Resid. Dev Pr(>Chi)
```

```
## NULL                         23     24.564
## Treatment  2   1.9219        21     22.642   0.3825
```

Even when comparing only the Drupella treatments against each other, no significant difference in infection rate could be found. Meaning that the BrB infection rate in the direct treatment is not significantly different from the infection rate in the 12h or 24h treatment.

So infection rate after 12h and 24h is similar to infection rate in the direct treatment.

Now we want to know if the presence of Drupella significantly affects the nubbins infection rate. To test this, we pooled all the Drupella treatments together and tested them against the combined controls.

Model with just Factor: Drupella (Direct, 12h and 24h) against Control (Injury, Water)

```
DRUP.glm2<-glm(Status~Factor,family=binomial, data=DRUP)
summary(DRUP.glm2)

##
## Call:
## glm(formula = Status ~ Factor, family = binomial, data = DRUP)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.68354  -0.68354  -0.00008  -0.00008   1.77122
##
## Coefficients:
##                Estimate Std. Error z value Pr(>|z|)
## (Intercept)      -19.57    2688.50  -0.007    0.994
## FactorDrupella    18.23    2688.50   0.007    0.995
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 30.142  on 39  degrees of freedom
## Residual deviance: 24.564  on 38  degrees of freedom
## AIC: 28.564
##
## Number of Fisher Scoring iterations: 18
```

Check for Goodness of fit and Overdispersion

```
DRUP2.resid<-sum(resid(DRUP.glm2,type="pearson")^2)
1-pchisq(DRUP2.resid,DRUP.glm2$df.resid)

## [1] 0.9625835

1-pchisq(DRUP.glm2$deviance,DRUP.glm2$df.resid)

## [1] 0.9548658

DRUP2.resid/DRUP.glm2$df.resid

## [1] 0.6315789

DRUP.glm2$deviance/DRUP.glm2$df.resid
```

```
## [1] 0.6464085

#No lack of fit and no overdispersion

anova(DRUP.glm2,test="LRT")

## Analysis of Deviance Table
##
## Model: binomial, link: logit
##
## Response: Status
##
## Terms added sequentially (first to last)
##
##
##        Df Deviance Resid. Df Resid. Dev Pr(>Chi)
## NULL                      39     30.142
## Factor  1   5.5781        38     24.564  0.01819 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Drupella has a significant effect on BrB infection rate. Meaning that the infection rate was significantly higher in the Drupella treatments than in the controls.

APPENDIX C: R script Chapter 4

Example of Ripley's K function for disease distribution

Quadrat 5

```
require(sp)

require(spatstat)
```

Create modified window of available space for Quadrat 5

```
GridMap5<-read.csv("/Users/kjnicolet/Desktop/Spatial Analysis
Paper/GIS Maps/Map5/Map5_Grid.csv", header=T, strip.white=T, dec=".")

summary(GridMap5)

##        x                y           Coral
##  Min.   :0.050   Min.   :0.050   Mode :logical
##  1st Qu.:2.525   1st Qu.:2.525   FALSE:4261
##  Median :5.000   Median :5.000   TRUE :5739
##  Mean   :5.000   Mean   :5.000   NA's :0
##  3rd Qu.:7.475   3rd Qu.:7.475
##  Max.   :9.950   Max.   :9.950

Map5<-as.owin(GridMap5)
plot(Map5)
```


```
summary(Map5)

## binary image mask
## 100 x 100 pixel array (ny, nx)
```

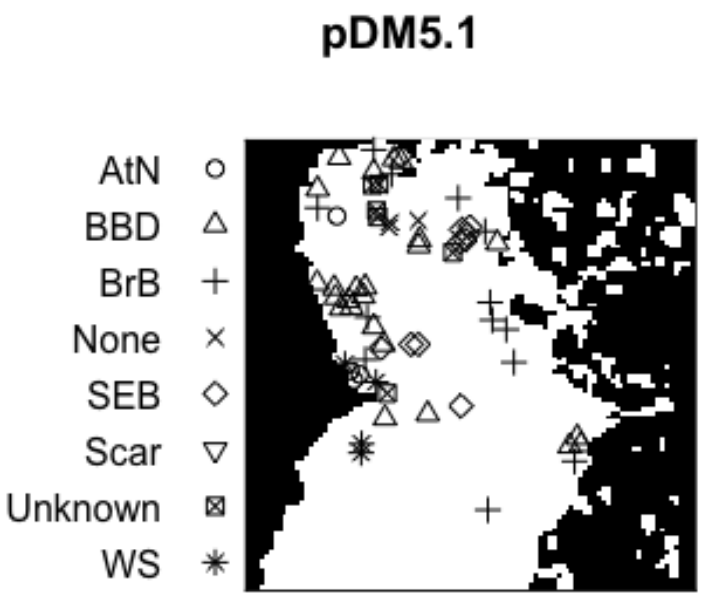
```
## pixel size: 0.1 by 0.1 units
## enclosing rectangle: [0, 10] x [0, 10] units
## Window area = 57.39 square units
## Fraction of frame area: 0.574
```

The file used containes x y coordinates of all grid cells with either "coral" or "other". The spatstat package transformes this into a window in which the spatial analysis can be run.

Sampling period 03-06.13

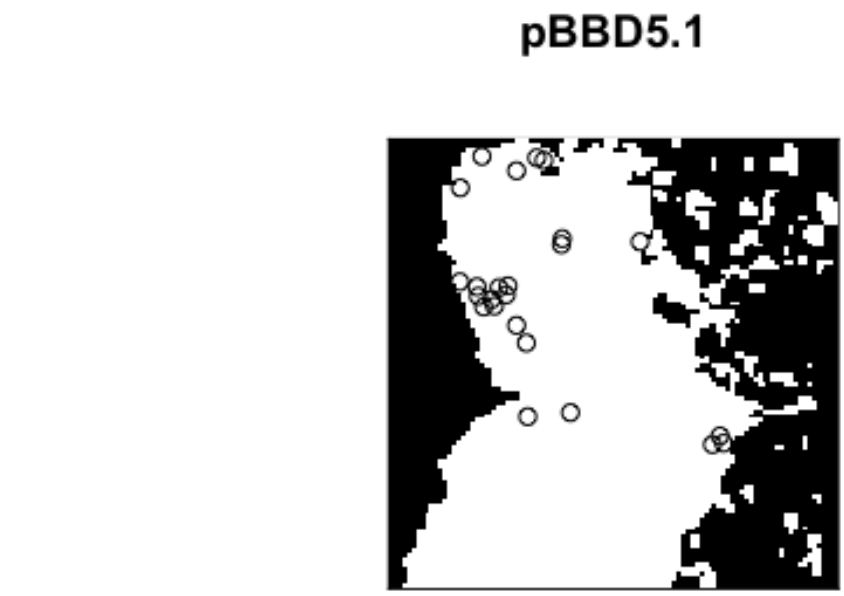
Create point pattern for disease

```
#All samples (scars and diseases)
M5.1<-read.csv("/Users/kjnicolet/Desktop/Spatial Analysis Paper/GIS Maps/Map5/Map5_Disease_030613.csv",header=T,strip.white=T,dec=".")
#M5.1

#All disease samples (-scars)
DM5.1<-subset(M5.1,Disease!="Scar")
#DM5.1

pDM5.1<-ppp(DM5.1$x,DM5.1$y,window=Map5,marks=DM5.1$Disease)

plot(pDM5.1,window=Map5)
```

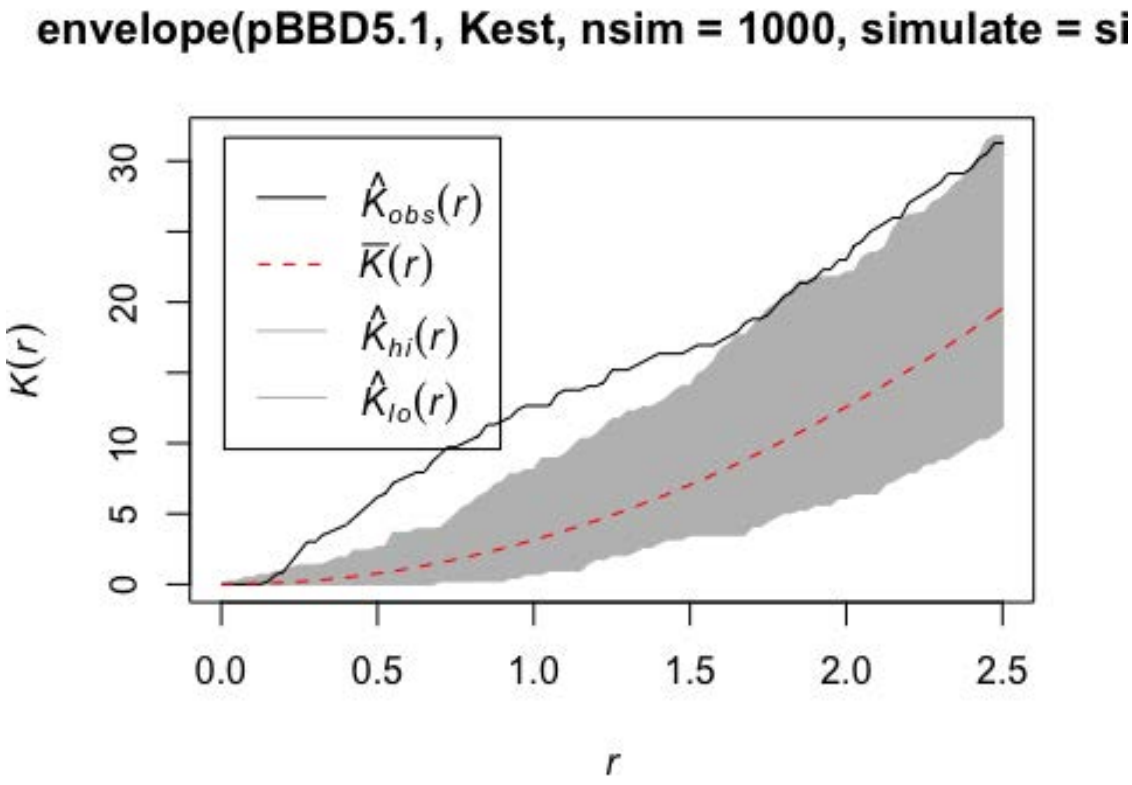


Spatial distribution analysis for BBD between mar and jun 2013

```
BBD5.1<-subset(DM5.1,Disease=="BBD")
#BBD5.1

pBBD5.1<-ppp(BBD5.1$x,BBD5.1$y,window=Map5)
plot(pBBD5.1,window=Map5)
```




```
sim<-expression(runifpoint(pBBD5.1$n,win=Map5))
plot(envelope(pBBD5.1,Kest,nsim=1000,simulate=sim))

## Generating 1000 simulations by evaluating expression  ..........
## Done.
```



Similar analysis were run for all diseases in all sampling periods

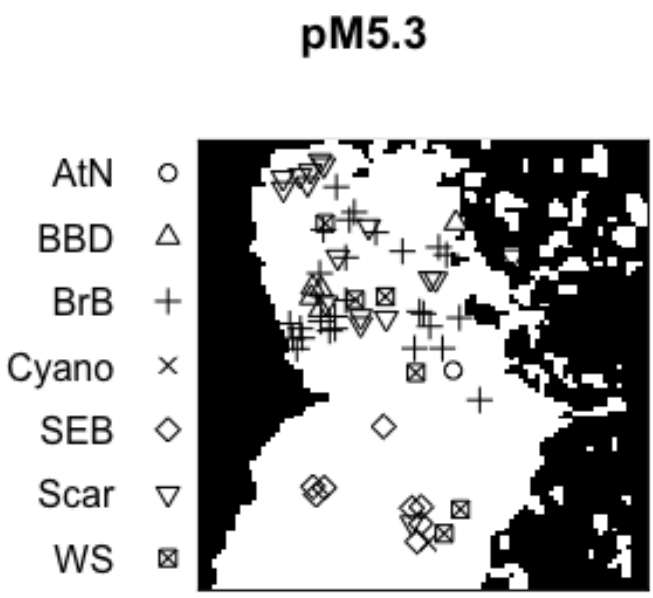
Example of K cross function

Tests whether distribution of disease and feeding scars are associated

Sampling period 01-03.14

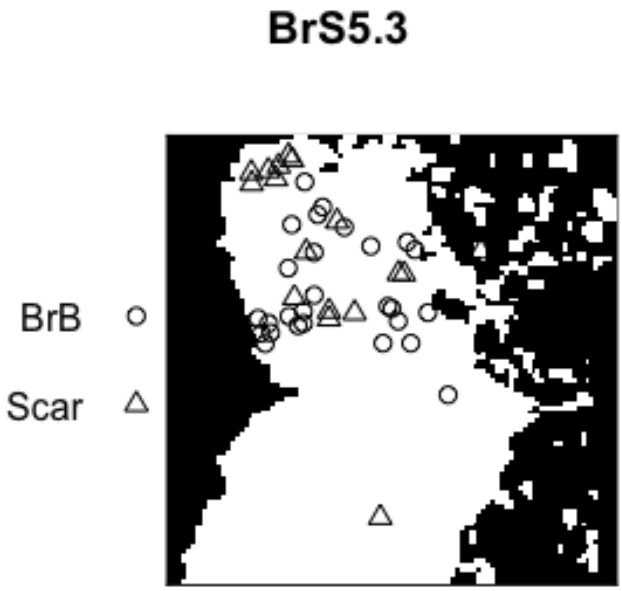
Create point pattern for disease

```
#All samples (scars and diseases)
M5.3<-read.csv("/Users/kjnicolet/Desktop/Spatial Analysis Paper/GIS Maps/Map5/Map5_Disease_010314.csv",header=T,strip.white=T,dec=".")
pM5.3<-ppp(M5.3$x,M5.3$y,window=Map5,marks=M5.3$Disease)

plot(pM5.3,window=Map5)
```

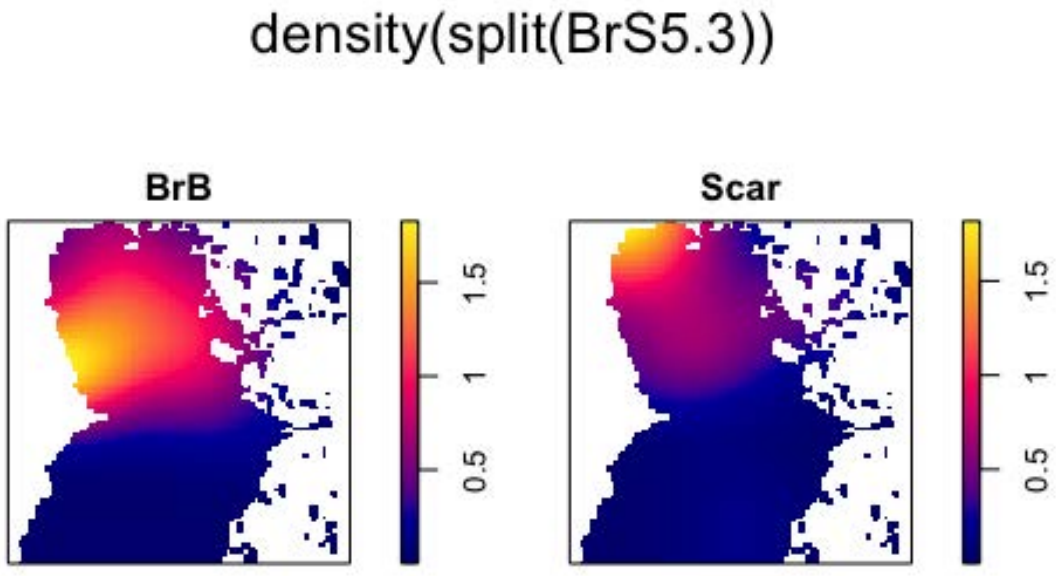


BrB

```
BrB5.3<-split(pM5.3)$BrB
Scar5.3<-split(pM5.3)$Scar

BrS5.3<-superimpose(BrB=BrB5.3,Scar=Scar5.3)
plot(BrS5.3)
```

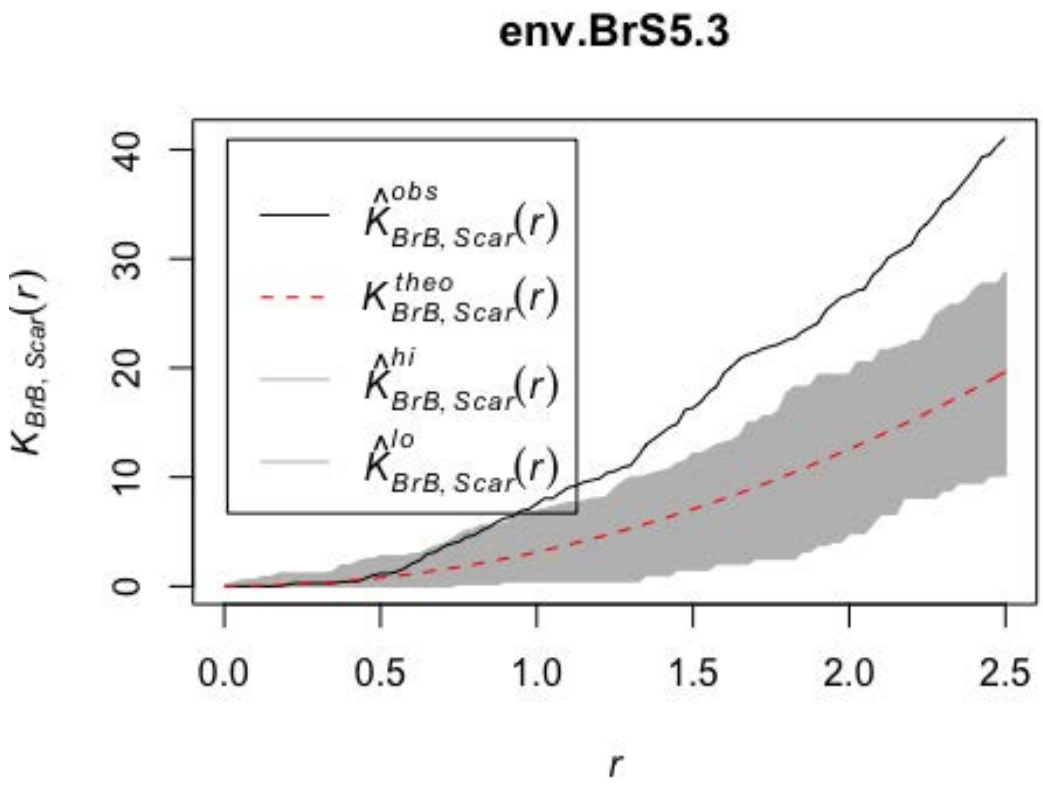



```
plot(density(split(BrS5.3)))
```



Run Kcross simulation

```
env.BrS5.3<-envelope(BrS5.3,Kcross,nsim=1000,i="BrB",j="Scar")

## Generating 1000 simulations of CSR  ...
## Done.

plot(env.BrS5.3)
```


env.BrS5.3
$K_{BrB,\ Scar}(r)$
$\hat{K}^{obs}_{BrB,\ Scar}(r)$
$K^{theo}_{BrB,\ Scar}(r)$
$\hat{K}^{hi}_{BrB,\ Scar}(r)$
$\hat{K}^{lo}_{BrB,\ Scar}(r)$
0
10
20
30
40
0.0
0.5
1.0
1.5
2.0
2.5
r

APPENDIX D: R script Chapter 5

PCA of environmental factors

```
data<-read.csv("/Users/kjnicolet/Desktop/Env Factors
Paper/Stats/PCA/xls csv/PCA.csv",header=T, strip.white=T)



data$Trip<-as.factor(data$Trip)
data$Map<-as.factor(data$Map)

summary(data)

##  Trip         Date           Map         Light            Temp
##  1:11   13.05.26: 2   Horseshoe:30   Min.   : 5026   Min.   :24.16
##  2:16   13.05.30: 2   Trawler  :35   1st Qu.:13534   1st Qu.:25.87
##  3:26   13.10.30: 2                  Median :17122   Median :27.26
##  4:12   13.11.01: 2                  Mean   :17470   Mean   :27.26
##         13.11.03: 2                  3rd Qu.:21904   3rd Qu.:29.12
##         13.11.05: 2                  Max.   :34787   Max.   :29.84
##         (Other) :53
##       TDP              TDN             DIN              Chla
##  Min.   :0.0460   Min.   :3.801   Min.   :0.1270   Min.   :0.0040
##  1st Qu.:0.0830   1st Qu.:5.261   1st Qu.:0.6908   1st Qu.:0.0900
##  Median :0.1050   Median :5.804   Median :0.9510   Median :0.1425
##  Mean   :0.1214   Mean   :6.051   Mean   :1.0536   Mean   :0.1740
##  3rd Qu.:0.1585   3rd Qu.:6.825   3rd Qu.:1.3094   3rd Qu.:0.2160
##  Max.   :0.2670   Max.   :9.470   Max.   :3.0630   Max.   :0.6520
##
##       PN              PC              PP                SS
##  Min.   :0.627   Min.   :11.63   Min.   :0.01800   Min.   :0.0000
##  1st Qu.:3.360   1st Qu.:20.92   1st Qu.:0.04200   1st Qu.:0.5350
##  Median :4.118   Median :24.63   Median :0.05100   Median :0.7100
##  Mean   :4.361   Mean   :25.86   Mean   :0.05375   Mean   :0.8381
##  3rd Qu.:5.235   3rd Qu.:29.64   3rd Qu.:0.06200   3rd Qu.:1.0600
##  Max.   :8.339   Max.   :49.43   Max.   :0.10800   Max.   :2.7500
##
```

PCA

```
library(vegan)

data.pca<-rda(data[,c(-1:-3)],scale=TRUE) #scale=T means using
correlation. Scale = F means using covariance.
summary(data.pca)

##
## Call:
## rda(X = data[, c(-1:-3)], scale = TRUE)
##
## Partitioning of correlations:
##               Inertia Proportion
## Total              10          1
```

```
## Unconstrained      10          1
##
## Eigenvalues, and their contribution to the correlations
##
## Importance of components:
##                            PC1    PC2    PC3    PC4     PC5     PC6
PC7
## Eigenvalue            4.2062 1.2995 1.1890 0.8770 0.80219 0.56019
0.41522
## Proportion Explained  0.4206 0.1300 0.1189 0.0877 0.08022 0.05602
0.04152
## Cumulative Proportion 0.4206 0.5506 0.6695 0.7572 0.83739 0.89341
0.93493
##                            PC8     PC9    PC10
## Eigenvalue            0.24543 0.22347 0.18179
## Proportion Explained  0.02454 0.02235 0.01818
## Cumulative Proportion 0.95947 0.98182 1.00000
##
## Scaling 2 for species and site scores
## * Species are scaled proportional to eigenvalues
## * Sites are unscaled: weighted dispersion equal on all dimensions
## * General scaling constant of scores:  5.029734
##
##
## Species scores
##
##           PC1     PC2      PC3      PC4       PC5     PC6
## Light -0.4643  0.5989  1.17713 -0.02602  0.547545 -0.3473
## Temp   1.0992  0.7261  0.22604  0.07268  0.531140  0.2760
## TDP   -1.1213 -0.2474  0.24394  0.67239  0.103199  0.6238
## TDN   -0.5212 -0.7913 -0.04655 -0.98613  0.762016  0.2677
## DIN    0.5432  0.2383 -1.09742  0.40833  0.783410 -0.2408
## Chla   1.2834 -0.4100  0.19653  0.20389 -0.007104  0.5773
## PN     1.3808 -0.3491  0.20116 -0.13485 -0.034273 -0.1867
## PC     1.2664 -0.5260  0.22535 -0.09541 -0.239303 -0.3460
## PP     1.3192 -0.2825  0.36159  0.33305  0.267694  0.1093
## SS    -0.7040 -1.0053  0.21145  0.66450  0.335071 -0.4492
##
##
## Site scores (weighted sums of species scores)
##
##             PC1        PC2      PC3      PC4       PC5      PC6
## sit1   0.153189  0.8252493  0.13859 -0.80547 -0.322505 -0.36179
## sit2   0.416794 -0.6616900  0.05653 -0.23264 -0.119938 -0.34669
## sit3   0.439190 -0.3567923  0.32105 -0.47073 -0.159614 -0.48170
## sit4  -0.125281  0.3851385 -0.72761 -0.55852 -0.659988  0.07649
## sit5   0.197270  0.2729322 -0.66491 -0.10018 -1.071219 -0.17779
## sit6  -0.091424  0.1508082 -0.28420  0.49761 -0.092434 -0.46578
## sit7  -0.203954 -0.1571519 -0.07368  0.54933 -0.535663 -0.09572
## sit8  -0.172338  0.0128447 -0.16763 -0.22065 -0.453440 -0.05389
## sit9  -0.256544 -0.4109286 -1.16845 -0.15141  0.063893  0.07133
## sit10  0.287700 -0.8227881 -0.88959 -0.31730 -0.331137 -0.27530
## sit11 -0.204539 -0.2564627 -0.56553 -0.72162 -0.143810 -0.98187
## sit12 -0.041409  0.4811635  0.60767 -0.91771  0.124144 -1.02235
```

```
## sit13  0.609473 -0.5332482  0.74895 -0.17747  0.363155 -1.05363
## sit14  0.585558  0.0868114  0.95434 -0.79682  0.072069  0.48803
## sit15  0.576986 -0.4789352 -0.09490 -1.01516  0.488422 -0.44256
## sit16  0.259491 -1.0789794  0.64790 -0.30879  0.261818 -1.11548
## sit17 -0.049532  0.1815756  0.43491 -0.84489  0.091891 -0.54299
## sit18  0.110968 -0.5541098  0.11859 -0.61863  0.080188 -0.50011
## sit19 -0.137774 -0.3113638  0.06308 -0.87962  0.174989 -0.58872
## sit20 -0.792710 -1.3887303  0.31195  1.20973  1.296370 -0.14879
## sit21 -0.387090  0.1864468  0.39097 -0.57805  0.516166  0.06346
## sit22 -0.344725  0.2208125 -1.29552 -0.65784  0.638195  0.22873
## sit23 -0.565117 -0.8880101 -0.56127 -0.12639  0.526884  0.04075
## sit24 -0.471938  0.8037711  0.34324 -0.13622  1.416086 -1.02960
## sit25  0.101440  0.6539508  0.64273  0.07505 -0.156365 -0.53507
## sit26 -0.289608  0.2142315 -0.34678 -0.43307 -0.948478 -0.72232
## sit27  0.274637 -0.7492092 -0.33752 -0.39055 -1.028721  0.08390
## sit28  0.027256  0.8305797 -0.28662 -0.84046 -0.398056  0.76231
## sit29  1.814516 -1.2259991  0.85420  0.21497 -0.769617  0.74920
## sit30  0.660299  0.8940536 -0.42647  0.30038  0.786318 -0.29673
## sit31  0.010726  0.6505356  0.40270 -0.08623 -0.092312 -0.19600
## sit32 -0.216432  0.6163556  0.40859  0.18245  0.049301 -0.30608
## sit33  0.462212  0.3006838  0.34895 -0.11528 -0.328205  0.10589
## sit34  1.428902 -0.5990984 -0.71152  0.34413  0.450957  0.03098
## sit35  0.709454 -0.0002432 -0.19634  0.30395 -0.910334  0.48793
## sit36  0.931606 -0.7720932  0.31797  0.62426 -0.149713  1.56359
## sit37  0.429288 -0.9700387 -0.20680 -0.27245  0.595765  1.47520
## sit38  0.228176  0.8177959 -0.06978  0.32185 -0.228509 -0.15724
## sit39  0.854136 -0.2198864  0.55766  0.39038 -0.121538  0.68249
## sit40 -0.113475  0.8213194  0.80063  0.04719  0.446389  0.69264
## sit41 -0.235950  0.7994485  0.08558  0.07803  0.213453 -0.02512
## sit42  1.038300 -0.1259018 -0.85284  1.19253  1.603901 -0.87885
## sit43  0.518445  0.6280169 -0.82188 -0.05293  0.085415 -0.09390
## sit44  1.153115 -0.1505968  0.91495  0.07003  0.498345  0.20775
## sit45  0.059966  0.0937562 -0.23133 -0.51792  0.830151  0.96052
## sit46 -0.356968  0.5116659  0.08839 -0.37741  1.252037  1.07052
## sit47  0.326034  0.6669245  0.18249  0.53611  0.449711  0.60028
## sit48 -0.454806  0.9035178  0.50117  0.82207  0.164733  1.40973
## sit49  0.242022  0.8845611 -0.32903  0.40778  0.252866 -0.16640
## sit50 -0.001585  1.1710740  0.71871  0.56014 -0.019913 -0.21230
## sit51  0.561035  1.0388889  0.14190  0.73979 -0.001498  0.06247
## sit52  0.155709  0.2207516 -0.67219  0.64388 -1.196446 -0.20687
## sit53  0.447740  0.5620741 -1.52731  0.50031 -0.913503 -0.16335
## sit54 -1.158330 -0.5231701  0.18190 -1.10281  0.754654  0.64490
## sit55 -0.927888 -0.2925446  0.07392 -1.37034 -0.508668  0.71835
## sit56 -1.050841  0.0992669  0.40264  0.55708 -0.579269  0.54939
## sit57 -0.930870 -0.1629608 -0.13723  0.01916 -0.819807  1.12163
## sit58 -1.063882 -0.0212532  0.53896  0.79850 -0.113260  0.28632
## sit59 -0.501029 -0.3112346  0.39744 -0.08197 -1.325362 -0.18761
## sit60 -0.888457 -0.0802293 -0.84549  0.92235  0.008666  0.03523
## sit61 -0.712931 -0.4036722 -1.36897  1.27789  0.412397 -0.38168
## sit62 -0.634792 -0.6914782  1.15860  0.90049 -0.159693 -0.49987
## sit63 -0.919083 -0.7269708 -0.79782 -0.46055  0.756510  0.66762
## sit64 -0.884118 -0.0684101  1.17821  0.35416 -0.445973 -0.48569
## sit65 -0.886211 -0.9928252  0.62316  1.29651 -0.620853 -0.73775
```

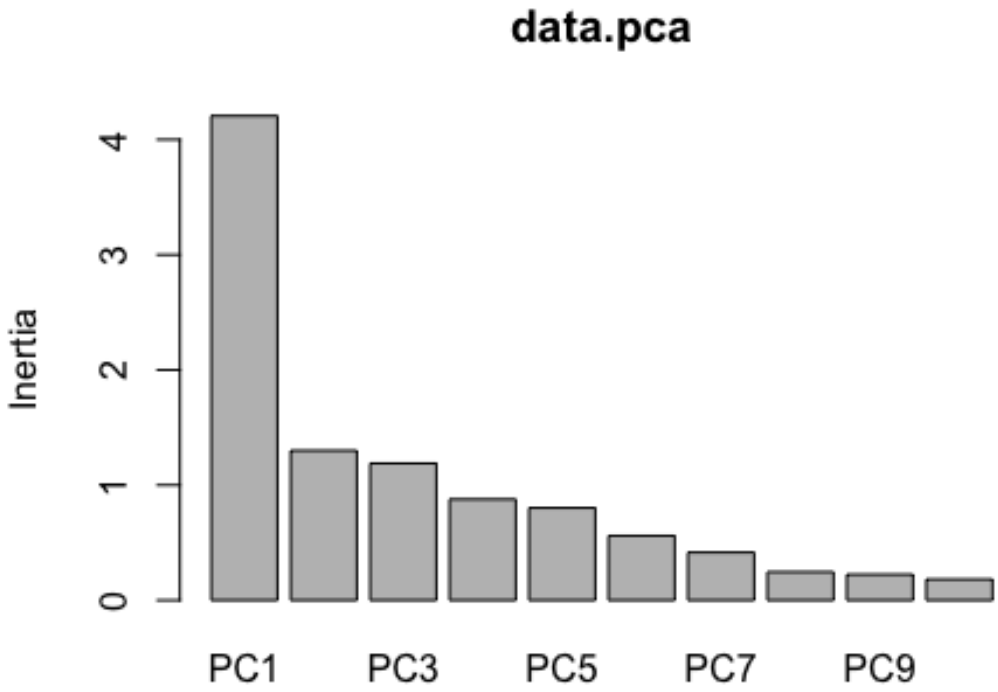
Eigenvalues: how much of the total variance is explained by each axis. If no correlation at all -> same anount of PC than nb of column and all with vallue = 1. If Eigenvalue greater than 1, it means that PC axis explains more than what would be expected by random.

Data reduction: 1) keep only PC axis that are more than 1 (when using correlation scale) 2) only keep axis that, cumulated, explain >80% (see cumulatice proportion values). 3) plot the eigenvalues and get a screen plot and find the "elbow" where line goes from steep to flat.

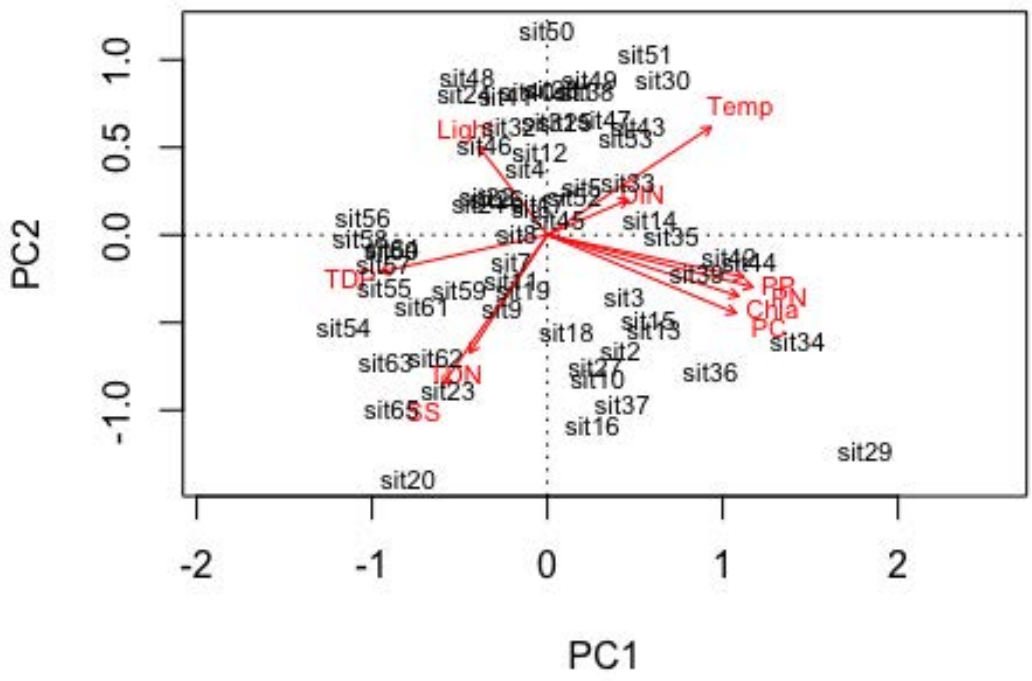
Component loadings: species (here env factors) and site (here date) scores. It's the contribution to each species/site to each PC axis. Look for high correlation (high absolute value).

So in this case, PC1 is the most important, explaining 42%. Temp, TDP, PC, PN, PP and Chla are the main drivers of PC1. For PC2 the main drivers are Temp and TDN.

```
screeplot(data.pca)
```




```
biplot(data.pca,scaling=2)
```



Boosted Regression Trees

Date Exploration

All Trips using data with average prog by colony

All Env variables

```
env<-read.csv("/Users/kjnicolet/Desktop/Env Factors
Paper/Stats/BRT/xls csv/BRT_Colony_Final.csv",header=T, strip.white=T)



env$Map<-as.factor(env$Map)
env$Colony<-as.factor(env$Colony)

env<-subset(env, DIN<4)
summary(env)
```

```
##         Trip           Date         Disease    Map          Colony
##  JanMar14:945   13.05.30: 112   AtN     :222   1:616   3.4.1  :  13
##  JulSep14:488   13.11.07: 107   BBD     :553   2:445   3.4.10 :  13
##  MarJun13:576   13.11.11: 100   BrB     :195   3:637   3.4.11 :  13
##  OctNov13:752   14.01.26: 100   Cyano   :186   4:263   3.4.12 :  13
##                 13.11.03:  99   SEB     :623   5:800   3.4.14 :  13
##                 13.11.05:  99   Unknown:148             3.4.8  :  13
```

```
##                 (Other) :2144   WS     :834           (Other):2683
##       Prog              Light             Temp             TDP
##  Min.   :0.00010   Min.   : 5026   Min.   :24.16   Min.   :0.0460
##  1st Qu.:0.03393   1st Qu.:13534   1st Qu.:25.87   1st Qu.:0.0833
##  Median :0.13347   Median :17755   Median :27.04   Median :0.1020
##  Mean   :0.31804   Mean   :17577   Mean   :27.17   Mean   :0.1225
##  3rd Qu.:0.40719   3rd Qu.:21904   3rd Qu.:29.01   3rd Qu.:0.1590
##  Max.   :4.09506   Max.   :34787   Max.   :29.84   Max.   :0.2670
##
##       TDN              DIN               Chla              PN
##  Min.   :3.801   Min.   :0.1270   Min.   :0.0040   Min.   :0.627
##  1st Qu.:5.293   1st Qu.:0.6908   1st Qu.:0.0900   1st Qu.:3.191
##  Median :5.901   Median :0.9510   Median :0.1390   Median :4.118
##  Mean   :6.122   Mean   :1.0296   Mean   :0.1661   Mean   :4.309
##  3rd Qu.:6.899   3rd Qu.:1.2674   3rd Qu.:0.2105   3rd Qu.:5.283
##  Max.   :8.823   Max.   :2.3430   Max.   :0.6520   Max.   :8.339
##
##        PC               PP                SS
##  Min.   :11.63   Min.   :0.01800   Min.   :0.0000
##  1st Qu.:20.69   1st Qu.:0.04200   1st Qu.:0.5100
##  Median :24.52   Median :0.04900   Median :0.7550
##  Mean   :25.71   Mean   :0.05182   Mean   :0.8432
##  3rd Qu.:29.64   3rd Qu.:0.05800   3rd Qu.:1.0750
##  Max.   :49.43   Max.   :0.10800   Max.   :2.7500
##
```

```
BBD<-subset(env, Disease=="BBD")
summary(BBD)
```

BRT

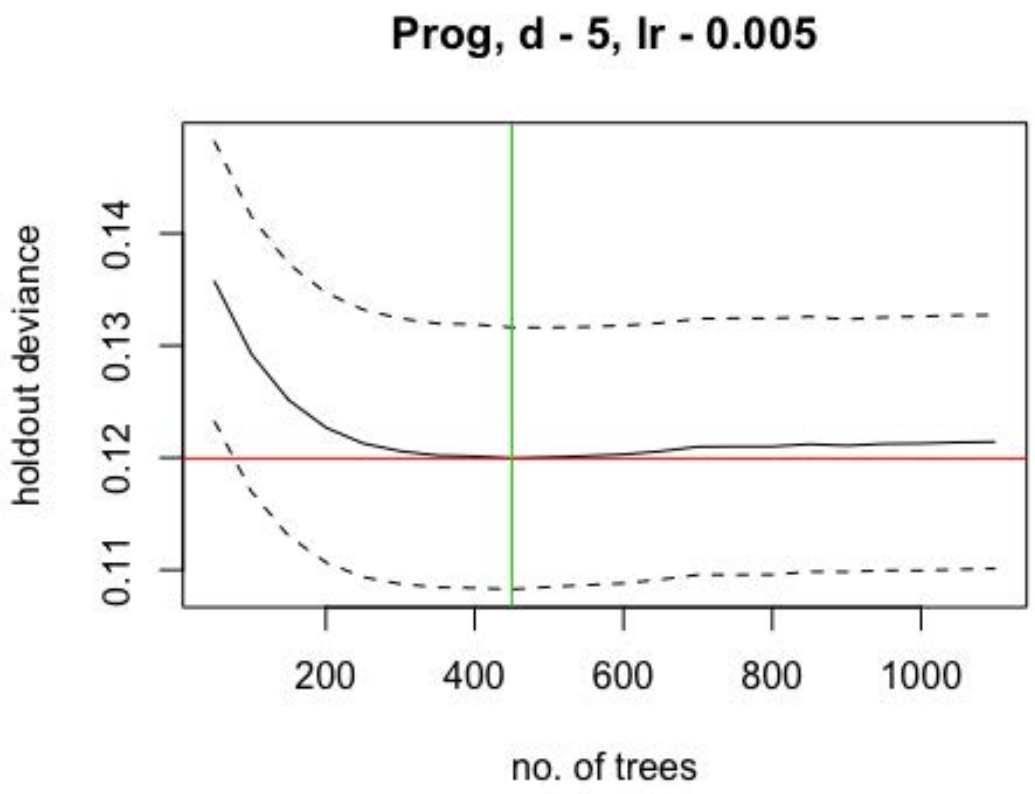
Using 7 variables

Examples of BBD disease

```
library(dismo)

BBD1<-
gbm.step(data=BBD,gbm.x=7:16,gbm.y=6,family="gaussian",tree.complexity
= 5, learning.rate = 0.005, bag.fraction = 0.5)
```

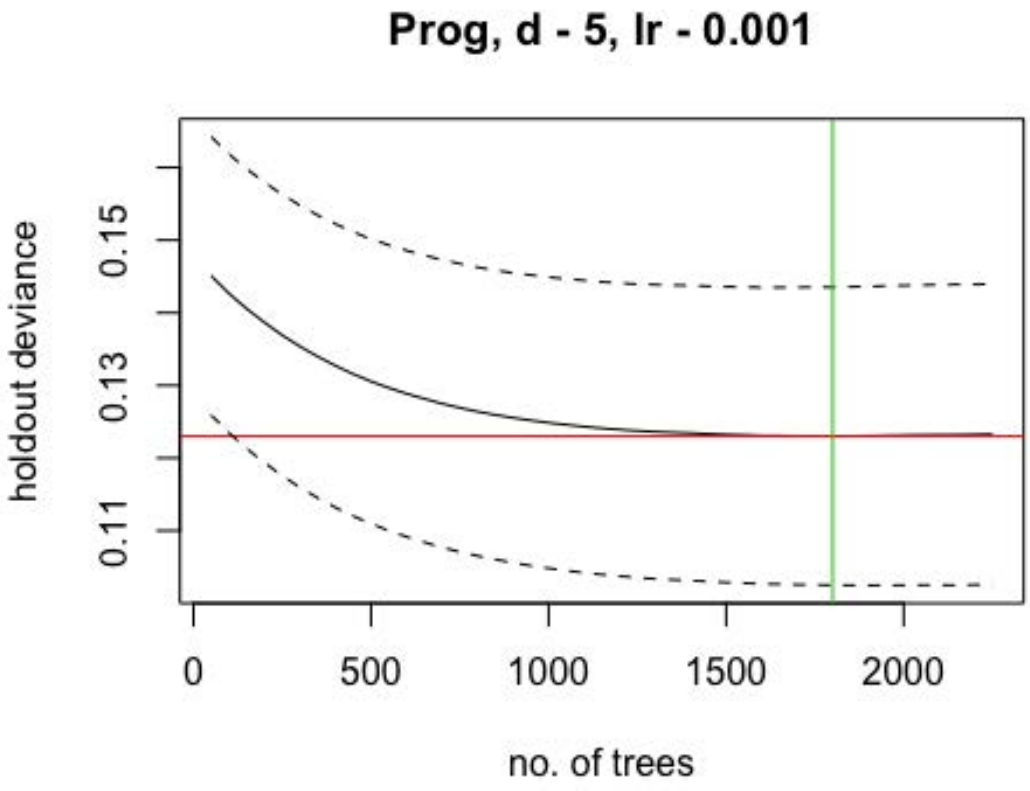
```
##
##
##  GBM STEP - version 2.9
##
## Performing cross-validation optimisation of a boosted regression
tree model
## for Prog and using a family of gaussian
## Using 553 observations and 10 predictors
## creating 10 initial models of 50 trees
##
##  folds are unstratified
## total mean deviance =  0.1465
## tolerance is fixed at  1e-04
## ntrees resid. dev.
```

```
## 50    0.1358
## now adding trees...
## 100   0.1292
## 150   0.1251
……

## fitting final gbm model with a fixed number of 450 trees for Prog
```



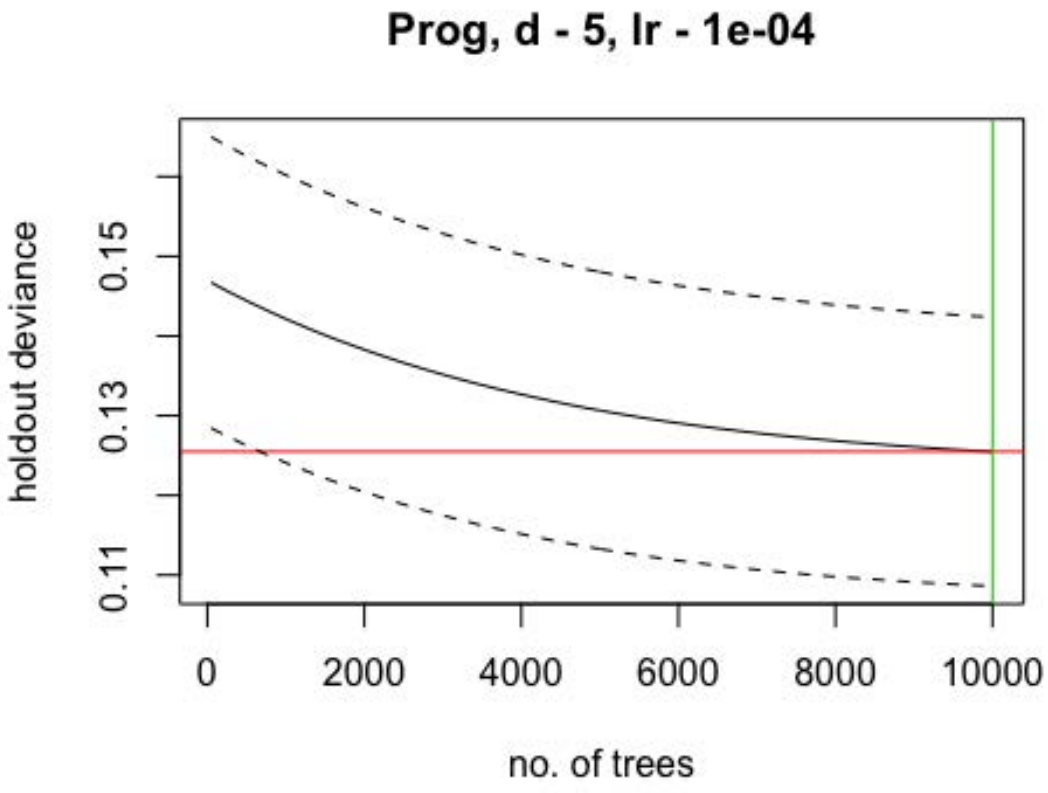

```
##
## mean total deviance = 0.147
## mean residual deviance = 0.107
##
## estimated cv deviance = 0.12 ; se = 0.012
##
## training data correlation = 0.526
## cv correlation =  0.441 ; se = 0.021
##
## elapsed time -  0.08 minutes

BBD2<-
gbm.step(data=BBD,gbm.x=7:16,gbm.y=6,family="gaussian",tree.complexity
= 5, learning.rate = 0.001, bag.fraction = 0.5)

##
##
##  GBM STEP - version 2.9
##
## Performing cross-validation optimisation of a boosted regression
```

```
tree model
## for Prog and using a family of gaussian
## Using 553 observations and 10 predictors
## creating 10 initial models of 50 trees
##
##  folds are unstratified
## total mean deviance =  0.1465
## tolerance is fixed at  1e-04
## ntrees resid. dev.
## 50    0.1451
## now adding trees...
……

## fitting final gbm model with a fixed number of 1800 trees for Prog
```



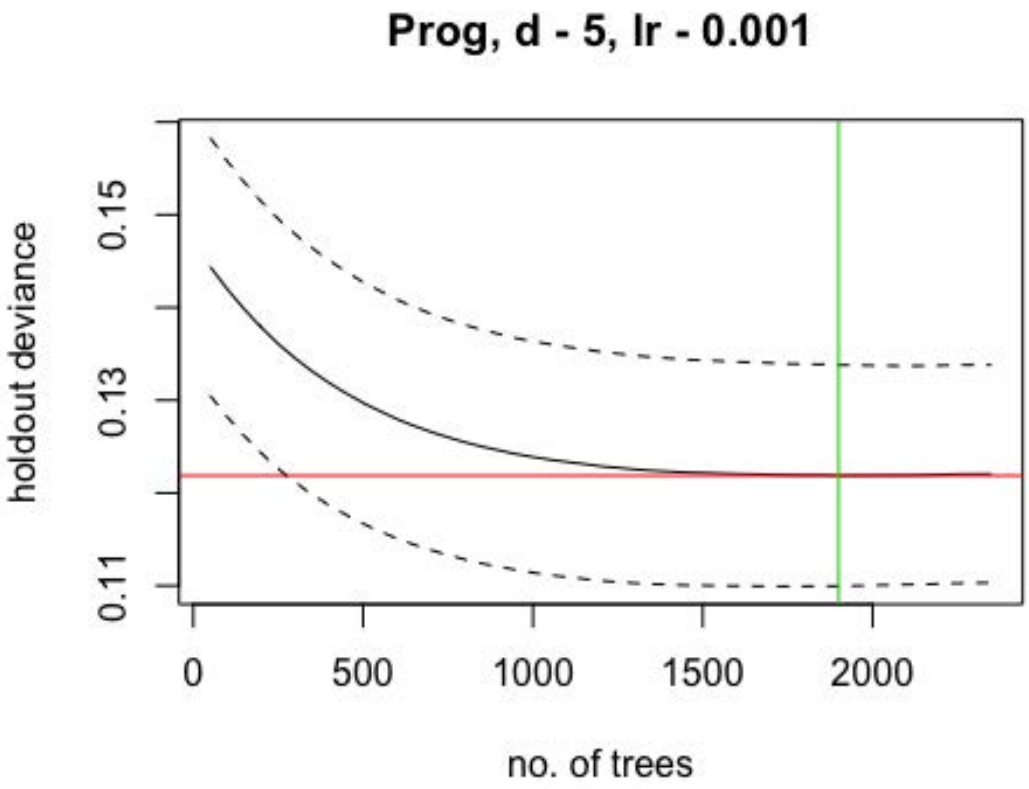

```
##
## mean total deviance = 0.147
## mean residual deviance = 0.109
##
## estimated cv deviance = 0.123 ; se = 0.021
##
## training data correlation = 0.524
## cv correlation =  0.423 ; se = 0.053
##
## elapsed time -  0.18 minutes
```

```
BBD3<-
gbm.step(data=BBD,gbm.x=7:16,gbm.y=6,family="gaussian",tree.complexity
= 5, learning.rate = 0.0001, bag.fraction = 0.5)

##
##
##  GBM STEP - version 2.9
##
## Performing cross-validation optimisation of a boosted regression
tree model
## for Prog and using a family of gaussian
## Using 553 observations and 10 predictors
## creating 10 initial models of 50 trees
##
##  folds are unstratified
## total mean deviance =  0.1465
## tolerance is fixed at  1e-04
## ntrees resid. dev.
## 50    0.1467
## now adding trees...
…….


## fitting final gbm model with a fixed number of 10000 trees for Prog
```




```
##
## mean total deviance = 0.147
## mean residual deviance = 0.115
```

```
##
## estimated cv deviance = 0.126 ; se = 0.017
##
## training data correlation = 0.516
## cv correlation =  0.407 ; se = 0.039
##
## elapsed time -  0.02 minutes
##


BBD4<-
gbm.step(data=BBD,gbm.x=7:16,gbm.y=6,family="gaussian",tree.complexity
= 5, learning.rate = 0.001, bag.fraction = 0.6)

##
##
##  GBM STEP - version 2.9
##
## Performing cross-validation optimisation of a boosted regression
tree model
## for Prog and using a family of gaussian
## Using 553 observations and 10 predictors
## creating 10 initial models of 50 trees
##
##  folds are unstratified
## total mean deviance =  0.1465
## tolerance is fixed at  1e-04
## ntrees resid. dev.
## 50    0.1444
## now adding trees...


## fitting final gbm model with a fixed number of 1900 trees for Prog
```

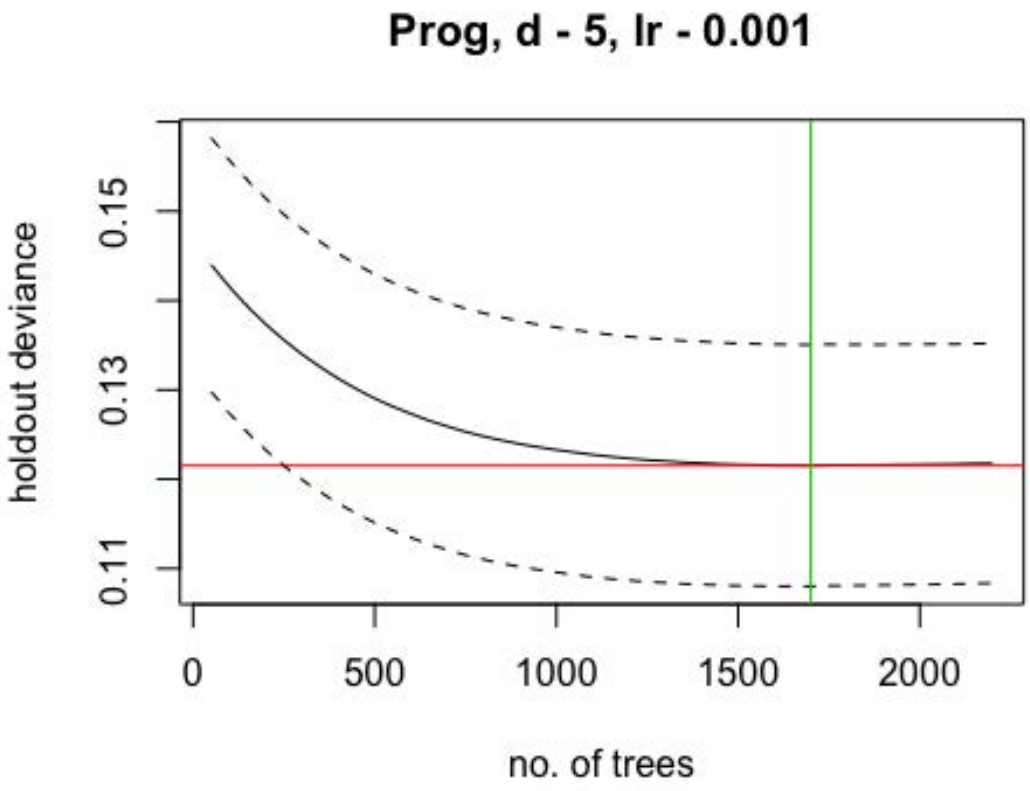



```
##
## mean total deviance = 0.147
## mean residual deviance = 0.108
##
## estimated cv deviance = 0.122 ; se = 0.012
##
## training data correlation = 0.525
## cv correlation =  0.425 ; se = 0.029
##
## elapsed time -  0.18 minutes
```

```
BBD5<-
gbm.step(data=BBD,gbm.x=7:16,gbm.y=6,family="gaussian",tree.complexity
= 5, learning.rate = 0.001, bag.fraction = 0.7)
```

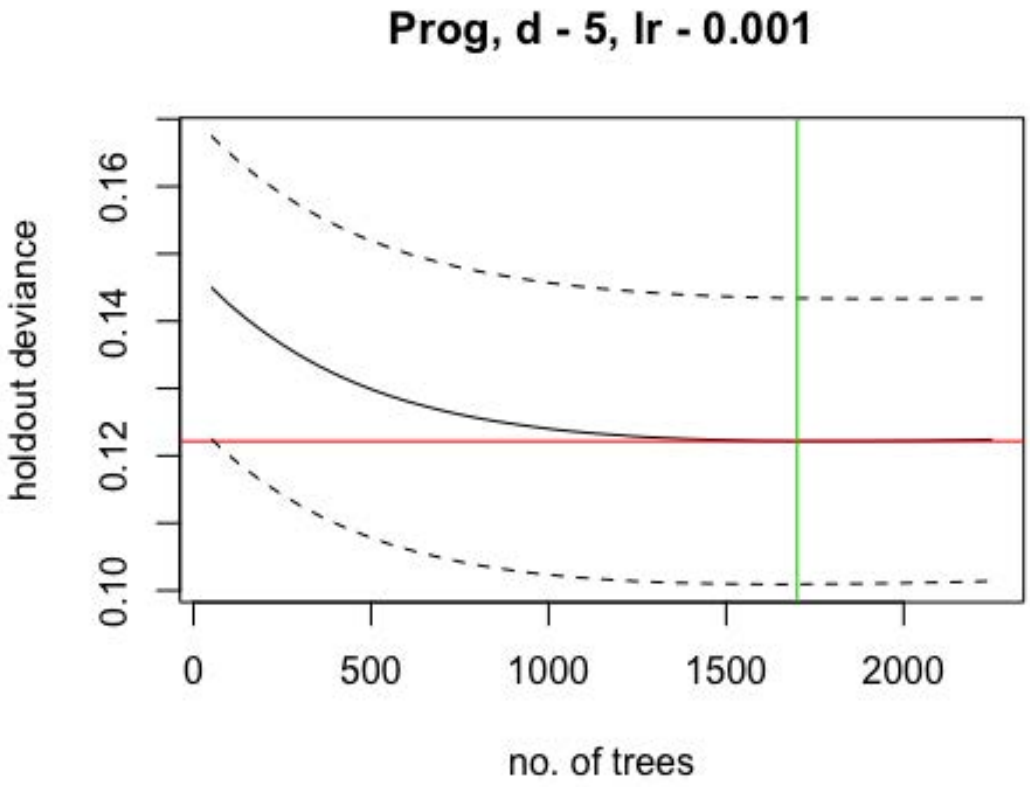
```
##
##
##  GBM STEP - version 2.9
##
## Performing cross-validation optimisation of a boosted regression
tree model
## for Prog and using a family of gaussian
## Using 553 observations and 10 predictors
## creating 10 initial models of 50 trees
##
##  folds are unstratified
## total mean deviance =  0.1465
## tolerance is fixed at  1e-04
```

```
## ntrees resid. dev.
## 50    0.144
## now adding trees...


## fitting final gbm model with a fixed number of 1700 trees for Prog
```




```
##
## mean total deviance = 0.147
## mean residual deviance = 0.109
##
## estimated cv deviance = 0.122 ; se = 0.014
##
## training data correlation = 0.523
## cv correlation =  0.423 ; se = 0.028
##
## elapsed time -  0.16 minutes

BBD6<-
gbm.step(data=BBD,gbm.x=7:16,gbm.y=6,family="gaussian",tree.complexity
= 5, learning.rate = 0.001, bag.fraction = 0.8)

##
##
##  GBM STEP - version 2.9
##
## Performing cross-validation optimisation of a boosted regression
tree model
```

```
## for Prog and using a family of gaussian
## Using 553 observations and 10 predictors
## creating 10 initial models of 50 trees
##
##  folds are unstratified
## total mean deviance =  0.1465
## tolerance is fixed at  1e-04
## ntrees resid. dev.
## 50    0.145
## now adding trees...


## fitting final gbm model with a fixed number of 1700 trees for Prog
```




```
##
## mean total deviance = 0.147
## mean residual deviance = 0.109
##
## estimated cv deviance = 0.122 ; se = 0.021
##
## training data correlation = 0.522
## cv correlation =  0.415 ; se = 0.037
##
## elapsed time -  0.16 minutes

100* (BBD1$self.statistics$mean.null -
BBD1$cv.statistics$deviance.mean) / BBD1$self.statistics$mean.null
```

```
## [1] 18.12873
```

```
100* (BBD2$self.statistics$mean.null -
BBD2$cv.statistics$deviance.mean) / BBD2$self.statistics$mean.null
```

```
## [1] 16.04067
```

```
100* (BBD3$self.statistics$mean.null -
BBD3$cv.statistics$deviance.mean) / BBD3$self.statistics$mean.null
```

```
## [1] 14.34013
```

```
100* (BBD4$self.statistics$mean.null -
BBD4$cv.statistics$deviance.mean) / BBD4$self.statistics$mean.null
```

```
## [1] 16.81802
```

```
100* (BBD5$self.statistics$mean.null -
BBD5$cv.statistics$deviance.mean) / BBD5$self.statistics$mean.null
```

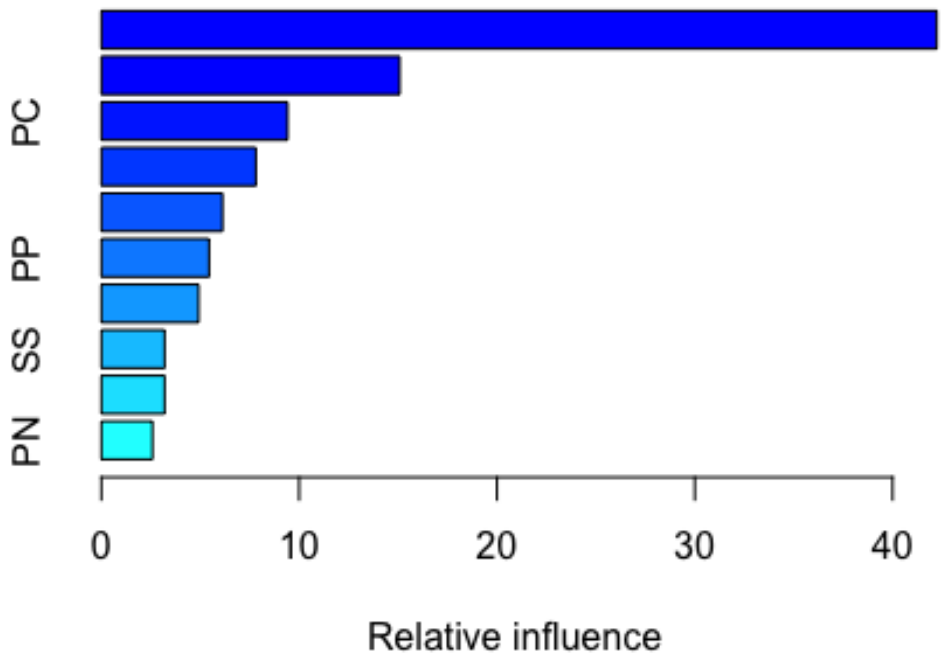
```
## [1] 17.05297
```

```
100* (BBD6$self.statistics$mean.null -
BBD6$cv.statistics$deviance.mean) / BBD6$self.statistics$mean.null
```

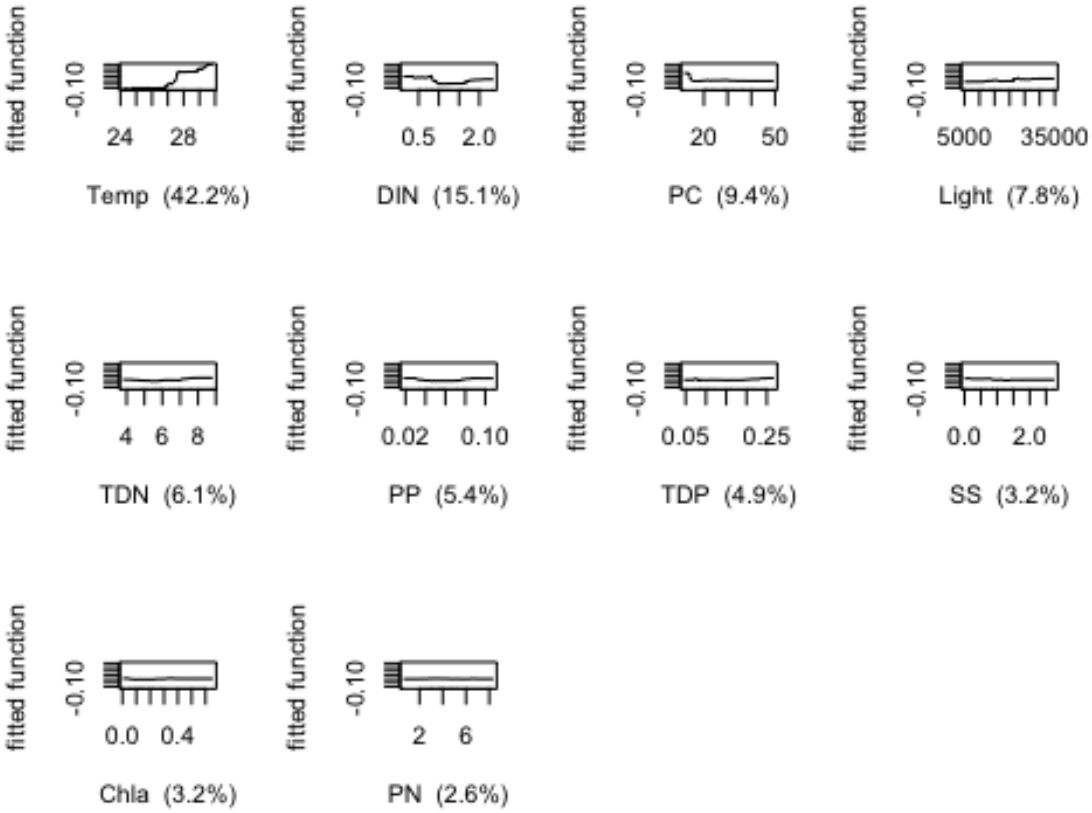
```
## [1] 16.6246
```

BBD3 [1] 14.34013

```
summary(BBD3)
```

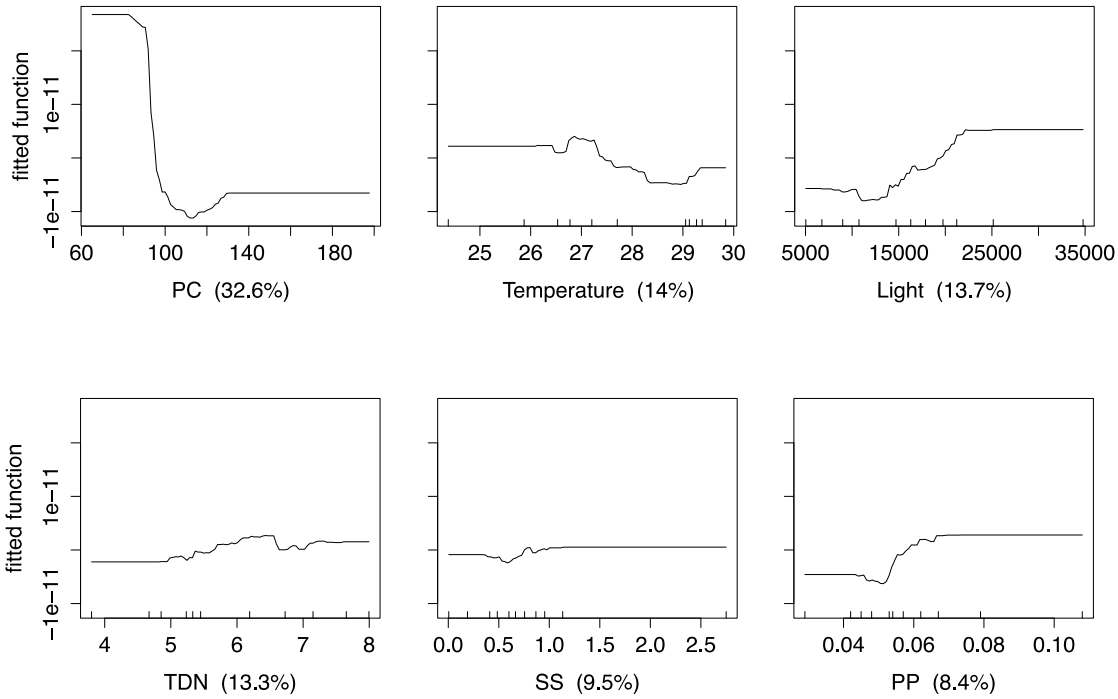


```
gbm.plot(BBD3, n.plots=10, write.title = F, rug = T)
```

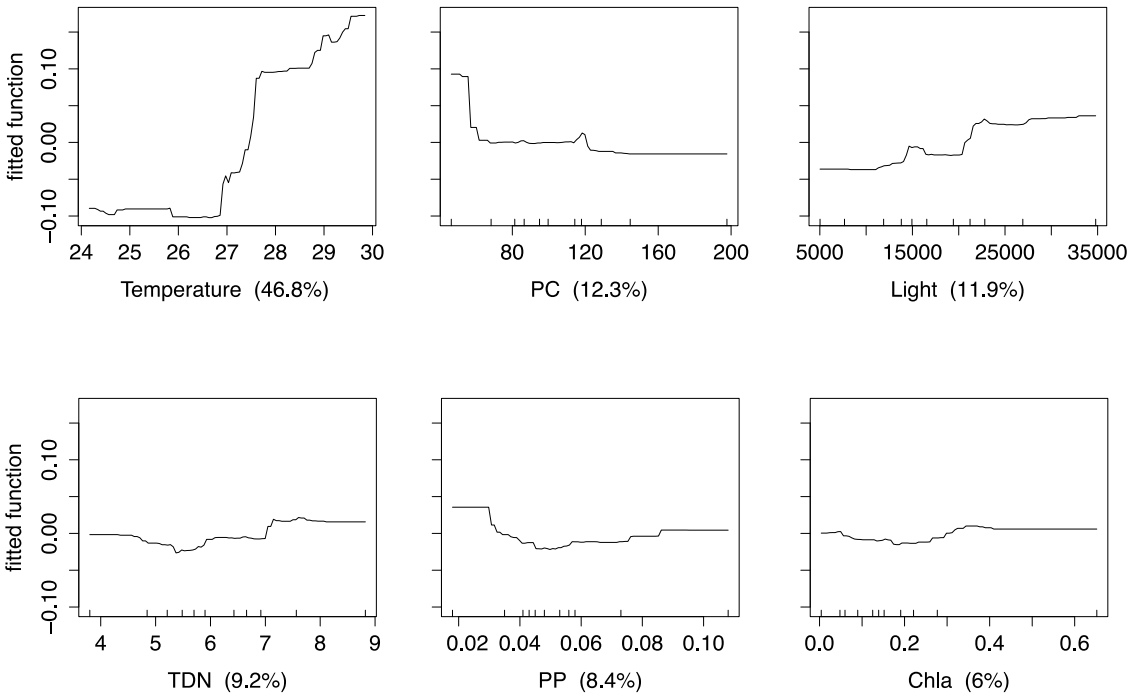


APPENDIX E: BOOSTED REGRESSION TREES GRAPHS

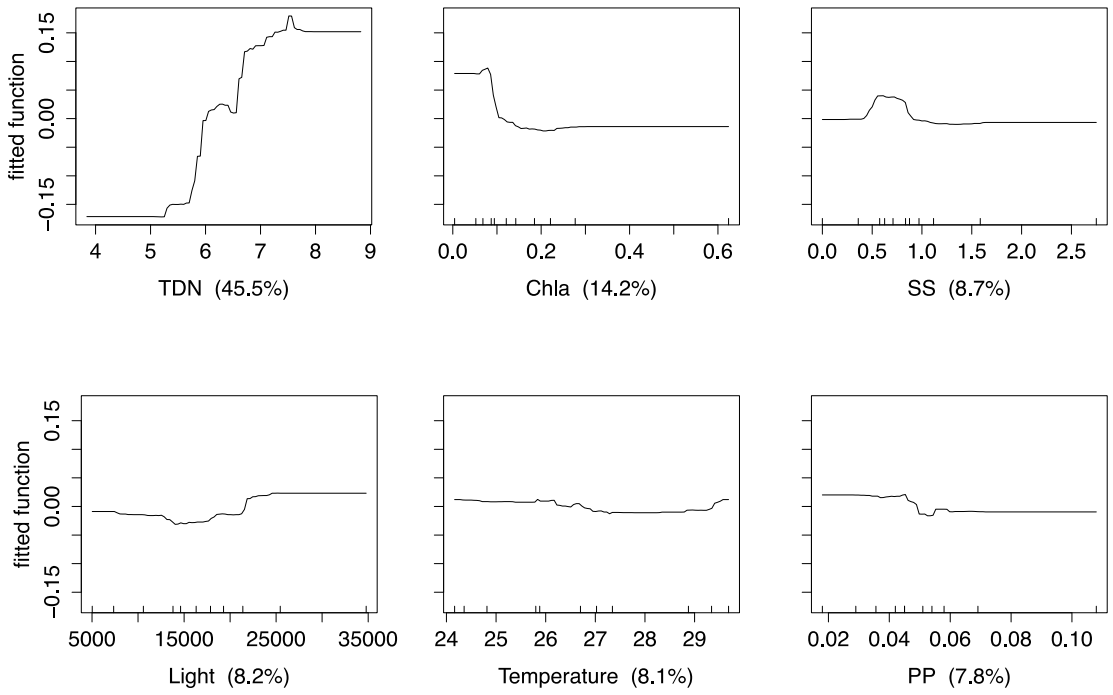
Atramentous necrosis



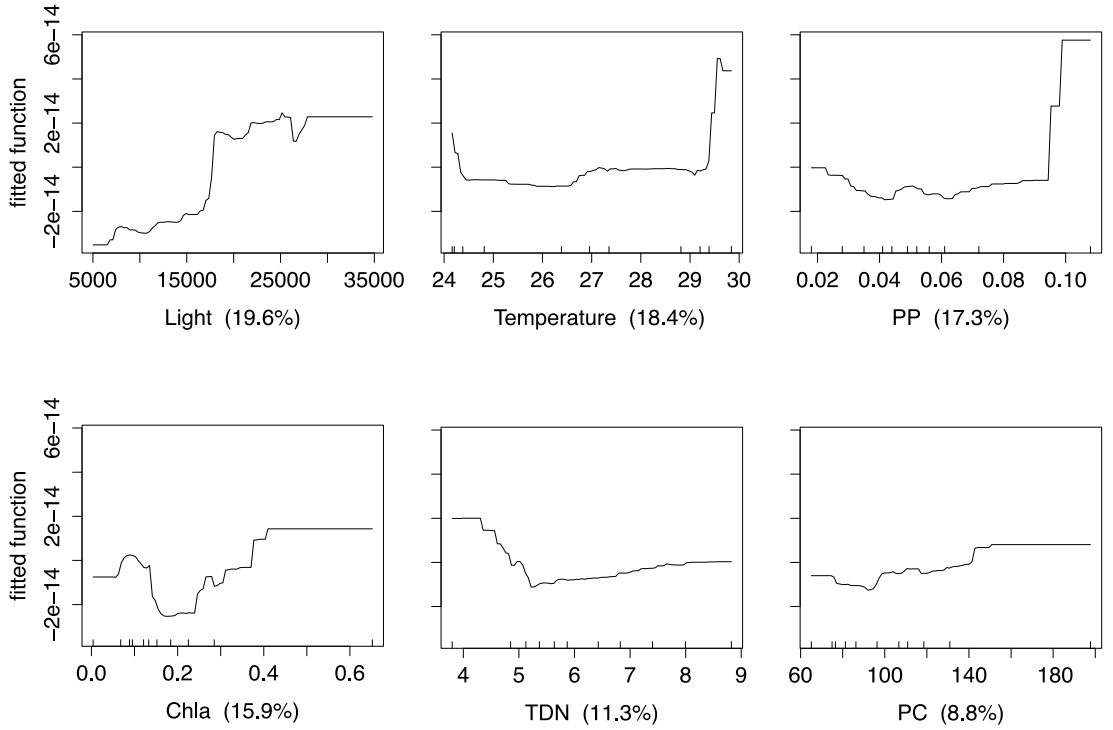
Black band disease



Brown band disease



Skeletal eroding band



White syndrome