School of Molecular and Life Science

Biological and molecular characterisation of growth anomalies affecting *Isopora palifera* at the Cocos Keeling Islands

Sophie Ogilvie Preston
0000-0002-3014-5775

This thesis is presented for the Degree of
Master of Research (Environmental Science)
of
Curtin University

July 2021

Declaration

To the best of my knowledge and belief, this thesis contains no material previously published by andother person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diplomain any University

SIGNED| Sophie Preston
DATED| July 2021

Abstract

The study of coral diseases has historically been hampered by a lack of comprehensive diagnostics for identifying diseases leading to ambiguous descriptions based on visual underwater observations. With advancing technology, the focus has shifted to describing the structure and role of microbial communities associated with the coral host and attempting to identify potential pathogens of coral diseases. This microbial research has proven challenging due to the difficulty of documenting the coral holobiont both *in situ* and in laboratory environments as a result of the plasticity of bacterial communities. Despite these challenges, the development of 16S rRNA gene sequencing technology has led to the identification of key bacterial agents in the etiology of some coral diseases. Preserving the health and productivity of coral reefs is a key management objective, hence it is important to continue to advance and expand the coral disease diagnostic toolkit. Classical characterisation of gross lesions in the field coupled with modern molecular techniques can be applied to examine coral diseases at all levels of biological complexity and inform reef management and mitigation plans. Here, I have applied an integrated approach to the characterisation of growth anomalies, a scleractinian coral disease that produces pale tumour-like masses and abnormal polyp development. In 2018, growth anomalies were observed to be affecting the reef-building coral species *Isopora palifera* at the Cocos (Keeling) Islands, a remote coral atoll in the Indian Ocean. This observation is unusual as the Cocos (Keeling) Islands have not been impacted by any recent known environmental disturbances and supports a small population with low levels of local pollution. Furthermore, this observation is the first report of growth anomalies affecting this species globally. In this study I utilise classical methods to describe the extent of the *I. palifera* population affected, quantify the distribution in comparison to two published outbreak thresholds, and characterise the biological effects of this disease on the functioning of *I. palifera.* I also apply modern molecular technologies to describe the bacterial associations of growth anomaly affected and healthy *I. palifera* and compare to communities found within the surrounding water column and at two possible point sources of aquatic pollution. Growth anomalies were reported at 75% of sites surveyed, affecting one third of the population, and qualifying as an outbreak at both site and regional level. Geochemical analyses of trace elements indicated a shift from aragonite to less dense calcite, indicating the division of energy to rapid skeletal development may have occurred at the detriment of reproductive and autotrophic capabilities. Histological analyses revealed reduced capacity for biological and reproductive functioning with implications for the future population viability and reef accretion potential. 16S rRNA gene metabarcoding indicated broad similarities in bacterial communities across all control, asymptomatic and diseased coral

samples, and that the bacterial communities found in these coral tissues were distinctly different to those found within the water column. Importantly there were no bacterial associations between potential sources of aquatic pollution and growth anomaly affected tissues, indicating that this outbreak is unlikely to be the result of local pollution. This research characterised a novel outbreak of growth anomalies, highlighting the significant impact of this disease on the current and future *I. palifera* population. Furthermore, the bacterial associations characterised in this research supports the growing evidence that growth anomalies are not caused by bacterial agents. To further investigate the dynamics of this disease, the role of corallivorous fish and non-bacterial agents should be investigated to clarify the transmission of growth anomalies across the Cocos (Keeling) Islands' reefs. Furthermore, I describe several directions for the wider research field which would advance the understanding of coral disease dynamics and implications, including rapid vector detection, improving the global marine bacteria database, and further understanding of the innate immune system. I also highlight the importance of a universal toolkit for not just growth anomalies, but the wider coral disease field to ensure standardised diagnostics for the effective management of global coral reefs.

Acknowledgements

This Master's project has provided me with the opportunity to explore one of Australia's most pristine coral reefs, an experience that has shaped the direction of my career. The challenges I have encountered during this project have enabled me to grow in confidence and ability, and I have been rewarded in the network of colleagues, collaborators, and friends I have made along the way. Firstly, I would like to thank my supervisorypanel of Dr. Zoe Richards, Dr. Kar-Chun Tan and Dr. Ben Saunders. Your advice, extensive knowledge, and unwavering support has shaped both myself as a scientist, and the path I want my career to follow. I would also like to thank everyone in the Trace and Environmental DNA (TrEnD) Laboratory, Curtin University, for their guidance and assistance over the course of this thesis.

To my co-authors: your input and advice over the course of my master's has been invaluable. For this I would like to acknowledge Dr. Megan Huggett, Dr. Merinda Nash, Dr. Nicole White, Dr. Claus Christopherson, Dr. Katrina West, Arne Adams, Matthew Heydenrych and Stephen Newman. I would also like to thank Megs Powell and Dieter Gerhard of Cocos Dive, Taj Powell, and the Water Corporation for their support during field work, the Curtin Health Innovation and Research Institute for guiding me through tissue work and the Microscopy and Microanalysis Facility, Curtin University for skeletal imagery and guidance.

Significant thankyous go towards the limitless support and advice of the TrEnD Lab. I would like to thank Nicole White for guiding me through the intimidating world of eDNA. To my friends and peers in the office: Katrina West, Matt Heydenrych, Arne Adams, David Juszkiewicz, Kristen Fernandes, Jason Alexander, Georgia Nester, Jackie Jones, Mieke van der Heyde, Matt Power, Alishum Ali; thank you for putting up with my endless supply of questions.

I would like to recognise the financial support for both fieldwork and career development, from the Australian Research Council, Western Australian Naturalist Society, Linnaean Society of New South Wales, and the Australian Coral Reef Society. I was supported personally by a Curtin University stipend for a substantially part of my Masters.

Finally, I would like to acknowledge and thank the enduring support of my parents (Joanna and Viv), who have always encouraged me to ask 'why?", my siblings (Alex, Doug, and Sam) and finally my friends (Kat, Matt, Meg, Patrick, and many more) for sharing coffee, snacks, and their friendship during my university career.

Table of Contents

Statement of Contributions

Chapter 2: Preston, S., Richards, Z. Biological consequences of an outbreak of growth anomalies on *Isopora*

palifera at the Cocos (Keeling) Islands. *Coral Reefs* (2020). https://doi.org/10.1007/s00338-020-02019-0

Author contributions: Funding acquisition, Z.R; conceptualisation and fieldwork, Z.R, S.P; laboratoryprocessing and analysis, S.P; co-writing and editing of manuscript, S.P, Z.R.

Chapter 3: Preston, S., Adams, A., Huggett, M.J., White, N., Christophersen, C., Tan, K.C., Richards, Z.

Bacterial associations of growth anomaly affected *Isopora palifera*. *[in preparation]*

Author contributions: Funding acquisition, Z.R, S.P; conceptualisation, Z.R, S.P; fieldwork, Z.R, S.P; laboratory processing, S.P, N.W; analysis, S.P, A.A, M.J.H, C.C; co-writing and editing of manuscript, S.P, A.A, M.J.H, C.C, K.C.T, Z.R.

Chapter 1&4: Preston, S., Heydenrych, M., West, K., Huggett, M.J., Tan, K.C., Richards, Z. Integrating

eDNA into the genomics age toolkit for characterising scleractinian coral diseases. *[in preparation]*

Author contributions: Conceptualisation, S.P, Z.R, K.C.T; co-writing and editing of manuscript, S.P,M.H, K.W, M.J.H, K.C.T, Z.R.

List of Figures

List of Tables

Abbreviations

ANOVA	Analysis of variance
ASV	Amplicon Sequence Variant
CKI	Cocos (Keeling) Islands
DNA	Deoxyribonucleic acid
eDNA	Environmental DNA
GA	Growth Anomaly
GTDB	Genome Taxonomy Database
NCBI	National Center for Biotechnology Information
PCO	Principal coordinate analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
PERMDISP	Permutational analysis of multivariate dispersions
qPCR	Qualitative PCR
RDP	Ribosomal Database Project
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SIMPER	Similarity percentage analysis
TrEnD	Trace and Environmental DNA Laboratory

Chapter 1

Introduction

1.1 Coral reefs in a changing world

1.1.1 The impacts of climate change on coral reefs

Coral reefs are the foundation of a sustainable and productive marine environment and globally are valued at over US$1 trillion in economic, cultural. and social currency (Costanza et al., 2014). Recent models estimate that all corals will be threatened by 2050 (Ainsworth et al., 2020), as a direct result of the increasing and cumulative impacts of climate change, and localised anthropogenic influences such as pollution and habitat degradation (Dixson et al., 2014; Rice et al., 2019). The loss of reef ecosystems as a consequence of climate change is estimated to cost US$500 billion per year by 2100 (Hoegh-Guldberg et al., 2015), and is expected to lead to ecological cascades which will impact marine biodiversity, erode reef landscapes, subsequently jeopardizing the livelihoods of an estimated 6 million people (Cinner, 2014). Since 1980, the average global temperature has increased by 1^{o}C, and in both 2016/17 and 2019/20 marine heatwaves swept the Indo-Pacific and wider Atlantic coral reefs (Burt et al., 2019; Eakin et al., 2019; Gilmour et al., 2019). These anomalous climate stress events resulted in mass coral mortality (Hughes et al., 2018), impaired stock recruitment (Hughes et al., 2019), and led to substantial changes in community composition (Vergés et al., 2019). The impact of climate change is pervasive and can lead to several outcomes including widespread bleaching (Suggett & Smith, 2020) and subsequent mortality (DeCarlo et al., 2017), physical damage from increasing cyclone frequency and intensity (Cheal et al., 2017), loss of diversity (Descombes et al., 2015; Richards & Day, 2018) and coral cover (Madin et al., 2018). Furthermore, directly, and indirectly resulting from changing environmental conditions and increasing anthropogenic pressure is the increased prevalence of coral diseases on global reefs (Woodley et al., 2015). Despite the threat diseases pose to the health and productivity to coral reefs, many data gaps still remain about the causes and consequences of coral disease.

1.2 Common tropical coral diseases

Over 40 tropical coral diseases have been described globally (Woodley et al., 2015), however several diseases have ambiguous characteristics when observed *in situ* (Ainsworth et al., 2007; Raymundo et al., 2008). Here, I have described six of the most common coral diseases found across the Indo-Pacific and Atlantic coral reefs (Woodley et al., 2015).

1.2.1 White syndromes

White syndromes (WS) refer to a group of diseases described *in situ* as displaying similar symptoms. They are the most widespread and destructive of coral diseases, affecting many taxa, however the most susceptible are reef building corals belong to the families Acroporidae, Pocilliporidae and Poritidae (Haapkyla et al., 2009; Hobbs et al., 2015). An outbreak typically results in total colony mortality and subsequent decimation of populations,and there are few reports of recovery from WS (Dalton & Smith, 2006). Over 13 variations of "white syndromes" have been reported from the Western Atlantic region (Bruckner, 2015), since first observations were made at the US Virgin Islands (Robinson, 1973). In the Wider Atlantic, white syndromes are characterised by tissue loss that manifests as spreading zones of exposed skeleton however they are almost exclusively differentiated by the rate and pattern of progression (Bruckner, 2015). The difference is often not seen in a single *in situ* survey, hence these lesions are often referred to collectively unless molecular work has been undertaken (Ainsworth et al., 2007; Bythell et al., 2004). In the Indo-Pacific, first reports of symptoms matching similar lesions observed in the West Atlantic, were made in 1985 in the Red Sea (Antonius,1985), and have now been reported across the region, from Indonesia (Sisney et al., 2018), to Japan (Wada et al., 2018), India (Thinesh et al., 2017) and the Great Barrier Reef (Willis et al., 2004). WSs in this region are grouped together, until the underlying etiology is known and a comprehensive characterisation of lesions and cellular processes is recorded (for example, *Montipora* WS) (Bourne et al., 2015).

1.2.2 Ulcerative White Spot

First described in 2003, ulcerative white spot (UWS) disease is found across the Indo-Pacific, manifesting as round or ovoid lesions that result in multifocal tissue loss patterns or colony mortality, (Raymundo et al., 2003).Initially thought to only exist on *Porites* species, it has since been recorded on *Montipora,* Faviids and *Heliopora*, hence the collective term Ulcerative White Spot (Raymundo et al., 2008). Despite often being classified under white syndromes, *Porites* ulcerative white spot is considered here separately, as the occurrenceof UWS on other species indicates further research is required to describe and characterise the disease. Additionally, "pigmentation response" symptoms can often be confused with ulcerative white spot syndrome, due to the multifocal patterns, however this disease produces a pink secretion (Weil et al., 2012).

1.2.3 Black band and other coloured band diseases

Black band disease (BBD) was first recorded in Belize in the 1970s and has since been reported across the Caribbean and Indo-Pacific (Antonius, 1985; Richardson, 2004; Séré et al., 2015; Willis et al., 2004). It manifests as a band of microbial assemblages that progresses across corals, killing healthy tissue, and leaving bare skeleton behind (Frias-Lopez et al., 2003; Sutherland et al., 2004). A unique characteristic is the active sulfuretum maintained by the microbial organisms; an anaerobic layer of sulfur across the coral tissue, and an aerobic layer of oxygen above (Brownell & Richardson, 2014; Carlton & Richardson, 2006). Black Band Disease exhibits a distinct seasonality and occurs at low prevalence (<1%) on reefs where active (Dinsdale, 2002; Johan et al., 2016), hence it is often regarded as a natural part of the environment (Page & Willis, 2006).Nonetheless it is seen to be a considerable threat to coral reefs, as affected colonies have not been observed to recover and exposed skeleton is only recolonised by coral recruits after an extended period of time (Edmunds, 2000; Richardson, 2004). Until present, much of the research on BBD has focused on describing the causal agent. Histological data indicates the presence of cyanobacterial filaments and coccoid bacterial cells were found in abundance in the affected host tissue (Bythell et al., 2002).

Other coloured band diseases are characterised by a distinct pigmented band across the disease front separatinghealthy tissue and exposed skeleton (Bruckner & Riegl, 2015) and include brown band, skeletal eroding band and yellow band diseases (Raymundo & Weil, 2016). The distinctive appearance and characteristic coloured banding mean these diseases are relatively easy to diagnose in the field however ambiguity still surrounds yellow band disease, with regards to the difference in symptoms across regions (Bruckner & Riegl, 2015). Band diseases often cause total colony mortality over a number of years, as the destruction of healthy tissue also removes the symbiotic zooxanthellae and the photosynthetic products corals feed on (Guerra et al., 2014; Hadaidi et al., 2018; Montano et al., 2015).

1.2.4 *Porites* trematodiasis

Porites trematodiasis manifests as discrete, enlarged nodules over abnormal skeletal protrusions, changing frombright pink to pale pink then white as the disease progresses (Aeby, 2015). The presence of *Porites* trematodiasis does not usually result in mortality, instead histological research shows a reduction in colony growth by up to 15% and impairment of the calcification mechanism in the infected polyps (Cheng & Wong, 1974). *Porites*

trematodiasis is the result of infection by a digenetic trematode (*Polypipapiliotrema stenometra*), where *Porites sp.* function as the intermediate host in the trematode's lifecycle, with the third host coral feeding fish (Aeby, 1998; Martin et al., 2018). Unlike other diseases, *Porites* trematodiasis is prevalent year round, but displays a positive association with warmer sea temperatures during summer months (Sudek et al., 2015). Historically thought to be confined to Hawaii, a recent observation of multifocal pink spots was recorded in New Caledonia in 2010, indicating a possible biogeographical range extension, however differences in histology between Hawaiian and New Caledonian trematodes indicates that there may be different species between regions of disease ecology (Aeby et al., 2016; Work et al., 2014).

1.2.5 Aspergillosis

Aspergillosis is a disease of sea fan coral, first documented in 1995, and now widespread in the Caribbean (Kim& Rypien, 2015; Smith et al., 1996), however is yet to be described upon scleractinian corals. Characterised bypatches of tissue loss and purple tissue surrounding the active edge of disease progression (Kim & Rypien, 2015). Aspergillosis can cause total colony mortality and reproductive failure in its host (Kim & Harvell, 2002)and the combined loss of large sea fans and reproductive failure can result in a decline in the population over time. The pathogen of aspergillosis was identified as a common soil fungus, *Aspergillus sydowsii*, (Geiser et al., 1998), however its initial occurrence in the Caribbean is yet to be substantiated.

1.2.6 Growth Anomalies

First recorded in 1965 on *Madrepora kauensis,* growth anomalies (GAs) have since been observed across the Caribbean and Indo-Pacific (Aeby, et al., 2011; Work et al., 2008). The term growth anomaly is used collectively for a range of reported symptoms (skeletal growth anomalies, coral tumours, skeletal tissue anomalies; Work et al., 2015), however the characteristics of these diseases are similar across literature. Growth anomalies are the most distinctive of lesions as there is no tissue loss (Aeby, Williams, Franklin, Haapkyla, et al., 2011), instead they appear to cause rapid growth and abnormal skeletal structure (Domart-Coulon et al., 2006), reduced fecundity (Burns & Takabayashi, 2011; Work et al., 2015), reduced immune response (Palmer & Baird, 2018), inhibited feeding ability and lowered autotrophic capacity (Irikawa et al., 2011). Generally, however, abnormalities are thought to be confined to lesions, meaning unaffected tissues surrounding the GA can continue to

function normally (Burns & Takabayashi, 2011; McClanahan et al., 2009).

1.3 The global distribution of coral diseases

Since the 1970s, coral diseases have been reported across numerous tropical coral reefs (Antonius, 1985; Moriarty et al., 2020) and their prevalence is an important parameter when monitoring reef health. With changing environmental conditions and anthropogenic pressure, it is predicted that conditions on temperate reefs will become more favourable for coral diseases and thermo-dependent bacteria (Bally & Garrabou, 2007; Garren et al., 2016) and the geographical range of tropical coral diseases will extend (Vergés et al., 2019). The increasing prevalence and spread of coral diseases have been highlighted as a growing risk to coral reef restoration efforts (Moriarty et al., 2020), and as such there is a growing need to establish baseline disease prevalence across tropical coral reefs in order to detect climate-induced changes.

1.3.1 Indo-Pacific

Coral reefs found in the Indian and Pacific Oceans encompass over 80% of all coral reef ecosystems (Bryant et al., 1998). Coral reefs in this region are exposed to abnormal thermal events, cyclones, and the severe impacts of tourism (Aeby, Williams, Franklin, Haapkyla, et al., 2011; Bourne et al., 2015; Willis et al., 2004). Long term coral reef monitoring programs in Hawaii, Japan, the Great Barrier Reef, and Indonesia has led to an increased number of reports, as well as providing an in-depth understanding of the spatial distribution, drivers and spatio-temporal variation of coral diseases across major Pacific reefs (Figure 1-1, Aeby et al., 2011; Bourne, Ainsworth, & Willis, 2015; Irikawa et al., 2011).

Little is known about coral diseases in the Indian Ocean, the only known reports from this region originate from the east coast of Africa, the Red Sea, and central Western Australia (Figure 1-1). The first coral disease in the Indo-Pacific was reported from the Red Sea. This region has long been recognised as an area of high biodiversity (Stehli & Wells, 1971), and is of increasing interest within the coral reef community due to the variable temperatures and high salinity characterising the environment (Edwards et al., 1987). Whilst coral diseases have been identified through the region (Antonius, 1985; Mohamed & Sweet, 2018; Zvuloni et al., 2009), there is limited research available due to permitting regulations, political climates, and limited marine research facilities (Berumen et al., 2013). In Western Australia, diverse coral reefs are found from the Perth metropolitan region to offshore oceanic reefs to

the north (10^{O}S) (Gilmour et al., 2019; Richards et al., 2016; Richards & Rosser, 2012), and thousands of kilometres offshore to the Cocos (Keeling) and Christmas Islands (Richards & Hobbs,2014). Despite the extent and diversity of corals within this region, coral diseases are often only reported as partof a larger environmental impact studies, such as in relation to the impacts of dredging (Pollock, Lamb, et al., 2016), or in small, high interest zones, such as the Ningaloo Reef World Heritage Area (Onton et al., 2011).

1.3.2 Caribbean and wider Atlantic

Only 8% of coral reefs globally are found within the wider Caribbean region (Spalding & Grenfell, 1997) however, in 2000, more than 66% of disease reports came from 38 countries within this region (Green & Bruckner, 2000; Figure 1-2). Reefs in this region are subject to numerous stress events, including hurricanes, bleaching events, pollution, and excessive nutrients (Randall & van Woesik, 2015; Weil & Rogers, 2011). Coral disease research throughout the Caribbean has been significantly more extensive than that in the Indo-Pacific (Bourne et al., 2015), leading to an in-depth record of diseases throughout the region (Weil, 2004). Limited paleontological records suggest the outbreak of white pox disease that occurred between 1996 to 1999 and resulted in the demise of the critically endangered elkhorn coral, *Acropora palmata,* was unparalleled over at least 3 millennia (Acosta, 2001).

Another major reef system in the Atlantic is the Brazilian offshore system that includes the Abrolhos Bank, and the recently discovered Great Amazon Reef (Francini-Filho et al., 2018). Brazilian reefs contain eight of the 18species found in South Atlantic, including one endemic to the region (*Mussismilia braziliensis)* (Francini-Filho et al., 2008). This reef system is characterised by the local fauna's adaptations to the unique sediment deposition environment created by river discharge (Tedesco et al., 2017). Outflow of Amazon and Orinoco rivers separateBrazilian and Caribbean reefs, a provide a mechanism that may aid in the dispersal of pathogens or other causalagents of coral diseases (Weil & Rogers, 2011). There are limited reports of disease in Brazil, but those that dooccur arise from the Abrolhos Bank (Francini-Filho et al., 2008). With the discovery of the Great Amazon ReefSystem in 2016 (Moura et al., 2016), it is likely that reports may increase as more research is undertaken on thissystem.

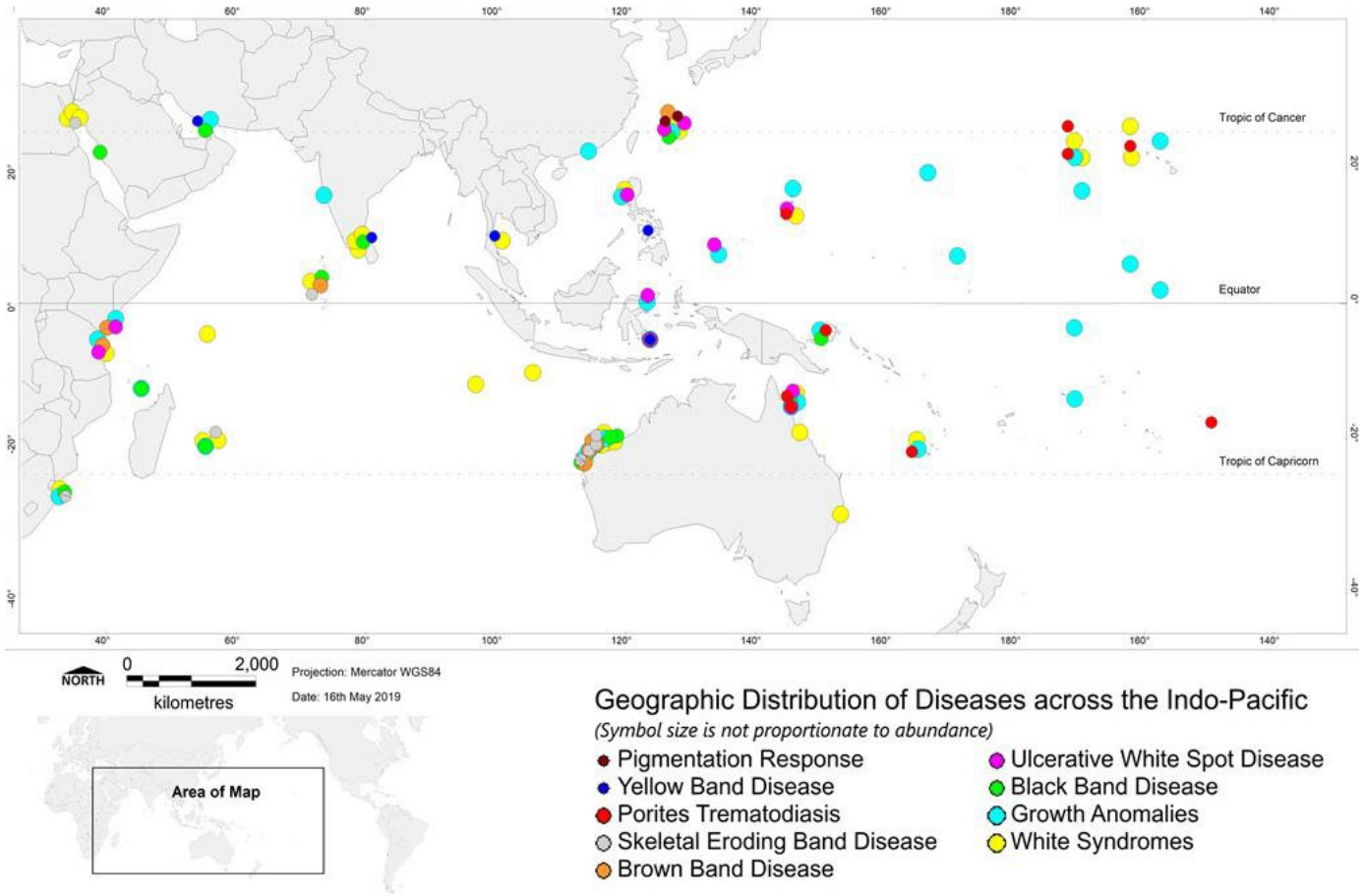

Figure 1-1: Distribution of major diseases reported across the Indo-Pacific (Woodley et al., 2015). Reports collected from a variety of peer-reviewed and published journals using [Disease Name] and [Location] as key search terms.

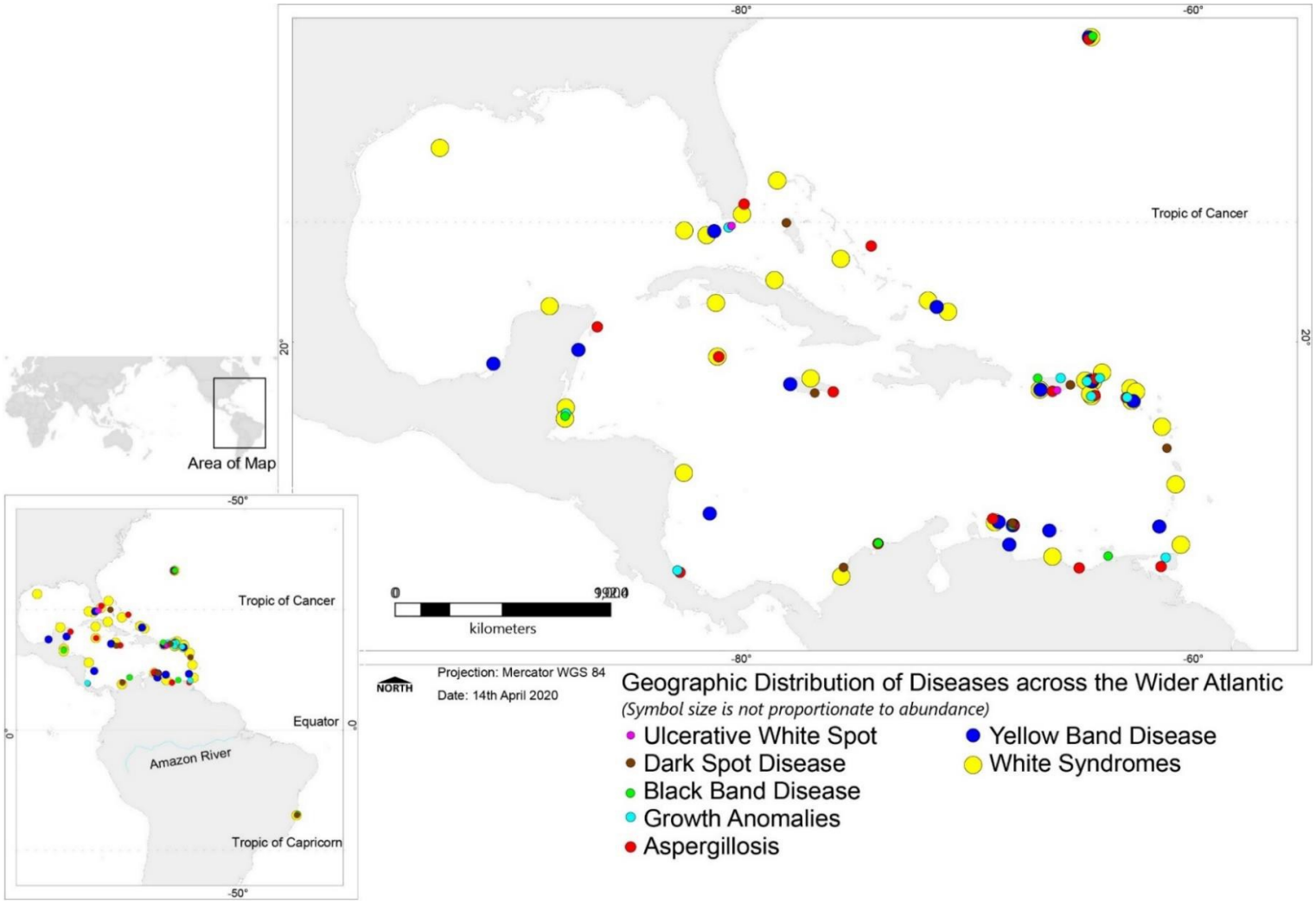

Figure 1-2: Distribution of major diseases reported across the Wider Atlantic (Woodley et al., 2015). Reports collected from a variety of peer-reviewed and published journals using [Disease Name] and [Caribbean] or [Atlantic] as key search terms

1.4 Classical coral disease diagnoses

Coral diseases are typically characterised by ecological surveys, examining macroscopic signs, such astissue loss, changes in colouration and exposure of the skeleton (Williams et al., 2011). Microscopic analyses utilise histology to examine biological and reproductive functioning, or scanning election microscopy and trace element analyses to measure skeletal architecture and chemical composition (Domart-Coulon et al., 2006; Gateño et al., 2003).Decades of research into the dynamics of coral diseasehas highlighted the difficulty of macroscopically characterising diseases *in situ*, and while frameworks exist for disease diagnosis (Raymundo et al., 2008), they have not been universally applied or adopted (Lozada-Misa et al., 2015). These difficulties also pertain to the fact that coral diseases are most often observed in the field on one-off surveys and described using ambiguous characteristics, which can oftenlead to a misdiagnosis (Bourne et al., 2015; Bythell et al., 2004). Many diseases are characterised by similar macroscopic signs which makes diagnosis a challenge to even the most experienced professionals (Hind-Ozan et al., 2008), and as such, more in depth investigations are required to give an accurate identification of disease.

1.5 The coral microbiome

Coral disease research has historically focused on macro- and microscopic characterisation and mapping outbreaks of diseases. This approach, however, struggles to identify the causal mechanisms behind disease onset and progression (Bourne et al., 2009). A complementary approach to coral diseasecharacterisation has been to utilise molecular techniques (Pollock, Wada, et al., 2016; Séré et al., 2013;Zhang et al., 2017) to describe the structure and function of the symbiotic bacterial community (Bourneet al., 2009; Muller-Parker et al., 2015; Stat et al., 2012), the molecular impacts of diseases (Wong et al., 2021; Zhang et al., 2017), and investigating potential microbial causal agents (Séré et al., 2013; Sunagawa et al., 2009; Sussman et al., 2008). This change in focus is facilitated by advances in high- throughput sequencing technologies and the increasing need to establish the etiology of diseases.

Coral microbial communities are some of the most intricate microbial biospheres, the diversity of which surpasses other model systems such as *Exiaptasia* and the human gut (Blackall et al., 2015; Huggett & Apprill, 2019). Corals are home to dinoflagellates, bacterial, fungal, viral, and archaeal associates (Bourne et al., 2016), collectively referred to as the ‘coral holobiont’. Given their structural complexity, corals host numerous microhabitats for bacteria (Ainsworth et al., 2010; Koren & Rosenberg, 2006; Sweet et al.,

2011). As parts of the coral host are an open system (Thompson et al., 2014), a significant proportion of the microbial community is transient (Hernandez-Agreda et al., 2018). This contributes to significant geographical, temporal, and species specific variability all of which must be considered when researching the coral microbiome (Hernandez-Agreda et al., 2017; Morrow et al., 2012).

The microbiome is also often the focus for investigations into the degradation of health. Over the last few decades, increased effort has been dedicated towards attempting to describe the microbial associations of corals under various stressors (Cárdenas et al., 2012; Morrow et al., 2012; Ziegler et al., 2019), but progress in this space has been hindered by the difficulty of replicating the spatial and temporal fluctuations of the natural environment within a laboratory environment (Ainsworth & Hoegh- Guldberg, 2009). Nevertheless, research to date on the structure and function of the coral microbiome has established that the onset of disease often results in a drastic change in the microbiome (Moriarty et al., 2020; Sweet & Bulling, 2017; Ziegler et al., 2019).

Coral microbes offer a significant contribution to host health by disrupting disease progression (Alagelyet al., 2011), participating in nutrient cycling (Raina et al., 2009), and creating diverse community structures essential to the innate immunity of corals (Pollock et al., 2019). To date, several bacteria have been identified that may play a role in disease resistance (Rosales et al., 2019) and lessen the effects ofbleaching. For example, taxa such as *Endozoicomonas* (Neave et al., 2017) are suggested to facilitate successful interactions between dinoflagellates and the host by providing access to essential nutrients and metabolic pathways (Ainsworth et al., 2015). Several taxa belonging to the predatory bacterial orderMyxocaccales (Welsh et al., 2016) have been identified as potentially playing a role in disease resistance (Rosales et al., 2019) by consuming opportunistic pathogens (Welsh et al., 2016). Another study found that manipulating the microbiome to include thermally tolerant bacteria could increase host resistance to bleaching (Rosado et al., 2019). Bacteria can potentially be used as bioindicators of reef health (Roitman et al., 2018), as they play an important role in host susceptibility and the proliferation of coral disease (O'Brien et al., 2019). Molecular tools such as 16S rRNA gene metabarcoding (here on referred to as 16S sequencing) have been applied extensively to many coral diseases in an effort to describe the bacterial communities associated with both healthy and compromised colonies. Environmental microbiological studies have attempted to describe shifts to the microbiome configuration under various environmental and anthropogenic stressors by applying the Anna Karenina Principle to test the hypothesis of dysbiotic shifts (aka imbalances) in coral microbiomes (Ricci et al., 2019; Zaneveld et al., 2017). Dysbiosis (or the imbalance of microbial communities) of diseased hosts (Egan & Gardiner, 2016) has

been proposed as a key avenue for investigation in coral health research where the changes in bacterial communities may allow for unchecked growth of pathogenic or opportunistic bacteria and shifts in the functioning of the host. In the case of white syndromes on *Acropora hyacinthus*, lesions were found to have distinct microbiomes compared to healthy tissues (Pollock, Wada, et al., 2016). Furthermore, whilst no causative agent was identified, a significant increase in rRNA sequencing reads from *Rhodobacteraceae*-affiliated bacteria at disease fronts was identified, indicating that these species play a potential role in pathogenesis and may possibly be useful as an diagnostic indicator for the disease (Pollock, Wada, et al., 2016). Interestingly, *Rhodobacteraceae* have also been reported to associate with healthy corals, predominately the genus *Roseobacter* (Apprill et al., 2009; Bernasconi et al., 2019), indicating that the functional role of bacteria may differentiate within families.

The level of diversity found within the microbiome is often a reflection of coral health (Zaneveld et al., 2017) hence monitoring shifts in diversity measures, such as β-diversity (change in species between samples, turnover), species richness (species diversity within a sample) and evenness (count distribution between multiple species), is useful in coral health assessments (Bourne et al., 2009; Roder et al., 2015). Contrasting this principle is the recent finding of significantly higher compositional homogeneity within the bacterial associations of diseased tissues, potentially as a result of strong competition between the healthy host microbiome and the pathogenic bacterial community (Sweet et al., 2019). Nevertheless, both the ideas of homogeneity and dysbiosis highlight the shift away from a one disease – one pathogen concept, that is, a disease is the result of a single pathogenic agent, towards a complex and dynamic system (Braga et al., 2016).

Uncovering the role of the microbial community within the coral holobiont, be it pathogenic or an immune response, is facilitated by improvements in DNA sequencing technology and reductions in molecular laboratory operating costs (Blackall et al., 2015). Metatranscriptomics (Arotsker et al., 2016), shotgun metagenomics (Thurber et al., 2009), targeted amplicon sequencing (Meyer et al., 2017; Wegley et al., 2007), including eDNA metabarcoding (Marcelino & Verbruggen, 2016) and 16S sequencing are all tools used to generate bacterial community assemblage data, and functionality linked to RNA expression. Collectively these approaches can provide a more holistic understanding of both the microbes associated with diseases, and the processes behind causality and transmission.

1.6 The dynamics of coral diseases

1.6.1 Transmission

There are a number of both abiotic and biotic factors that facilitate the spread of coral diseases across a reef. Abiotic mechanisms include physical injury, host density and waterborne transmission. Host density and specifically high coral cover have been directly linked to the transmission of black band disease (Zvuloni et al., 2009), growth anomalies (GAs) (Aeby, Williams, Franklin, Haapkyla, et al., 2011), and white syndromes (Bruno et al., 2007). Furthermore, coral reefs with high biodiversity are thought to be less susceptible to disease due to lower prevalence of the host organism (Aeby, Bourne, et al., 2011; Ward et al., 2006).

Biotic transmission factors include over 190 corallivores identified as possible vectors of coral diseases, including crustaceans, fish, echinoderms, and molluscs (Rice et al., 2019). These putative vectors are linked as either direct carriers, by creating putative infection sites around feeding scars (Bruckner & Bruckner, 2015; Rice et al., 2019), or influencing the composition of the microbiome (Aeby & Santavy, 2006; Ainsworth et al., 2020). For example, the crown-of-thorns starfish *Acanthaster planci* was foundto be directly associated with facilitating the spread of brown band disease on *Acropora cytherea* (Nugues & Bak, 2009).Other cases are more complex. For example, a butterflyfish species (*Chaetodon multicinctus*) was found to be the 3rd host in the lifecycle of the trematode that is responsible for *Porites* trematodiasis (Aeby, 2015). Collectively, butterflyfish have demonstrated selective feeding preference for diseased tissues (Chong-Seng et al., 2011), however may transfer a potential pathogen to otherwise healthy corals through faeces or mouthparts (Aeby & Santavy, 2006). The variety of methods in which coral disease could possibly be transmitted in diverse coral reefs is vast and many questions remain about transmission in disease etiology.

1.7 Causal mechanisms

The incidence of coral disease is often the result of complex interactions between abiotic and biotic factors. Causal mechanisms of coral diseases also vary spatio-temporally and taxonomically (Precht et al., 2016; Shore-Maggio et al., 2018) and as such, all of these potential factors must be considered whenattempting to characterise the etiology of a disease (Goyen et al., 2019).

Abiotic factors of coral diseases include anthropogenic influences, such as sedimentation,

excessive nutrient loading and plastics (Lamb et al., 2018; Pollock, Lamb, et al., 2016; Rice et al., 2019), in addition to environmental conditions, such as extreme climatic events, seasonal temperature fluctuations, or tropicalisation via the introduction of new species (Aeby et al., 2020; Heron et al., 2018; Vergés et al., 2019). The link between thermal stress events and the onset of disease has been widely reported (Aeby et al., 2020; Aeby, Williams, Franklin, Kenyon, et al., 2011; Maynard et al., 2011). It is apparent thermal stress compromises the immune systems of coral, providing opportunities for invasion by pathogenic/opportunistic microbes or instigation of thermophilic bacteria (Bally & Garrabou, 2007; Randall & van Woesik, 2015).

Identifying the causal agent of a disease can be difficult. For determining biotic disease agents, various studies have tried to incorporate the pathological microbiological method of using Koch's postulates, criteria designed to elucidate the microbial causal agent of a disease, to unequivocally identify coral pathogens (Palić et al., 2015; Richardson, 1998). Whilst a useful method to validate the cause-and- effect of a microbe-host interaction, there are limitations to this method that are recognised within the pathology field and extend to the ecological sphere (Garcion et al., 2009; Milligan-Myhre et al., 2011). These include the difficulty in replicating the natural reef environment within a laboratory setting (Shore & Caldwell, 2019), the role of non-culturable pathogens or consortiums in disease etiology (Richardson et al., 2015) and the requirement for pathogens to only be present in the compromised host (Mera & Bourne, 2018). Several adaptations of Koch's postulates have been proposed to account for the various limitations, such as sequence-based (Fredricks & Relman, 1996), ecological (Vonaesch et al., 2018), and microecological (Huang et al., 2020) postulates. Each version however still falls short when applied to marine diseases, especially when applied to the complex case of the coral microbiome (Mera & Bourne, 2018). Further contradiction to utilising even a modified Koch's postulates to detect changes to bacterial communities in health-compromised corals lie in the recent finding of homogenous species composition in the pathobiome (host associated organisms associated with a compromised health status) when compared to apparently healthy hosts (Sweet et al., 2019). To move the field forward, marine microbiologists have recommended using a combination of classical tools, such as the postulates, combined with novel molecular techniques to determine marine diseases (Burge et al., 2016; Egan & Gardiner, 2016).

1.8 An integrated approach to coral disease characterisation

Whilst coral diseases are increasingly reported across coral reefs, there are distinct discrepancies in characterisation techniques leading to coral reef researchers failing to

adopt a standardised tool kit for the comprehensive diagnosis of a disease outbreak. Numerous marine disease studies have demonstrated success in rapid disease diagnostics by combining traditional and modern techniques (reviewed in Burge et al., 2016). For example, both growth anomalies and *Porites* trematodiasis resultin an abnormal swelling of polyps (Williams et al., 2011; Work et al., 2014) that can only be reliably distinguished at a macroscopic and cellular level (Work et al., 2015). An integrated or total evidence approach would involve investigating coral diseases at different levels of biological complexity, from broad scale ecological surveys of disease incidence and extent at individual to population and community levels, through to cellular changes, geochemical changes, and microbial shifts within the host.

1.9 Research questions and thesis layout

1.9.1 Background

In 2018, growth anomalies (GAs) were reported to affect *Isopora palifera*, a locally dominant scleractinian coral, at the Cocos Keeling Islands, 2,900km north west of Perth, Western Australia (Richards & Newman, 2019). This putative outbreak is the first published record of growth anomalies on *I. palifera* and occurred without obvious environmental or anthropogenic triggers. Therefore, confirming the extent of this disease, and its impact on this reef building species is essential for reef management and understanding the future of the population.

The overarching aim of this thesis is to apply an integrated approach for the characterisation of growth anomalies (GAs), a skeletal coral disease widespread across the Indo-Pacific and wider Atlantic regions. This will be achieved across two data chapters, addressing the classical characterisation approach (Chapter 2), and complementing it with modern molecular techniques (Chapter 3); see Figure 1-3. Each data chapter has a specific set of research questions and comprises of an abstract, introduction, methods, results, discussion, conclusion, references, and supplementary information. These chapters are written and formatted as journal articles and descriptions of each are located below. The final discussion (Chapter 4) collates the thesis' research and examines the significance and limitations of the work. The future questions arising from this thesis, and the directions for coral disease research are explored in thecontext of an integrated approach.

1.9.2 Chapter 2: Biological consequences of an outbreak of growth anomalies on *Isopora palifera* at the Cocos (Keeling) Islands

In chapter two I utilise traditional diagnostic methodology to characterise a putative outbreak of growth anomalies on *Isopora palifera* at the Cocos (Keeling) Islands. I conducted ecological surveys to quantify the distribution of GAs across the atoll and quantify the proportion of the population affected.I apply histological methods to examine the effects of this disease on the biological and reproductive functioning of the host and discuss findings in the context of future population viability. Finally, x-ray fluorescence analysis is used to examine the trace elemental composition of affected skeletons to investigate changes to skeletal growth processes. The results of this study add to the current understanding of the impact of GAs on reef building corals, highlight the implications for the future population, and identifies the changes to skeletal growth processes as a key avenue for the investigation

of this disease.

1.9.3 Chapter 3: Bacterial associations of growth anomaly affected *Isopora palifera.*

In chapter three, I utilise 16S rRNA gene sequencing to investigate the bacterial associations of *I. palifera* samples for evidence of dysbiosis or common indicator taxa for diseased health states. I account for spatial differences and discuss the influence of environmental variables in microbiome composition. The microbiomes of both coral samples and the surrounding water column are compared, as well as samples collected from possible point sources of pollution. The results of this study are compared to prior research on bacterial associations of GA-affected corals and add to the current understanding of coral microbiome dynamics.

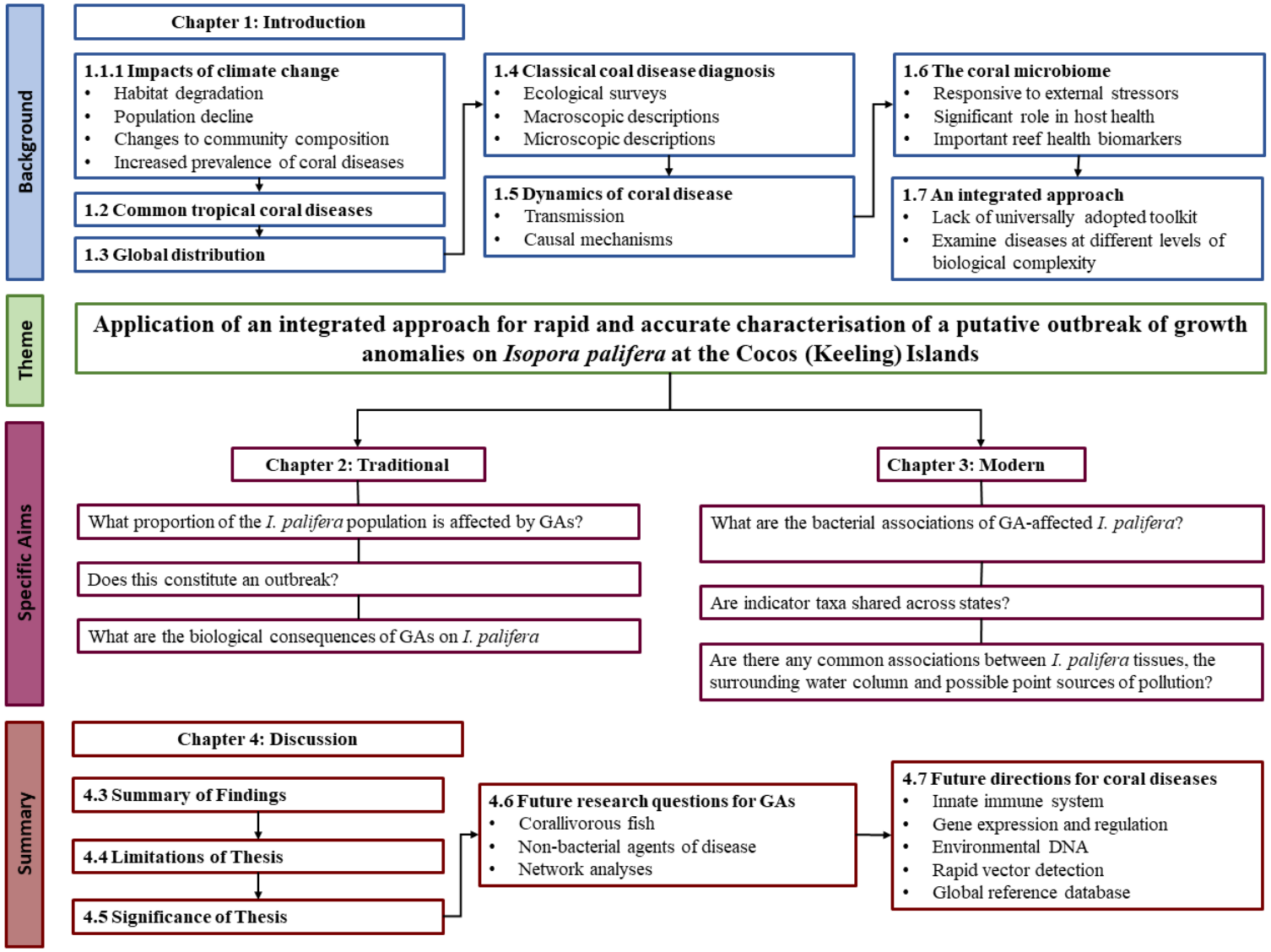

Figure 1-3: Conceptual diagram of thesis rationale, theme, specific aims, and conclusions

1.9.4 References

The owners of copyright material used in this thesis have been acknowledged with every fair and reasonable effort. I would be grateful to hear from any copyright owner should they feel they have beenomitted or acknowledged incorrectly.

Acosta, A. (2001). Disease in Zoanthids: Dynamics in space and time. *Hydrobiologia*, *460*(1), 113–130. https://doi.org/10.1023/A:1013135702430

Aeby, G. S. (1998). A digenean metacercaria from the reef coral, Porites compressa, experimentally identified as Podocotyloides stenometra. *Journal of Parasitology*, *84*(6), 1259–1261. https://www.jstor.org/stable/3284684

Aeby, G. S. (2015). Porites trematodiasis. In C. M. Woodley, C. Downs, A. W. Bruckner, J. W. (James W. Porter, & S. B. Galloway (Eds.), *Diseases of coral* (pp. 387–390). John Wiley &Sons, Inc. https://doi.org/10.1002/9781118828502.ch28

Aeby, G. S., Bourne, D. G., Wilson, B., & Work, T. M. (2011). Coral Diversity and the Severity of Disease Outbreaks: A Cross-Regional Comparison of Acropora White Syndrome in a Species- Rich Region (American Samoa) with a Species-Poor Region (Northwestern Hawaiian Islands) . *Journal of Marine Biology*, *2011*, 1–8. https://doi.org/10.1155/2011/490198

Aeby, G. S., Howells, E., Work, T., Abrego, D., Williams, G. J., Wedding, L. M., Caldwell, J. M.,Moritsch, M., & Burt, J. A. (2020). Localized outbreaks of coral disease on Arabian reefs are linked to extreme temperatures and environmental stressors. *Coral Reefs*, 1–18. https://doi.org/10.1007/s00338-020-01928-4

Aeby, G. S., & Santavy, D. L. (2006). Factors affecting susceptibility of the coral Montastraea faveolata to black-band disease. *Marine Ecology Progress Series*, *318*, 103–110. https://doi.org/10.3354/meps318103

Aeby, G. S., Tribollet, A., Lasne, G., & Work, T. M. (2016). Assessing threats from coral andcrustose coralline algae disease on the reefs of New Caledonia. *Marine and Freshwater Research*, *67*(4), 455. https://doi.org/10.1071/MF14151

Aeby, G. S., Williams, G. J., Franklin, E. C., Haapkyla, J., Harvell, C., Neale, S., Page, C. A., Raymundo, L., Vargas-Ángel, B., Willis, B. L., Work, T. M., & Davy, S. K. (2011). Growth anomalies on the coral genera Acropora and Porites are strongly associated with host density andhuman population size across the Indo-Pacific. *PLoS ONE*, *6*(2), e16887. https://doi.org/10.1371/journal.pone.0016887

Aeby, G. S., Williams, G. J., Franklin, E. C., Kenyon, J., Cox, E. F., Coles, S., & Work, T.

M. (2011). Patterns of Coral Disease across the Hawaiian Archipelago: Relating Disease to Environment. *PLoS ONE*, *6*(5), e20370. https://doi.org/10.1371/journal.pone.0020370

Ainsworth, T. D., & Hoegh-Guldberg, O. (2009). Bacterial communities closely associated with coral tissues vary under experimental and natural reef conditions and thermal stress. *Aquatic Biology*, *4*(3), 289–296. https://doi.org/10.3354/ab00102

Ainsworth, T. D., Kramasky-Winter, E., Loya, Y., Hoegh-Guldberg, O., & Fine, M. (2007). Coral disease diagnostics: what's between a plague and a band? *Applied and Environmental Microbiology*, *73*(3), 981–992. https://doi.org/10.1128/AEM.02172-06

Ainsworth, T. D., Krause, L., Bridge, T., Torda, G., Raina, J.-B., Zakrzewski, M., Gates, R. D., Padilla-Gamiño, J. L., Spalding, H. L., Smith, C., Woolsey, E. S., Bourne, D. G., Bongaerts, P., Hoegh-Guldberg, O., & Leggat, W. (2015). The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *The ISME Journal*, *9*(10), 2261–2274.https://doi.org/10.1038/ismej.2015.39

Ainsworth, T. D., Renzi, J. J., & Silliman, B. R. (2020). Positive Interactions in the Coral Macro and Microbiome. *Trends in Microbiology*, *28*(8), 602–604. https://doi.org/10.1016/j.tim.2020.02.009

Ainsworth, T. D., Thurber, R. V., & Gates, R. D. (2010). The future of coral reefs: a microbial perspective. In *Trends in Ecology and Evolution* (Vol. 25, Issue 4, pp. 233–240). https://doi.org/10.1016/j.tree.2009.11.001

Alagely, A., Krediet, C. J., Ritchie, K. B., & Teplitski, M. (2011). Signaling-mediated cross-talk modulates swarming and biofilm formation in a coral pathogen Serratia marcescens. *ISMEJournal*, *5*(10), 1609–1620. https://doi.org/10.1038/ismej.2011.45

Antonius, A. (1985). Coral Diseases in the Indo-Pacific: A First Record. *Marine Ecology*, *6*(3), 197–218. https://doi.org/10.1111/j.1439-0485.1985.tb00322.x

Apprill, A., Marlow, H. Q., Martindale, M. Q., & Rappé, M. S. (2009). The onset of microbial associations in the coral Pocillopora meandrina. *ISME Journal*, *3*(6), 685–699. https://doi.org/10.1038/ismej.2009.3

Arotsker, L., Kramarsky-Winter, E., Ben-Dov, E., & Kushmaro, A. (2016). Microbial transcriptome profiling of black band disease in a Faviid coral during a seasonal disease peak. *Diseases of Aquatic Organisms*, *118*(1), 77–89. https://doi.org/10.3354/dao02952

Bally, M., & Garrabou, J. (2007). Thermodependent bacterial pathogens and mass mortalities in temperate benthic communities: a new case of emerging disease linked to climate change. *Global Change Biology*, *13*(10), 2078–2088.

https://doi.org/10.1111/j.1365-2486.2007.01423.x

Bernasconi, R., Stat, M., Koenders, A., Paparini, A., Bunce, M., & Huggett, M. J. (2019). Establishment of coral-bacteria symbioses reveal changes in the core bacterial community with host ontogeny. *Frontiers in Microbiology*, *10*(JULY), 1529. https://doi.org/10.3389/fmicb.2019.01529

Berumen, M. L., Hoey, A. S., Bass, W. H., Bouwmeester, J., Catania, D., Cochran, J. E. M., Khalil, M. T., Miyake, S., Mughal, M. R., Spaet, J. L. Y., & Saenz-Agudelo, P. (2013). The status of coral reef ecology research in the Red Sea. *Coral Reefs*, *32*(3), 737–748. https://doi.org/10.1007/s00338-013-1055-8

Blackall, L. L., Wilson, B., & van Oppen, M. (2015). Coral-the world's most diverse symbiotic ecosystem. *Molecular Ecology*, *24*(21), 5330–5347. https://doi.org/10.1111/mec.13400

Bourne, D. G., Ainsworth, T. D., & Willis, B. L. (2015). White Syndromes of Indo-Pacific Corals. In C. M. Woodley, C. Downs, A. W. Bruckner, J. W. (James W. Porter, & S. B. Galloway (Eds.), *Diseases of coral* (pp. 300–315). John Wiley & Sons, Inc. https://onlinelibrary.wiley.com/doi/pdf/10.1002/9781118828502#page=311

Bourne, D. G., Garren, M., Work, T. M., Rosenberg, E., Smith, G. W., & Harvell, C. (2009). Microbial disease and the coral holobiont. In *Trends in Microbiology* (Vol. 17, Issue 12, pp.554–562). https://doi.org/10.1016/j.tim.2009.09.004

Bourne, D. G., Morrow, K. M., & Webster, N. S. (2016). Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. *Annual Review of Microbiology*,*70*(1), 317–340. https://doi.org/10.1146/annurev-micro-102215-095440

Braga, R. M., Dourado, M. N., & Araújo, W. L. (2016). Microbial interactions: ecology in a molecular perspective. *Brazilian Journal of Microbiology*, *47*, 86–98. https://doi.org/10.1016/j.bjm.2016.10.005

Brownell, A. C., & Richardson, L. L. (2014). Sulfate reducing bacteria as secondary and necessary pathogens in black band disease of corals. In *Rev. Biol. Trop. (Int. J. Trop. Biol. ISSN* (Vol. 62).https://www.scielo.sa.cr/pdf/rbt/v62s3/a09v62s3.pdf

Bruckner, A. W. (2015). White syndromes of western Atlantic reef-building corals. In *Diseases of Coral* (pp. 316–332). John Wiley & Sons. https://onlinelibrary.wiley.com/doi/pdf/10.1002/9781118828502#page=327

Bruckner, A. W., & Bruckner, R. . (2015). Mechanical lesions and corallivory. In C. M. Woodley, C. A. Downs, A. W. Bruckner, J. W. Porter, & S. B. Galloway (Eds.), *Diseases*

of Coral (pp. 242– 265). John Wiley adn Sons Inc. https://onlinelibrary.wiley.com/doi/pdf/10.1002/9781118828502#page=253

Bruckner, A. W., & Riegl, B. (2015). Yellow-band diseases. In C. M. Woodley, C. A. Downs, A. W. Bruckner, J. W. Porter, & S. B. Galloway (Eds.), *Diseases of coral* (pp. 376–386). John Wiley &Sons, Inc. https://doi.org/10.1002/9781118828502.ch27

Bruno, J. F., Selig, E. R., Casey, K. S., Page, C. A., Willis, B. L., Harvell, C., Sweatman, H., & Melendy, A. M. (2007). Thermal Stress and Coral Cover as Drivers of Coral Disease Outbreaks. *PLoS Biology*, *5*(6), e124. https://doi.org/10.1371/journal.pbio.0050124

Bryant, D., Burke, L., & Mcmanus, J. (1998). Reefs at risk: A map-based indicator of threats to the world's coral reefs. In *World Resources Institute*. http://agris.fao.org/agris-search/search.do?recordID=US201300101707

Burge, C. A., Friedman, C. S., Getchell, R., House, M., Lafferty, K. D., Mydlarz, L. D., Prager, K. C., Sutherland, K., Renault, T., Kiryu, I., & Thurber, R. V. (2016). Complementary approaches to diagnosing marine diseases: A union of the modern and the classic. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1689). https://doi.org/10.1098/rstb.2015.0207

Burns, J. H. R., & Takabayashi, M. (2011). Histopathology of growth anomaly affecting the coral, Montipora capitata: implications on biological functions and population viability. *PLoS ONE*,*6*(12), e28854. https://doi.org/10.1371/journal.pone.0028854

Burt, J. A., Paparella, F., Al-Mansoori, N., Al-Mansoori, A., & Al-Jailani, H. (2019). Causes and consequences of the 2017 coral bleaching event in the southern Persian/Arabian Gulf. *CoralReefs*, *38*(4), 567–589. https://doi.org/10.1007/s00338-019-01767-y

Bythell, J., Barer, M. R., Cooney, R. P., Guest, J. R., O'Donnell, A. G., Pantos, O., & Le Tissier, M. D. A. (2002). Histopathological methods for the investigation of microbial communities associated with disease lesions in reef corals. *Letters in Applied Microbiology*, *34*(5), 359–364.https://doi.org/10.1046/j.1472-765X.2002.01097.x

Bythell, J., Pantos, O., & Richardson, L. L. (2004). White plague, white band, and other "white"diseases. In *Coral health and disease* (pp. 351–365). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-06414-6_20

Cárdenas, A., Rodriguez-R, L. M., Pizarro, V., Cadavid, L. F., & Arévalo-Ferro, C. (2012). Shifts in bacterial communities of two caribbean reef-building coral species affected by white plague disease. *ISME Journal*, *6*(3), 502–512. https://doi.org/10.1038/ismej.2011.123

Carlton, R. G., & Richardson, L. L. (2006). Oxygen and sulfide dynamics in a horizontally

migrating cyanobacterial mat: Black band disease of corals. *FEMS Microbiology Ecology*, *18*(2), 155–162.https://doi.org/10.1111/j.1574-6941.1995.tb00173.x

Cheal, A. J., MacNeil, M. A., Emslie, M. J., & Sweatman, H. (2017). The threat to coral reefs from more intense cyclones under climate change. *Global Change Biology*, *23*(4), 1511–1524. https://doi.org/10.1111/gcb.13593

Cheng, T. C., & Wong, A. K. L. (1974). Chemical, histochemical, and histopathological studies on corals, Porites spp., parasitized by trematо de metacercariae. *Journal of Invertebrate Pathology*, *23*(3), 303–317. https://doi.org/10.1016/0022-2011(74)90095-0

Chong-Seng, K. M., Cole, A. J., Pratchett, M. S., & Willis, B. L. (2011). Selective feeding by coral reef fishes on coral lesions associated with brown band and black band disease. *Coral Reefs*, *30*(2), 473–481. https://doi.org/10.1007/s00338-010-0707-1

Cinner, J. (2014). Coral reef livelihoods. *Current Opinion in Environmental Sustainability*, *7*, 65–71.https://doi.org/10.1016/J.COSUST.2013.11.025

Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S. J., Kubiszewski, I., Farber, S., & Turner, R. K. (2014). Changes in the global value of ecosystem services. *Global Environmental Change*, *26*(1), 152–158. https://doi.org/10.1016/j.gloenvcha.2014.04.002

DeCarlo, T. M., Cohen, A. L., Wong, G. T. F., Davis, K. A., Lohmann, P., & Soong, K. (2017). Mass coral mortality under local amplification of 2 °c ocean warming. *Scientific Reports*, *7*(1), 1–9. https://doi.org/10.1038/srep44586

Descombes, P., Wisz, M. S., Leprieur, F., Parravicini, V., Heine, C., Olsen, S. M., Swingedouw, D.,Kulbicki, M., Mouillot, D., & Pellissier, L. (2015). Forecasted coral reef decline in marine biodiversity hotspots under climate change. *Global Change Biology*, *21*(7), 2479–2487. https://doi.org/10.1111/gcb.12868

Dinsdale, E. A. (2002). Abundance of black-band disease oncorals from one location on the Great Barrier Reef: a comparison with abundance in the Caribbean region. *Proceedings of the NinthInternational Coral Reef Symposium, Bali, 23-27 October 2000*, 1239–1243.

Dixson, D. L., Abrego, D., & Hay, M. E. (2014). Reef ecology. Chemically mediated behavior ofrecruiting corals and fishes: a tipping point that may limit reef recovery. *Science (New York,N.Y.)*, *345*(6199), 892–897. https://doi.org/10.1126/science.1255057

Domart-Coulon, I. J., Traylor-Knowles, N., Peters, E., Elbert, D., Downs, C. A., Price, K., Stubbs, J.,McLaughlin, S., Cox, E., Aeby, G. S., Brown, P. R., & Ostrander, G. K. (2006). Comprehensive characterization of skeletal tissue growth anomalies of the finger coral

Porites compressa. *Coral Reefs*, *25*(4), 531–543. https://doi.org/10.1007/s00338-006-0133-6

Eakin, C. M., Sweatman, H. P. A., & Brainard, R. E. (2019). The 2014–2017 global-scale coral bleaching event: insights and impacts. *Coral Reefs*, *38*(4), 539–545. https://doi.org/10.1007/s00338-019-01844-2

Edmunds, P. J. (2000). Recruitment of scleractinians onto the skeletons of corals killed by black banddisease. *Coral Reefs*, *19*(1), 69–74. https://doi.org/10.1007/s003380050229

Edwards, A., Head, S., Braithwaite, C., Edwards, F., Karbe, L., Weikert, H., Thiel, H., Walker, D.,Jones, D., Ghamrawy, M., & Wahbeh, M. (1987). *Key Environments; Red Sea*.

Egan, S., & Gardiner, M. (2016). Microbial dysbiosis: Rethinking disease in marine ecosystems. *Frontiers in Microbiology*, *7*(JUN), 991. https://doi.org/10.3389/fmicb.2016.00991

Francini-Filho, R. B., Asp, N. E., Siegle, E., Hocevar, J., Lowyck, K., D'Avila, N., Vasconcelos, A. A., Baitelo, R., Rezende, C. E., Omachi, C. Y., Thompson, C. C., & Thompson, F. L. (2018). Perspectives on the Great Amazon Reef: Extension, biodiversity, and threats. *Frontiers in Marine Science*, *5*(APR). https://doi.org/10.3389/fmars.2018.00142

Francini-Filho, R. B., Moura, R. L., Thompson, F. L., Reis, R. M., Kaufman, L., Kikuchi, R. K. P., &Leão, Z. M. A. N. (2008). Diseases leading to accelerated decline of reef corals in the largest South Atlantic reef complex (Abrolhos Bank, eastern Brazil). *Marine Pollution Bulletin*, *56*(5), 1008–1014. https://doi.org/10.1016/j.marpolbul.2008.02.013

Fredricks, D. N., & Relman, D. A. (1996). Sequence-based identification of microbial pathogens: A reconsideration of Koch's postulates. In *Clinical Microbiology Reviews* (Vol. 9, Issue 1, pp. 18– 33). American Society for Microbiology. https://doi.org/10.1128/cmr.9.1.18-33.1996

Frias-Lopez, J., Bonheyo, G. T., Jin, Q., & Fouke, B. W. (2003). Cyanobacteria Associated with Coral Black Band Disease in Caribbean and Indo-Pacific Reefs. *Applied and Environmental Microbiology*, *69*(4), 2409–2413. https://doi.org/10.1128/AEM.69.4.2409-2413.2003

Garcion, E., Naveilhan, P., Berger, F., & Wion, D. (2009). Cancer stem cells: Beyond Koch's postulates. In *Cancer Letters* (Vol. 278, Issue 1, pp. 3–8). Elsevier Ireland Ltd. https://doi.org/10.1016/j.canlet.2008.09.006

Garren, M., Son, K., Tout, J., Seymour, J. R., & Stocker, R. (2016). Temperature-induced

behavioral switches in a bacterial coral pathogen. *ISME Journal*, *10*(6), 1363–1372. https://doi.org/10.1038/ismej.2015.216

Gateño, D., León, A., Barki, Y., Cortés, J., & Rinkevich, B. (2003). Skeletal tumor formations in themassive coral Pavona clavus. *Marine Ecology Progress Series*, *258*, 97–108. https://doi.org/10.3354/meps258097

Geiser, D., Taylor, J., Ritchie, K., Nature, G. S.-, & 1998, undefined. (1998). Cause of sea fan death in the West Indies. *Nature*, *394*(66689), 137–138. https://idp.nature.com/authorize/casa?redirect_uri=https://www.nature.com/articles/BF28079&c asa_token=pX14fC0izjoAAAAA:cm8cYPmRLZk5gl-6_B6p8EzpZAqA6QI2kE5tVS- NkaP06mfE4NzsDTU6vIZds8TLBLKUmfkiBPR94Av2Njw

Gilmour, J. P., Cook, K. L., Ryan, N. M., Puotinen, M. L., Green, R. H., Shedrawi, G., Hobbs, J.-P., Thomson, D. P., Babcock, R. C., Buckee, J., Foster, T., Richards, Z. T., Wilson, S. K., Barnes, P. B., Coutts, T. B., Radford, B. T., Piggott, C. H., Depczynski, M., Evans, S. N., … Oades, D. (2019). The state of Western Australia's coral reefs. *Coral Reefs*, 1–17. https://doi.org/10.1007/s00338-019-01795-8

Goyen, S., Camp, E. F., Fujise, L., Lloyd, A., Nitschke, M. R., LaJeunensse, T., Kahlke, T., Ralph, P.J., & Suggett, D. (2019). Mass coral bleaching of P. versipora in Sydney Harbour driven by the 2015–2016 heatwave. *Coral Reefs*, 1–16. https://doi.org/10.1007/s00338-019-01797-6

Green, E. P., & Bruckner, A. W. (2000). The significance of coral disease epizootiology for coral reef conservation - Curtin Alma-Primo. *Biological Conservation*, *96*(3), 347–361. https://doi.org/10.1016/S0006-3207(00)00073-2

Guerra, M., López, M., Estéves, I., Zubillaga, A., & Cróquer, A. (2014). Fourier-transformed infrared spectroscopy: a tool to identify gross chemical changes from healthy to yellow band disease tissues. *Diseases of Aquatic Organisms*, *107*(3), 249–258. https://doi.org/10.3354/dao02680

Haapkyla, J., Unsworth, R. K. F., Seymour, A. S., Melbourne-Thomas, J., Flavell, M., Willis, B. L., & Smith, D. J. (2009). Spatio-temporal coral disease dynamics in the Wakatobi Marine National Park, South-East Sulawesi, Indonesia. *Diseases of Aquatic Organisms*, *87*, 105–115. https://doi.org/10.3354/dao02160

Hadaidi, G., Ziegler, M., Shore-Maggio, A., Jensen, T., Aeby, G. S., & Voolstra, C. R. (2018). Ecological and molecular characterization of a coral black band disease outbreak in

the Red Seaduring a bleaching event. *PeerJ*, *6*, e5169. https://doi.org/10.7717/peerj.5169

Hernandez-Agreda, A., Gates, R. D., & Ainsworth, T. D. (2017). Defining the core microbiome in corals' microbial soup. In *Trends in Microbiology* (Vol. 25, Issue 2, pp. 125–140). Elsevier Ltd.https://doi.org/10.1016/j.tim.2016.11.003

Hernandez-Agreda, A., Leggat, W., Bongaerts, P., Herrera, C., & Ainsworth, T. D. (2018). Rethinking the coral microbiome: Simplicity exists within a diverse microbial biosphere. *MBio*,*9*(5). https://doi.org/10.1128/mBio.00812-18

Heron, S., Liu, G., Caldwell, J., Donahue, M., Greene, A., Leggat, W., Ainsworth, T., Vargas-Angel, B., Couch, C., Monger, B., & Lamb, J. (2018). Improving Seasonal Forecasting of Coral Disease Outbreak Risk. *2018 Ocean Sciences Meeting*. https://agu.confex.com/agu/os18/mediafile/Handout/Paper306123/Heron_OSM2018_poster 36x45.pdf

Hind-Ozan, E. J., Lindop, A. M. M., & Bythell, J. C. (2008). The unknowns in coral disease identification: An experiment to assess consensus of opinion amongst experts. *Proceedings of the 11th International Coral Reef Symposium*, *7*. http://orca.cf.ac.uk/98009/1/M07-04 (2).pdf

Hobbs, J.-P. A., Frisch, A. J., Newman, S. J., & Wakefield, C. B. (2015). Selective impact of disease on coral communities: outbreak of white syndrome causes significant total mortality of Acropora plate corals. *PLOS ONE*, *10*(7), e0132528. https://doi.org/10.1371/journal.pone.0132528

Hoegh-Guldberg, O., Beal, D., Chaudhry, T., Elhaj, H., Abdullat, A., Etessy, P., & Smits, M. (2015). *Reviving the Ocean Economy: the case for action - 2015*. www.coralcoe.org.au

Huang, Z., Zeng, S., Xiong, J., Hou, D., Zhou, R., Xing, C., Wei, D., Deng, X., Yu, L., Wang, H., Deng, Z., Weng, S., Kriengkrai, S., Ning, D., Zhou, J., & He, J. (2020). Microecological Koch'spostulates reveal that intestinal microbiota dysbiosis contributes to shrimp white feces syndrome. *Microbiome*, *8*(1), 1–13. https://doi.org/10.1186/s40168-020-00802-3

Huggett, M. J., & Apprill, A. (2019). Coral microbiome database: Integration of sequences reveals high diversity and relatedness of coral-associated microbes. *Environmental Microbiology Reports*, *11*(3), 372–385. https://doi.org/10.1111/1758-2229.12686

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Chase, T. J., Dietzel, A., Hill, T., Hoey, A. S., Hoogenboom, M. O., Jacobson, M., Kerswell, A., Madin, J. S., Mieog, A., Paley, A. S., Pratchett, M. S., Torda, G., & Woods, R. M. (2019). Global warming impairs

stock–recruitmentdynamics of corals. *Nature*. https://doi.org/10.1038/s41586-019-1081-y

Hughes, T. P., Kerry, J. T., & Simpson, T. (2018). Large-scale bleaching of corals on the GreatBarrier Reef. *Ecology*, *99*(2), 501–501. https://doi.org/10.1002/ecy.2092

Irikawa, A., Casareto, B. E., Suzuki, Y., Agostini, S., Hidaka, M., & van Woesik, R. (2011). Growth anomalies on Acropora cytherea corals. *Marine Pollution Bulletin*, *62*(8), 1702–1707. https://doi.org/10.1016/J.MARPOLBUL.2011.05.033

Johan, O., Zamany, N., Smith, D., & Sweet, M. J. (2016). Prevalence and Incidence of Black Band Disease of Scleractinian Corals in the Kepulauan Seribu Region of Indonesia. *Diversity*, *8*(4),11. https://doi.org/10.3390/d8020011

Kim, K., & Harvell, C. (2002). Aspergillosis of sea fan corals: disease dynamics in the Florida Keys. In J. Porter & K. Porter (Eds.), *The Everglades, Florida Bay, and coral reefs of the Florida Keys: an ecosystem sourcebook.* (1st ed., Vol. 1, pp. 813–824). CRC Press, Boca Raton, FL.http://www.reefbase.org/resource_center/publication/pub_13844.aspx

Kim, K., & Rypien, K. (2015). Aspergillosis of Caribbean Sea Fan Corals, Gorgonia spp. In C. M. Woodley, C. A. Downs, A. W. Bruckner, J. W. Porter, & S. B. Galloway (Eds.), *Diseases of Corals* (Vol. 1, pp. 236–241). John Wiley & Sons Inc. https://doi.org/10.1002/9781118828502.ch16

Koren, O., & Rosenberg, E. (2006). Bacteria associated with mucus and tissues of the coral Oculina patagonica in summer and winter. *Applied and Environmental Microbiology*, *72*(8), 5254–5259.https://doi.org/10.1128/AEM.00554-06

Lamb, J. B., Willis, B. L., Fiorenza, E. A., Couch, C. S., Howard, R., Rader, D. N., True, J. D., Kelly, L. A., Ahmad, A., Jompa, J., & Harvell, C. (2018). Plastic waste associated with disease on coral reefs. *Science*, *359*(6374), 460–462. https://doi.org/10.1126/science.aar3320

Lozada-Misa, P., Kerr, A., & Raymundo, L. (2015). Contrasting lesion dynamics of white syndrome among the scleractinian corals Porites spp. *PLOS ONE*, *10*(6), e0129841. https://doi.org/10.1371/journal.pone.0129841

Madin, J., Baird, A., Bridge, T., Connolly, S., Zawada, K., & Dornelas, M. (2018). Cumulative effectsof cyclones and bleaching on coral cover and species richness at Lizard Island. *Marine Ecology Progress Series*, *604*, 263–268. https://doi.org/10.3354/meps12735

Marcelino, V. R., & Verbruggen, H. (2016). Multi-marker metabarcoding of coral skeletons reveals a rich microbiome and diverse evolutionary origins of endolithic algae. *Scientific Reports*, *6*(1), 1–9. https://doi.org/10.1038/srep31508

Martin, S. B., Sasal, P., Cutmore, S. C., Ward, S., Aeby, G. S., & Cribb, T. H. (2018). Intermediate host switches drive diversification among the largest trematode family: evidence from the Polypipapiliotrematinae n. subf. (Opecoelidae), parasites transmitted to butterflyfishes via predation of coral polyps. *International Journal for Parasitology*, *48*(14), 1107–1126. https://doi.org/10.1016/j.ijpara.2018.09.003

Maynard, J. A., Anthony, K. R. N., Harvell, C., Burgman, M. A., Beeden, R., Sweatman, H., Heron, S. F., Lamb, J. B., & Willis, B. L. (2011). Predicting outbreaks of a climate-driven coral disease in the Great Barrier Reef. *Coral Reefs*, *30*(2), 485–495. https://doi.org/10.1007/s00338-010- 0708-0

McClanahan, T. R., Weil, E., & Maina, J. (2009). Strong relationship between coral bleaching and growth anomalies in massive Porites. *Global Change Biology*, *15*(7), 1804–1816. https://doi.org/10.1111/j.1365-2486.2008.01799.x

Mera, H., & Bourne, D. G. (2018). Disentangling causation: complex roles of coral-associated microorganisms in disease. *Environmental Microbiology*, *20*(2), 431–449. https://doi.org/10.1111/1462-2920.13958

Meyer, J. L., Paul, V. J., Raymundo, L., & Teplitski, M. (2017). Comparative Metagenomics of the Polymicrobial Black Band Disease of Corals. *Frontiers in Microbiology*, *8*(APR), 618. https://doi.org/10.3389/fmicb.2017.00618

Milligan-Myhre, K., Charette, J. R., Phennicie, R. T., Stephens, W. Z., Rawls, J. F., Guillemin, K., & Kim, C. H. (2011). Study of Host-Microbe Interactions in Zebrafish. In *Methods in Cell Biology* (Vol. 105, pp. 87–116). Academic Press Inc. https://doi.org/10.1016/B978-0-12-381320- 6.00004-7

Mohamed, A. R., & Sweet, M. J. (2018). Current Knowledge of Coral Diseases Present Within the Red Sea. In N. Rasul & I. Stewart (Eds.), *Oceanographic and Biological Aspects of the Red Sea* (pp. 387–400). Springer, Cham. https://doi.org/10.1007/978-3-319-99417-8_21

Montano, S., Strona, G., Seveso, D., Maggioni, D., & Galli, P. (2015). Slow progression of black band disease in Goniopora cf. columna colonies may promote its persistence in a coral community. *Marine Biodiversity*, *45*(4), 857–860. https://doi.org/10.1007/s12526-014-0273-9

Moriarty, T., Leggat, W., Huggett, M. J., & Ainsworth, T. D. (2020). Coral Disease Causes, Consequences, and Risk within Coral Restoration. *Trends in Microbiology*, *0*(0). https://doi.org/10.1016/j.tim.2020.06.002

Morrow, K. M., Moss, A. G., Chadwick, N. E., & Liles, M. R. (2012). Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. *Applied and Environmental Microbiology*, *78*(18), 6438–6449. https://doi.org/10.1128/AEM.01162-12

Moura, R. L., Amado-Filho, G. M., Moraes, F. C., Brasileiro, P. S., Salomon, P. S., Mahiques, M. M., Bastos, A. C., Almeida, M. G., Silva, J. M., Araujo, B. F., Brito, F. P., Rangel, T. P., Oliveira, B. C. V., Bahia, R. G., Paranhos, R. P., Dias, R. J. S., Siegle, E., Figueiredo, A. G., Pereira, R. C., Thompson, F. L. (2016). An extensive reef system at the Amazon River mouth. *Science Advances*, *2*(4), e1501252. https://doi.org/10.1126 /sciadv.1501252

Muller-Parker, G., D'Elia, C. F., & Cook, C. B. (2015). Interactions between corals and their symbiotic algae. In *Coral Reefs in the Anthropocene* (pp. 99–116). Springer Netherlands. https://doi.org/10.1007/978-94-017-7249-5_5

Neave, M. J., Michell, C. T., Apprill, A., & Voolstra, C. R. (2017). Endozoicomonas genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts. *Scientific Reports*, *7*(1), 1–12. https://doi.org/10.1038/srep40579

Nugues, M. M., & Bak, R. P. M. (2009). Brown-band syndrome on feeding scars of the crown-of- thorn starfish Acanthaster planci. *Coral Reefs*, *28*(2), 507–510. https://doi.org/10.1007/s00338-009-0468-x

O'Brien, P. A., Webster, N. S., Miller, D. J., & Bourne, D. G. (2019). Host-Microbe Coevolution: Applying Evidence from Model Systems to Complex Marine Invertebrate Holobionts. In *mBio* (Vol. 10, Issue 1). NLM (Medline). https://doi.org/10.1128/mBio.02241-18

Onton, K., Page, C., Wilson, S., Neale, S., & Armstrong, S. (2011). Distribution and drivers of coraldisease at Ningaloo reef, Indian Ocean. *Marine Ecology Progress Series*, *433*, 75–84. https://doi.org/10.3354/meps09156

Page, C. A., & Willis, B. L. (2006). Distribution, host range and large-scale spatial variability in blackband disease prevalence on the Great Barrier Reef, Australia. *Diseases of Aquatic Organisms*, *69*(1), 41–51. https://doi.org/10.3354/dao069041

Palić, D., Warg, J., & Work, T. (2015). Biocontainment practices for coral disease research. In C. M.Woodley, C. A. Downs, A. W. Bruckner, J. W. Porter, & S. B. Galloway (Eds.), *Diseases of Coral* (1st ed., Vol. 1). John Wiley and Sons Inc.

https://doi.org/10.1002/9781118828502

Palmer, C. V., & Baird, A. (2018). Coral tumor-like growth anomalies induce an immune responseand reduce fecundity. *Diseases of Aquatic Organisms*, *130*(1), 77–81.

Pollock, F. J., Lamb, J. B., Field, S. N., Heron, S. F., Schaffelke, B., Shedrawi, G., Bourne, D. G., &Willis, B. L. (2016). Correction: Sediment and turbidity associated with offshore dredging increase coral disease prevalence on nearby reefs. *PLOS ONE*, *11*(11), e0165541. https://doi.org/10.1371/journal.pone.0165541

Pollock, F. J., Lamb, J. B., Van De Water, J. A. J. M., Smith, H. A., Schaffelke, B., Willis, B. L., & Bourne, D. G. (2019). Reduced diversity and stability of coral-associated bacterial communities and suppressed immune function precedes disease onset in corals. *Royal Society Open Science*, *6*(6). https://doi.org/10.1098/rsos.190355

Pollock, F. J., Wada, N., Torda, G., Willis, B. L., & Bourne, D. G. (2016). White Syndrome-Affected Corals Have a Distinct Microbiome at Disease Lesion Fronts. *Applied and Environmental Microbiology*, *83*(2). https://doi.org/10.1128/AEM.02799-16

Precht, W. F., Gintert, B. E., Robbart, M. L., Fura, R., & van Woesik, R. (2016). Unprecedented disease-related coral mortality in southeastern Florida. *Scientific Reports*, *6*(1), 31374. https://doi.org/10.1038/srep31374

Raina, J. B., Tapiolas, D., Willis, B. L., & Bourne, D. G. (2009). Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. *Applied and Environmental Microbiology*, *75*(11),3492–3501. https://doi.org/10.1128/AEM.02567-08

Randall, C. J., & van Woesik, R. (2015). Contemporary white-band disease in Caribbean corals driven by climate change. *Nature Climate Change*, *5*(4), 375–379. https://doi.org/10.1038/nclimate2530

Raymundo, L., Harvell, C., & Reynolds, T. L. (2003). Porites ulcerative white spot disease: description, prevalence, and host range of a new coral disease affecting Indo-Pacific reefs. *Diseases of Aquatic Organisms*, *56*(2), 95–104. https://doi.org/10.3354/dao056095

Raymundo, L., & Weil, E. (2016). Indo-Pacific Coloured Band Diseases of Coral. In C. M. Woodley, C. Downs, A. W. Bruckner, J. W. (James W. Porter, & S. B. Galloway (Eds.), *Diseases of Coral* (1st ed., Vol. 1, pp. 333–344). John Wiley & Sons, Inc. https://ebookcentral.proquest.com/lib/curtin/reader.action?docID=4039023

Raymundo, L., Work, T. M., Bruckner, A. W., & Willis, B. L. (2008). A decision tree for describing coral lesions in the field. In Laurie Raymundo, C. S. Couch, & C. D. Harvell

(Eds.), *Coral diseases handbook: guidelines for assessment, monitoring and management* (pp. 17–32). Coral reef targeted research and capacity building for management program. https://researchonline.jcu.edu.au/27750/

Ricci, F., Rossetto Marcelino, V., Blackall, L. L., Kühl, M., Medina, M., & Verbruggen, H. (2019). Beneath the surface: Community assembly and functions of the coral skeleton microbiome. *Microbiome*, *7*(1), 1–10. https://doi.org/10.1186/s40168-019-0762-y

Rice, M. M., Ezzat, L., & Burkepile, D. E. (2019). Corallivory in the anthropocene: interactive Effects of Anthropogenic Stressors and Corallivory on Coral Reefs. *Frontiers in Marine Science*, *5*, 525.https://doi.org/10.3389/fmars.2018.00525

Richards, Z. T., & Day, J. C. (2018). Biodiversity of the Great Barrier Reef—how adequately is it protected? *PeerJ*, *6*(5), e4747. https://doi.org/10.7717/peerj.4747

Richards, Z. T., & Hobbs, J.-P. A. (2014). The status of hard coral diversity at Christmas Island and Cocos (Keeling) Islands. *Raffles Bulletin of Zoology*, *30*, 376–398. http://zoobank.org/urn:lsid:zoobank.org:pub:B5C964DB-5B98-448A-A504-7122E8CDD37E

Richards, Z. T., Kirkendale, L., Moore, G., Hosie, A., Huisman, J., Bryce, M., Marsh, L., Bryce, C., Hara, A., Wilson, N., Morrison, S., Gomez, O., Ritchie, J., Whisson, C., Allen, M., Betterridge, L., Wood, C., Morrison, H., Salotti, M., … Fromont, J. (2016). Marine Biodiversity in Temperate Western Australia: Multi-Taxon Surveys of Minden and Roe Reefs. *Diversity*, *8*(4), 7. https://doi.org/10.3390/d8020007

Richards, Z. T., & Newman, S. J. (2019). Outbreak of growth anomalies in Isopora palifera at Cocos (Keeling) Islands. *Marine Biodiversity*, 1–2. https://doi.org/10.1007/s12526-019-00967-7

Richards, Z. T., & Rosser, N. (2012). Abundance, distribution and new records of scleractinian corals at Barrow Island and the Southern Montebello Islands, Pilbara (offshore) bioregion. *Journal of the Royal Society of Western Australia*, *95*, 155. https://scholar.google.com/scholar_lookup?title=Abundance%2C distribution and new records of scleractinian corals at Barrow Island and Southern Montebello Islands%2C Pilbara %28Offshore%29 Bioregion&journal=J R Soc West Aust&volume=95&pages=155-165&public

Richardson, L. L. (1998). Coral diseases: What is really known? In *Trends in Ecology and Evolution,* 13, (11), pp. 438–443. Elsevier Ltd. https://doi.org/10.1016/S0169-5347(98)01460-8

Richardson, L. L. (2004). Black band disease. In *Coral health and disease* (pp. 325–336). SpringerBerlin Heidelberg. https://doi.org/10.1007/978-3-662-06414-6_18

Richardson, L. L., Miller, A. W., Blackwelder, P. L., & Al-Sayegh, H. (2015). Cyanobacterial- Associated Colored-Band Diseases of the Atlantic/Caribbean. In C. M. Woodley, C. A. Downs, A. W., Bruckner, J. W., Porter, & S. B. Galloway (Eds.), *Diseases of Coral* (pp. 345–353). JohnWiley & Sons, Inc. https://doi.org/10.1002/9781118828502

Robinson, A. (1973). *Natural vs visitor-related damage to shallow water corals: recommendationsfor visitor management and the design of underwater nature trails in the Virgin Islands*.

Roder, C., Bayer, T., Aranda, M., Kruse, M., & Voolstra, C. R. (2015). Microbiome structure of the fungid coral *Ctenactis echinata* aligns with environmental differences. *Molecular Ecology*, *24*(13), 3501–3511. https://doi.org/10.1111/mec.13251

Roitman, S., Joseph Pollock, F., & Medina, M. (2018). Coral microbiomes as bioindicators of reef health. In M. Oleksiak & O. Rajora (Eds.), *Population genetics: marine organisms* (pp. 39–57).Springer, Cham. https://doi.org/10.1007/13836_2018_29

Rosado, P. M., Leite, D. C. A., Duarte, G. A. S., Chaloub, R. M., Jospin, G., Nunes da Rocha, U., P. Saraiva, J., Dini-Andreote, F., Eisen, J. A., Bourne, D. G., & Peixoto, R. S. (2019). Marine probiotics: increasing coral resistance to bleaching through microbiome manipulation. *ISME Journal*, *13*(4), 921–936. https://doi.org/10.1038/s41396-018-0323-6

Rosales, S. M., Miller, M. W., Williams, D. E., Traylor-Knowles, N., Young, B., & Serrano, X. M. (2019). Microbiome differences in disease-resistant vs. susceptible Acropora corals subjected to disease challenge assays. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019- 54855-y

Séré, M. G., Chabanet, P., Turquet, J., Quod, J., & Schleyer, M. H. (2015). Identification and prevalence of coral diseases on three Western Indian Ocean coral reefs. *Diseases of AquaticOrganisms*, *114*(3), 249–261. https://doi.org/10.3354/dao02865

Séré, M. G., Tortosa, P., Chabanet, P., Turquet, J., Quod, J.-P., & Schleyer, M. H. (2013). Bacterial communities associated with Porites white patch syndrome (PWPS) on three Western Indian Ocean (WIO) coral reefs. *PLoS ONE*, *8*(12), e83746. https://doi.org/10.1371/journal.pone.0083746

Shore-Maggio, A., Callahan, S. M., & Aeby, G. S. (2018). Trade-offs in disease and bleaching susceptibility among two color morphs of the Hawaiian reef coral, Montipora capitata. *CoralReefs*, *37*(2), 507–517. https://doi.org/10.1007/s00338-018-1675-0

Shore, A., & Caldwell, J. M. (2019). Modes of coral disease transmission: how do diseases spread between individuals and among populations? *Marine Biology*, *166*(4), 45. https://doi.org/10.1007/s00227-019-3490-8

Sisney, M. A., Cummins, R. H., & Wolfe, C. R. (2018). Incidence of black band disease, brown band disease, and white syndrome in branching corals on the Great Barrier Reef. *Estuarine, Coastal and Shelf Science*, *214*, 1–9. https://doi.org/10.1016/j.ecss.2018.09.005

Smith, G., Ives, L., Nagelkerken, I., & Ritchie, K. (1996). Caribbean sea-fan mortalities. *Nature*,*383*(6600), 487–487. https://link.springer.com/content/pdf/10.1038/383487a0.pdf

Spalding, M. D., & Grenfell, A. M. (1997). New estimates of global and regional coral reef areas. *Coral Reefs*, *16*(4), 225–230. https://doi.org/10.1007/s003380050078

Stat, M., Baker, A. C., Bourne, D. G., Correa, A. M. S., Forsman, Z., Huggett, M. J., Pochon, X., Skillings, D., Toonen, R. J., van Oppen, M. J. H., & Gates, R. D. (2012). Molecular Delineationof Species in the Coral Holobiont. In *Advances in Marine Biology* (Vol. 63, pp. 1–65). Academic Press. https://doi.org/10.1016/B978-0-12-394282-1.00001-6

Stehli, F. G., & Wells, J. W. (1971). Diversity and age patterns in hermatypic corals. *SystematicZoology*, *20*(2), 115. https://doi.org/10.2307/2412052

Sudek, M., Williams, G. J., Runyon, C., Aeby, G. S., & Davy, S. (2015). Disease dynamics of Poritesbleaching with tissue loss: prevalence, virulence, transmission, and environmental drivers.
Diseases of Aquatic Organisms, *113*(1), 59–68. https://doi.org/10.3354/dao02828

Suggett, D. J., & Smith, D. J. (2020). Coral bleaching patterns are the outcome of complex biological and environmental networking. *Global Change Biology*, *26*(1), 68–79. https://doi.org/10.1111/gcb.14871

Sunagawa, S., Desantis, T. Z., Piceno, Y. M., Brodie, E. L., Desalvo, M. K., Voolstra, C. R., Weil, E., Andersen, G. L., & Medina, M. (2009). Bacterial diversity and white Plague disease-associated community changes in the caribbean coral montastraea faveolata. *ISME Journal*, *3*(5), 512–521. https://doi.org/10.1038/ismej.2008.131

Sussman, M., Willis, B. L., Victor, S., & Bourne, D. G. (2008). Coral pathogens identified for white syndrome (WS) epizootics in the Indo-Pacific. *PLoS ONE*, *3*(6), e2393. https://doi.org/10.1371/journal.pone.0002393

Sutherland, K., Porter, J., & Torres, C. (2004). Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. *Marine Ecology Progress Series*, *266*, 273–302. https://doi.org/10.3354/meps266273

Sweet, M. J., & Bulling, M. T. (2017). On the importance of the microbiome and pathobiome in coral health and disease. In *Frontiers in Marine Science* (Vol. 4). Frontiers Media S. A. https://doi.org/10.3389/fmars.2017.00009

Sweet, M. J., Burian, A., Fifer, J., Bulling, M., Elliott, D., & Raymundo, L. (2019). Compositional homogeneity in the pathobiome of a new, slow-spreading coral disease. *Microbiome*, *7*(1), 1–14.https://doi.org/10.1186/s40168-019-0759-6

Sweet, M. J., Croquer, A., & Bythell, J. C. (2011). Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs*, *30*(1), 39–52. https://doi.org/10.1007/s00338-010-0695-1

Tedesco, E. C., Segal, B., Calderon, E. N., & Schiavetti, A. (2017). Conservation of Brazilian coral reefs in the Southwest Atlantic Ocean: A change of approach. In *Latin American Journal of Aquatic Research* (Vol. 45, Issue 2, pp. 228–245). Escuela de Ciencias del Mar. https://doi.org/10.3856/vol45-issue2-fulltext-1

Thinesh, T., Mathews, G., Diraviya Raj, K., & Edward, J. (2017). Outbreaks of Acropora white syndrome and Terpios sponge overgrowth combined with coral mortality in Palk Bay, southeast coast of India. *Diseases of Aquatic Organisms*, *126*(1), 63–70. https://doi.org/10.3354/dao03155

Thompson, J. R., Rivera, H. E., Closek, C. J., & Medina, M. (2014). Microbes in the coral holobiont: Partners through evolution, development, and ecological interactions. *Frontiers in Cellular andInfection Microbiology*, *4*(DEC). https://doi.org/10.3389/fcimb.2014.00176

Thurber, R. V., Willner-Hall, D., Rodriguez-Mueller, B., Desnues, C., Edwards, R. A., Angly, F., Dinsdale, E., Kelly, L. W., & Rohwer, F. (2009). Metagenomic analysis of stressed coral holobionts. *Environmental Microbiology*, *11*(8), 2148–2163. https://doi.org/10.1111/j.1462-2920.2009.01935.x

Vergés, A., McCosker, E., Mayer-Pinto, M., Coleman, M. A., Wernberg, T., Ainsworth, T. D., & Steinberg, P. D. (2019). Tropicalisation of temperate reefs: implications for ecosystem functions and management actions. *Functional Ecology*. https://doi.org/10.1111/1365-2435.13310

Vonaesch, P., Anderson, M., & Sansonetti, P. J. (2018). Pathogens, microbiome and the host: Emergence of the ecological Koch's postulates. In *FEMS Microbiology Reviews* (Vol. 42, Issue3, pp. 273–292). Oxford University Press. https://doi.org/10.1093/femsre/fuy003

Wada, N., Ohdera, A., & Mano, N. (2018). Coral Disease in Japan. In A. Iguchi & C. Hongo (Eds.),*Coral reef studies of Japan. Coral reefs of the world* (Vol. 13, pp. 41–62). Springer.

https://doi.org/10.1007/978-981-10-6473-9_4

Ward, J., Rypien, K., Bruno, J., Harvell, C., Jordán-Dahlgren, E., Mullen, K., Rodríguez-Martínez, R., Sánchez, J., & Smith, G. (2006). Coral diversity and disease in Mexico. *Diseases of Aquatic Organisms*, *69*(1), 23–31. https://doi.org/10.3354/dao069023

Wegley, L., Edwards, R., Rodriguez-Brito, B., Liu, H., & Rohwer, F. (2007). Metagenomic analysis of the microbial community associated with the coral Porites astreoides. *Environmental Microbiology*, *9*(11), 2707–2719. https://doi.org/10.1111/j.1462-2920.2007.01383.x

Weil, E. (2004). Coral reef diseases in the wider Caribbean. In *Coral health and disease* (pp. 35–68). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-06414-6_2

Weil, E., Irikawa, A., Casareto, B., & Suzuki, Y. (2012). Extended geographic distribution of several Indo-Pacific coral reef diseases. *Diseases of Aquatic Organisms*, *98*(2), 163–170. https://doi.org/10.3354/dao02433

Weil, E., & Rogers, C. S. (2011). Coral reef diseases in the Atlantic-Caribbean. In *Coral Reefs: An Ecosystem in Transition* (pp. 465–491). Springer Netherlands. https://doi.org/10.1007/978-94-007-0114-4_27

Welsh, R. M., Zaneveld, J. R., Rosales, S. M., Payet, J. P., Burkepile, D. E., & Thurber, R. V. (2016). Bacterial predation in a marine host-associated microbiome. *ISME Journal*, *10*(6), 1540–1544. https://doi.org/10.1038/ismej.2015.219

Williams, G. J., Work, T. M., Aeby, G. S., Knapp, I. S., & Davy, S. K. (2011). Gross and microscopic morphology of lesions in Cnidaria from Palmyra Atoll, Central Pacific. *Journal of Invertebrate Pathology*, *106*(2), 165–173. https://doi.org/10.1016/J.JIP.2010.08.002

Willis, B. L., Page, C. A., & Dinsdale, E. A. (2004). Coral disease on the Great Barrier Reef. In *Coral health and disease* (pp. 69–104). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-06414-6_3

Wong, Y. H., Zhang, Y., Lun, J. C. Y., & Qiu, J. W. (2021). A proteomic analysis of skeletal tissue anomaly in the brain coral Platygyra carnosa. *Marine Pollution Bulletin*, *164*, 111982. https://doi.org/10.1016/j.marpolbul.2021.111982

Woodley, C. M., Downs, C. A., Bruckner, A. W., Porter, J. W., & Galloway, S. B. (2015). Diseases of coral. In *Diseases of Coral*. John Wiley and Sons Inc. https://doi.org/10.1002/9781118828502

Work, T. M., Aeby, G. S., & Coles, S. (2008). Distribution and morphology of growth anomalies in Acropora from the Indo-Pacific. *Diseases of Aquatic Organisms*, *78*(3), 255–264. https://doi.org/10.3354/dao01881

Work, T. M., Aeby, G. S., Lasne, G., & Tribollet, A. (2014). Gross and microscopic pathology of hard and soft corals in New Caledonia. *Journal of Invertebrate Pathology*, *120*, 50–58. https://doi.org/10.1016/J.JIP.2014.05.007

Work, T. M., Kaczmarsky, L., & Peters, E. (2015). Skeletal growth anomalies in corals. In C. M. Woodley, C. Downs, A. W. Bruckner, J. W. Porter, & S. B. Galloway (Eds.), *Diseases of coral* (pp. 291–299). John Wiley & Sons, Inc. https://books.google.com.au/books?hl=en&lr=&id=bCqwCgAAQBAJ&oi=fnd&pg=PA291&dq=Skeletal+Growth+Anomalies+in+Corals+work+&ots=4k0c0i9L6E&sig=5Uv0zvlefvwUClK9- WKJmm7Arbo

Zaneveld, J. R., McMinds, R., & Thurber, Rebecca VegaThurber, R. V. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nature Microbiology*, *2*(9), 17121. https://doi.org/10.1038/nmicrobiol.2017.121

Zhang, Y., Sun, J., Mu, H., Lun, J. C. Y., & Qiu, J.-W. (2017). Molecular pathology of skeletal growth anomalies in the brain coral Platygyra carnosa: A meta-transcriptomic analysis. *Marine Pollution Bulletin*, *124*(2), 660–667. https://doi.org/10.1016/J.MARPOLBUL.2017.03.047

Ziegler, M., Grupstra, C. G. B., Barreto, M. M., Eaton, M., BaOmar, J., Zubier, K., Al-Sofyani, A.,Turki, A. J., Ormond, R., & Voolstra, C. R. (2019). Coral bacterial community structure responds to environmental change in a host-specific manner. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-10969-5

Zvuloni, A., Artzy-Randrup, Y., Stone, L., Kramarsky-Winter, E., Barkan, R., & Loya, Y. (2009). Spatio-temporal transmission patterns of black-band disease in a coral community. *PLoS ONE*, *4*(4), e4993. https://doi.org/10.1371/journal.pone.0004993

Chapter 2

Biological consequences of an outbreak of growth anomalies affecting *Isopora palifera* at the Cocos (Keeling) Islands

2.1 Pre word

This chapter resulted in a manuscript that was published in *Coral Reefs* (Preston & Richards, 2021, DOI:10.1007/s00338-020-02019-0), a copy of which can be found at the end of the Appendices. This chapter is a reproduction of the manuscript. As first author, permission to reproduce this copyright material is automatically granted if it constitutes less than half the total material in the publication.

2.2 Acknowledgements

This project was funded by ARC Linkage project (LP160101508). Coral samples were collected under Fisheries Exemption No. 3080. For fieldwork I thank Meg Weir and Deiter Gerhard from CocosDive and Taj Powell. I thank Dr. Merinda Nash and Dr. Jens Zinke for their guidance on geochemical analysis and interpretation. For advice and edits I give thanks to Dr. Kar-Chun Tan, Dr. Nicola Browne, Dr. Claus Christopherson, Dr. Stephen Newman, and the Australian Coral Reef Society Student Writing Retreat, in particular Natalia Andrade Rodriguez. Finally, I acknowledge the valuable assistance from the TrEnD Laboratory, especially Dr. Katrina West and the Coral CORE group.

2.3 Abstract

Growth anomalies (GAs), a tumour-like disease affecting scleractinian corals, have been reported across the major reef systems of the Indo-Pacific and wider Atlantic regions, predominantly affecting *Acropora* and *Porites* species. In 2018, GAs were observed for the first time on *Isopora palifera* from the isolated Cocos (Keeling) Islands in the East Indian Ocean. This is an important observation because this species is a key reef building coral at the atoll. In this study, the local distribution and abundance of GAs was quantified to determine if this occurrence could be classified as an outbreak, and the effects of this disease on *I. palifera* on reproductive potential and growth was described using histological and geochemical analysis. Growth anomalies were documented at 75% of sites and affected approximately one third of the *I. palifera* colonies examined. This disease compromises the biological and reproductive functioning of the host, as evidenced by a significant reduction in the density of oocytes, mesenteries, polyps, and zooxanthellae in diseased samples in comparison to healthy. Furthermore, geochemical analysis indicates changes to key trace elements may be the result of bioerosion processes by infecting bacteria and the reprecipitation of calcite. The results of this study indicate the division of energy to the rapid skeletal development that characterises the disease, may have occurred at the detriment of the future reproductive potential of the population.

2.4 Introduction

Growth anomalies (GAs) are pale, tumour-like disease that commonly affect important reef building species across the Indo-Pacific and Atlantic coral reefs (Aeby et al., 2011; Weil, 2004). First described on *Madrepora kauaiensis* (revised *Madrepora oculata* (Hoeksema & Cairns, 2020)) in 1965 (Squires, 1965), they have been reported to affect over 14 genera, however species belonging to *Acroporidae* and *Poritidae* are disproportionately affected (17 and 7 species respectively; Aeby et al., 2011). GAs have been defined as tumours (Gateño et al., 2003), calicoblastic neoplasms (Peters et al., 1986), neoplasia (Squires, 1965) or skeletal tissue anomalies (Domart-Coulon et al., 2006) resulting in ambiguity in the literature. Despite this, GAs are generally characterised using histological methods, by reduced fecundity, zooxanthellae density and fewer polyps (Burns & Takabayashi, 2011; Work et al., 2015). Recent analyses on the skeletons of growth anomalies have demonstrated a reduced density and abnormal development of structures (Domart-Coulon et al., 2006). Molecular work has identified changes to gene expression (Zhang et al., 2017), immune response (Palmer & Baird, 2018) and growth processes (Andersson et al., 2020). The global presence of GAs has led to a framework for diagnosis (Pollock et al., 2011; Raymundo, Work, et al., 2008), however there is much still unknown about this disease.

The consequences of GAs on the functioning of the host has been described consistently through literature (Burns & Takabayashi, 2011; Williams et al., 2011), and the effect on skeletal structures is of increasing interest (Andersson et al., 2020; Domart-Coulon et al., 2006), however transmission and causal mechanism of the disease is yet to be established. The instigation of GAs has been linked to everything from environmental and anthropogenic factors (Aeby et al., 2011), to microbial agents (Domart-Coulon et al., 2006; Work et al., 2015), and transmission of the disease to host density, coral cover and direct contact (Kaczmarsky & Richardson, 2007). It is likely that the etiology and progression of GAs is the result of a combination of factors and establishing the mechanisms of this disease is challenging without understanding the natural prevalence of GAs on coral reefs (Aeby, 2006; Harvell et al., 1999).

To understand the level of threat that a disease may pose to an ecosystem, it is necessary to understand what constitutes as an outbreak. A disease outbreak is technically defined as an R_0 value greater than one, where R_0 is 'the average number of secondary infections produced when one affected individual is introduced into a population of susceptible hosts' and is measured over time (Raymundo, Couch, et al., 2008). However, coral disease outbreaks are often seasonal (Sato et al., 2009), or baseline levels of coral disease upon the reef are unknown (Aeby, 2006; Harvell et al., 1999), so alternative methods of determining the

threshold to define an outbreak have also been used. Natural prevalence of coral diseases on reefs is approximately <1%, become of concern at 3% prevalence, and qualified as an outbreak at >5% (Willis et al., 2004). An alternative threshold used is the abundance of cases, where 60 cases per 1500m^2 qualifies as an outbreak, and 100 cases per 1500m^2 as severe (Maynard et al., 2011). The conflicting methods inhibit the ability to compare between regions and highlights the need for standardised thresholds as to what constitutes as an outbreak. Furthermore, coral disease observations are typically made over a single survey, leading to ambiguous descriptions of similar diseases (Bruckner, 2015; Bythell et al., 2004). Often defining characteristics, such as rate and pattern of progression, require temporal studies or microscope analyses to correctly identify the disease.

In 2018, a putative outbreak of growth anomalies was reported on *Isopora palifera* (Richards & Newman, 2019), a key reef building species on the Cocos (Keeling) Islands. The occurrence of GAs on *I. palifera* has only been mentioned once in literature (Irikawa et al., 2011), however no details were provided about the extent, causality or consequences of the disease on this species. Whilst CKI has experienced anoxic events and extreme weather events, the reefs have escaped significant stress events in recent history (Gilmour et al., 2019), and the potential triggers of this unique occurrence of GAs are yet to be established. This study aims to investigate the local distribution and abundance of *I. palifera* and to quantify the proportion of colonies affected with GAs to establish whether this occurrence constitutes an outbreak. Secondly, this study aims to establish the biological consequences of growth anomalies on *Isopora palifera* by comparing changes to the skeletal, biological, and reproductive status of diseased and healthy colonies. The results of this study establish the local extent and significance of the disease and contribute to the global understanding of growth anomalies.

2.5 Methods

2.5.1 Specimen collection, ecological surveys, and analysis

The Cocos (Keeling) Islands (CKI) is a remote atoll of approximately 600 inhabitants, situated in the Indian Ocean 2,100km north west of Exmouth, Western Australia (Lavers et al., 2019). CKI is a key marine hybrid zone (Hobbs et al., 2009), located at the junction between the Indian and Pacific Oceans,and has been reported to host marine species typically affiliated with both bioregions (Hobbs & Allen,2014). The corals of this region escaped the severe bleaching events that impacted reefs across the north west of Australia during the 2016/2017 marine heatwave, and coral cover was either preserved or increased (Gilmour et al., 2019).

Ecological surveys and specimen collections were undertaken on 17th – 24th May 2019, at 9 sites to document the distribution and abundance of diseased and healthy *I. palifera* colonies (Figure 2-1). Growth anomalies were identified by pale, tumorous masses protruding from the colony (Figure 2-2). Sites ranged in depth from 1m to 14m and chosen based on sites where GAs were observed in a previous study (Richards & Newman, 2019), prevailing weather conditions, boat accessibility and time constraints. Strong south-easterly trade winds rendered the east to south-east side of the atoll inaccessible, and the majority of southern lagoon (blue holes area) was too shallow to access safely. At each site, the abundance of healthy and diseased *I. palifera* colonies was measured along three 25m long by 10m wide belt transects. At four of these sites, healthy and diseased specimens were collected (Table S2-1). Diseased and adjacent healthy samples were collected from three replicate branches on three diseased colonies, in addition to three healthy samples from three healthy control colonies at eachsite (n = 12 per site). Samples were split into three subsamples which were preserved in the following ways - 100% ethanol (for subsequent genetic analysis), 10% formalin for histological analysis and for geochemical analysis, subsamples were bleached in 30% sodium hypochlorite solution, rinsed in freshwater water and air-dried.Prior to subsampling, the proportion of surface area covered in GAs on individual colonies was calculated using 10cmx10cm cards, premade with 6 randomly punched holes (0.5cm diameter). A card was randomly chosen and applied to 3 random branches and the presence/absence of GAs was counted under each point. To estimate the percentage contribution that *Isopora* makes to the overall benthic community, coral cover was surveyed across the 9 sites using three 25m point intersect transects that were randomly deployed at each site. Substrate was recorded every 50 cm and hard corals were identified to a genus level.

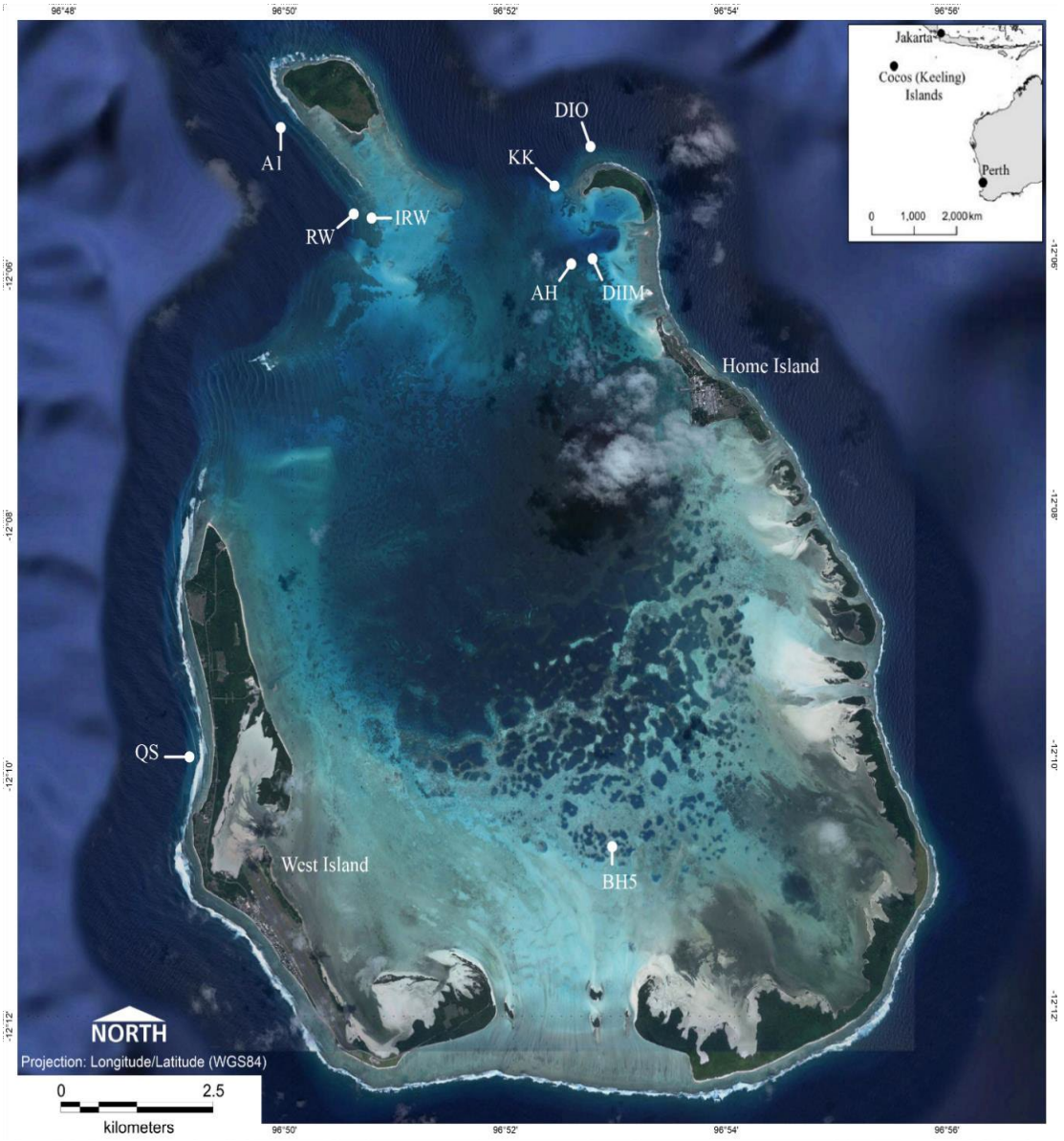

Figure 2-1: Sample sites at Cocos (Keeling) Islands (CKI) as well as main settlements (Home and West Islands). For detailed site information and specimen collection, see Table S2-1.

Figure 2-2: *Isopora palifera* field photos. a) healthy colony, b) growth anomaly (GA) affected colony, c) closeup of GA-affected colony, d) fish scrape on growth anomaly. Green arrow denotes healthy *I. palifera* skeleton, red denotes GA tumour.

To examine the abundance and distribution of growth anomalies, data was analysed by a 2-way analysis of variance (ANOVA) assessing site and health status as factors, after meeting assumptions of normality and variance. Tukeys HSD post hoc analysis was used to identify any significance between levels (Health Status, Site). All analysis was completed in R (v3.6.1) and RStudio (v1.2.500). Presence/absence of GAs was calculated per colony and averaged to establish proportion of surface area of individuals diseased across sites. The percentage mean and SE of overall hard coral cover and scleractinian genera cover was calculated at a site level.

2.5.2 Geochemical methodology and analysis

To test differences in trace element composition of diseased *I. palifera* skeletons, 10 replicate branches were analysed at 6 diseased points, and 6 adjacent healthy points per skeleton (n=120) (Andersson et al., 2020). Branches were 8-12cm in length, and featured multiple tumours of over 2cm in diameter, to ensure independence between samples. Samples were subject to X-ray fluorescence analysis using a ThermoScientific Niton^{TM} XL3t Ultra Analyser (Thermo Fisher), an increasingly used technique for accurate and rapid results in marine, environmental and geological contexts (Arenas-Islas et al., 2019; Liao et al., 2017). The percentage composition of 8 trace elements was quantified (Ca, Sr, Ba, Mg, K, P, U and Al) (Andersson et al., 2020; Domart-Coulon et al., 2006). The instrument used an Ag anode (6-50kV, 0-200 μA max) with a count time of 240s per sample to deliver analytical precision of <1% and 3 reference materials (Table S2-2) were used between samples to standardise results, as per manufacturers protocol (Schatzlein, 2015).

To describe changes in chemical composition of skeletons, normalised data was converted into a Euclidean distance resemblance matrix. A 2 way PERMANOVA main test was performed to examine the null hypothesis that there is a significant difference in chemical composition between healthy and diseased skeletons and between sites, using unrestricted permutation of raw data, 9999 permutations and type III sum of squares. A pairwise test using the same parameters was performed to test to address the interaction effect between health status and site. Separate permutational analyses of multivariate dispersion (PERMDISPs) was performed on the same matrices, with 9999 permutations, for both Health Status and Site to ascertain the significance of variation. The percentage contribution of each element was assessed using similarity percentage analysis (SIMPER), with a cut off of 100%. Data analysis was carried out in Primer 7 v7.0.13 (Primer-e, Quest Research Limited)

2.5.3 Histological methods and analysis

Corals preserved in 10% formalin and seawater were decalcified using 8% HCl and stored in 2% formalin solution for dissection and staining. Six pairs of diseased and adjacent healthy tissue, and four control healthy samples (n= 16) were trimmed and processed using a Leica TP1020 automated tissue processor (Leica Microsystems, Mt Waverly, Australia) and embedded in paraffin on a Leica EG1150 H Heated Paraffin Embedding Module (Leica Microsystems). Histological sections were prepared following a modified protocol outlined in Peters et al. (2005). Serial histological sections (6-8um) were cut on a cryostat and stained with Gill's hematoxylin and eosin (H&E). Slides were passed through 100% ethanol for 30 seconds three times for rehydration, as per core facility protocols. Slides were viewed on an Olympus BX51 upright microscope with DP70 digital microscope system (Olympus Life Science Solutions, Victoria, Australia). To measure changes in reproductive and biological functioning, presence/absence of ova, number of mesenteries and polyps were recorded per $5mm^2$ and density of zooxanthellae per $1mm^2$ were counted on three sections of each tissue sample, using the Olympus cellSens Standard v1.8 software (Olympus), and the mean taken. To investigate changes in biological and reproductive components, between control, adjacent healthy and diseased tissues, variables were standardised, and data square-root transformed to meet assumptions of normality and variance. A one-way PERMANOVA main test was applied to a Bray-Curtis Similarity matrix, to test for significant differences between individuals (ID) and health status (Diseased, Healthy, Control; fixed, nested in ID). The PERMANOVA used the following parameters: Type 1 sums of squares, 9999 permutations and unrestricted permutations of raw data. A pairwise PERMANOVA was used to test for significant differences between health status, using the same parameters. Descriptive statistics and data analysis were carried out in Primer 7.

2.6 Results

2.6.1 Local distribution and abundance of *Isopora palifera* and growth anomalies

A total 1322 *Isopora palifera* colonies were recorded on belt transects across 9 sites covering a total area survey of $6750m^2$. Coral cover surveys revealed 17 scleractinian coral genera present across 9 sites, with hard coral comprising of 44 ± 2.49% of substrate (Table 2-1). Per site, 7 genera accounted for approximately 95% of hard coral cover, proportional to site level coral cover (Figure 2-3). *Isopora* was the sixth most prevalent genera of coral, accounting for 5.78 ± 0.93% to the total hard coral cover (Figure S2-1). Comparatively, susceptible

Acropora and *Porites* colonies were the two dominant genera, comprising of 24.89 ± 3.38% and 18.81 ± 2.04% of hard coral. *I. palifera* was present at all sites, however there was a significant difference between number of colonies per site (p = 6.963e-12, df: 8,36, F = 4.82; Figure 2-4) whereby the highest mean number of colonies was found on the outer reef slope at Direction Island (DIO = 67.17 ± 8.23) and the lowest on the outer reef slope at Quarantine Station (QS = 6.83 ± 3.43).

Table 2-1: Substrate type (mean ± SE) calculated from point intersect transects across 9 sites.

Substrate Type	**Contribution (%)**
Hard coral	44.00 ± 2.4
Turf	19.70 ± 2.65
Soft coral	11.19 ± 2.49
Sand	10.00 ± 1.87
Coralline	9.11 ± 1.89
Rubble	2.44 ± 1.04
Macroalgae	1.85 ± 1.08
Red foliose algae	0.52 ± 0.52
Sponge	0.52 ± 0.25
Fresh dead coral	0.37 ± 0.24
Halimeda	0.22 ± 0.12
Clam	0.15 ± 0.10
Silt	0.07 ± 0.07
Encrusting algae	0.00
Holothurian	0.00

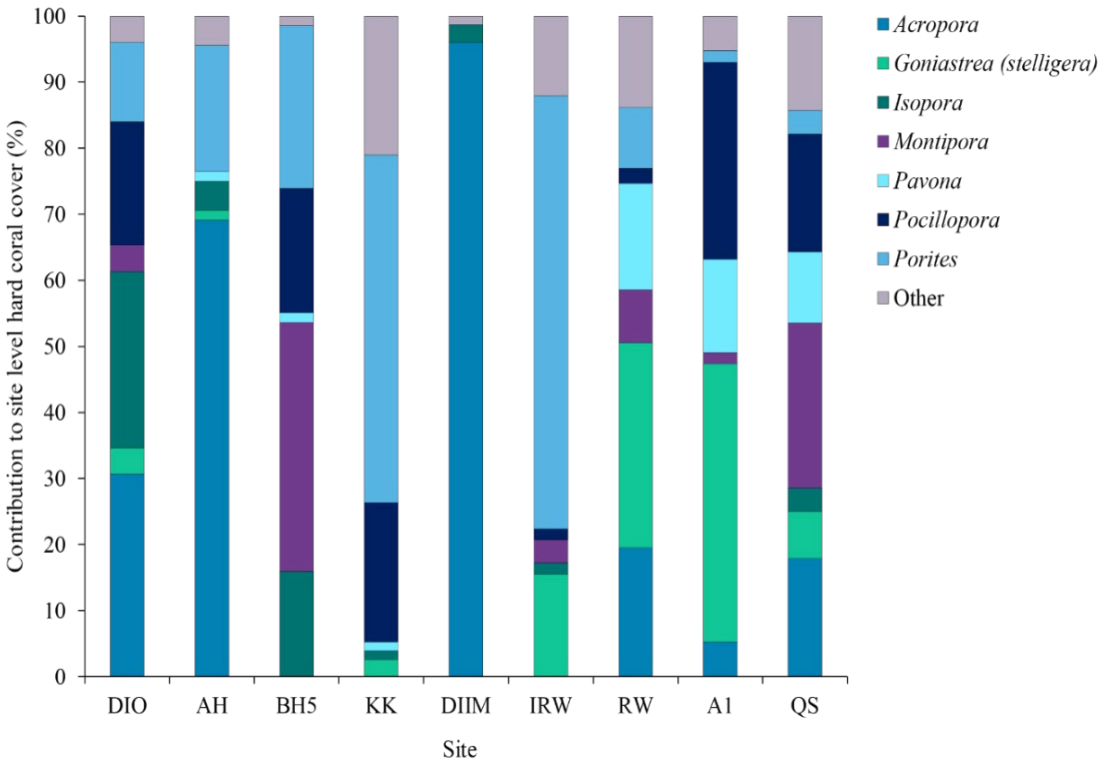

Figure 2-3: Contribution of major scleractinian genera (>5%) to the composition of hard coral cover at each site. Percentage as proportion of total coral cover per site. Coral cover surveyed by3x 25m point intersect transect method (50cm). Other (*Astreopora, Cyphastrea, Diploastrea, Favites, Gardinoseris, Leptastrea, Lobactis, Lobophyllia, Pachyseris and Psammocora*).

At reef-wide scale, there was a significant difference in the number of diseased and healthy colonies ($p = 2.632^{e-06}$, df:1,36, F = 30.99), where 67% (n = 883) of colonies were healthy and 33% (n = 439) of colonies were diseased with GAs. The average number of cases was reported at 16.26 per 750m^2 (± 8.57 SE). Diseased colonies were reported all survey sites except for Quarantine Station and Aquarium 1, however these sites exhibited lower numbers of *I. palifera* colonies (41 and 79 colonies respectively).

At the site scale, the mean number of healthy colonies per site was greater than the number of diseased(32.70 ± 4.12 and 16.26 ± 5.09 colonies respectively). Direction Island Outside was the only site where the mean number of diseased colonies was greater than the number of healthy colonies (78.33 ± 14.19 and 56.00 ± 3.51 respectively; Figure 2-4a). Six of nine sites recorded over 5%of the population diseased, however sites that recorded greater than 30 cases per 750m^2 were only Direction Island Outside (78.33 ± 14.19) and Anchor Hole (30.33 ± 9.87; Figure 2-4b). At a colony scale, growth anomalies were found on 41.25 ± 0.04% of the surface area of colonies diseased.

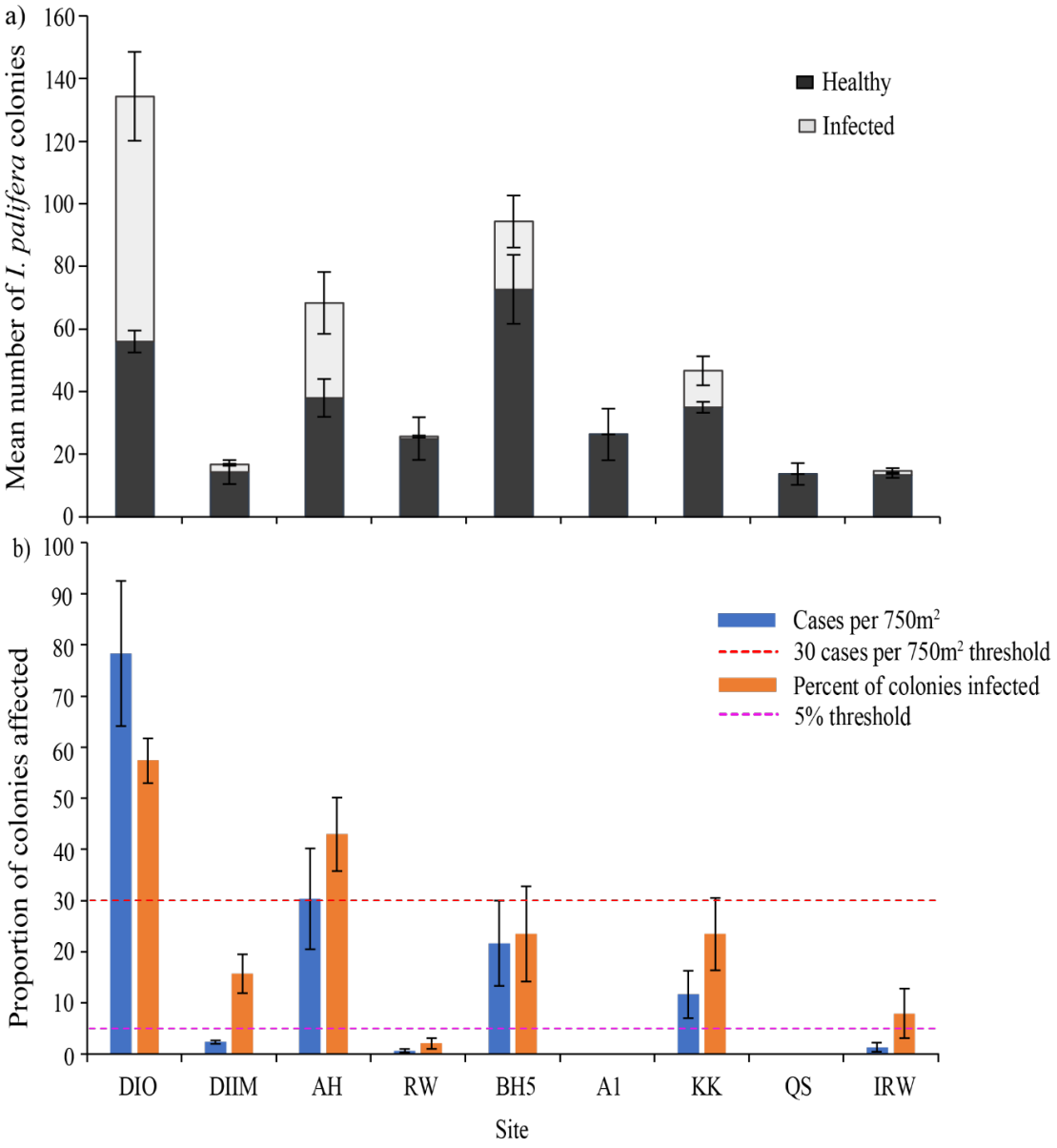

Figure 2-4: a) Number of healthy and affected *I. palifera* colonies per site (mean ± SE), b) proportion of affected *I. palifera* colonies only, in comparison to published outbreak threshold levels (---) of either 5% (Willis et al., 2004) or 30 cases per 75.

2.6.2 Geochemical differences between healthy and diseased skeletons

There was a significant interaction effect in chemical composition between site and healthy and diseased colonies ($p < 0.05$; Table 2-2). On further analysis, it was found that the trace element composition of diseased skeletons differed significantly between all sites, which was not represented in healthy skeletons (Table 2-3). At site level only, there was a significant difference between Kats Kable and both Direction Island Outside, and Anchor Hole with regards to chemical composition (Table 2-4).

Table 2-2: Two-way PERMANOVA of the difference in trace element composition between healthy and GA diseased skeletons * p<0.05 in response to both Health Status and Site. Data was normalised before analysis.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique Perms
Health Status	1	54.765	54.765	7.5805	0.0001*	9920
Site	2	12.962	12.962	1.7941	0.0297*	9939
Health Status x Site	2	17.267	17.267	2.3901	0.0025*	9913
Res	114	823.59	7.2245			
Total	119	952				

Table 2-3: Pairwise PERMANOVA investigating interaction effects between Health Status and Site for trace element analysis between healthy and diseased skeletons* p<0.05. Data was normalised prior to analysis.

Within level 'Healthy' of factor 'Health Status'			
Groups	**t**	**P(perm)**	**Unique Perms**
DIO, AH	0.80886	0.7626	9934
DIO, KK	1.3175	0.078	9934
AH, KK	1.1927	0.1578	9930
Within level 'Diseased' of factor 'Health Status'			
Groups	**t**	**P(perm)**	**Unique Perms**
DIO, AH	1.7958	0.0069*	9933
DIO, KK	1.548	0.0316*	9937
AH, KK	2.5304	0.0001	9940

Table 2-4: Pairwise PERMANOVA investigating the difference in trace element composition between sites only * $p<0.05$. Data normalised prior to analysis.

Groups	t	P(perm)	Unique Perms
DIO, AH	0.89523	0.5794	9936
DIO, KK	1.4328	0.0345*	9938
AH, KK	1.8121	0.0015*	9932

Mean barium, calcium, phosphorous and aluminium concentrations were greater in growth anomaly diseased skeletons whereas magnesium, uranium and potassium were higher in healthy skeletons (Table 2-5). Potassium, followed by barium, provided the greatest contribution to overall dissimilarity in the chemical composition of healthy and diseased colonies (Table S2-3). Within healthy colonies, potassium and uranium had the highest contribution to similarity between samples (22.34%, 17.89%), whereas strontium and calcium had the greatest contribution to the similarity within diseased colonies.

Table 2-5: Percentage concentration of key trace elements in either healthy or GA-affected skeleton samples (mean ± SE). n=120 (60 points each).

Element	Diseased	Healthy
Ca (%)	33.45 ± 0.50	31.81 ± 0.40
Sr (%)	0.66 ± 0.01	0.66 ± 0.01
Mg (ppm)	5535.23 ± 220.22	6298.24 ± 288.63
U (ppm)	169567.33 ± 21711.45	176809.28 ± 48181.73
K (ppm)	379.75 ± 16.58	543.00 ± 17.36
P (%)	0.15 ± 0.01	10.28 ± 10.16
Ba (ppm)	698.69 ± 19.32	562.56 ± 20.91
Al (ppm)	880.50 ± 26.55	806.01 ± 29.80

2.6.3 Biological differences between healthy and diseased tissue

The density of mesenteries, polyps and zooxanthellae, and presence of oocytes in tissues was significantly affected by health status ($p = 0.002$, df = 2,47, Pseudo-F = 3.966). Diseased tissues recorded the least quantities of the biological and reproductive components (Figure 2-5), specifically oocytes were only present in one sample, in contrast to both sets of healthy tissues (Figures 2-5, 2-6).

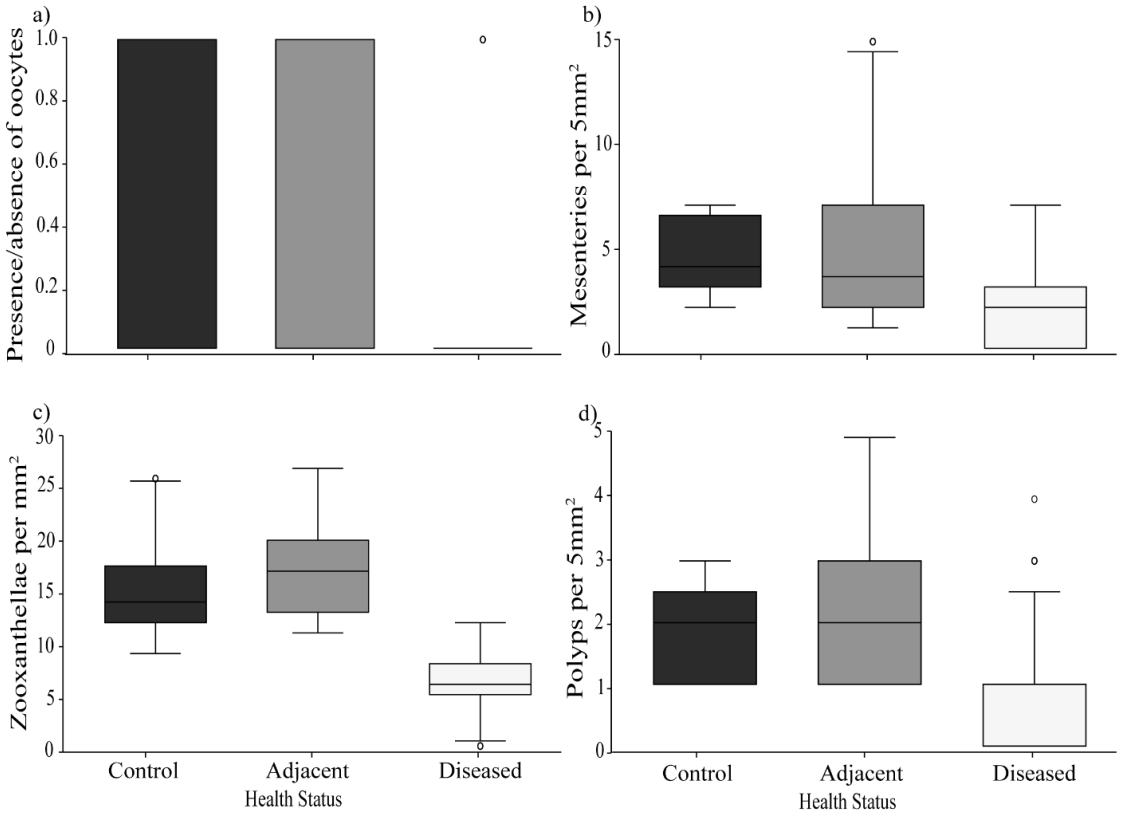

Figure 2-5: Comparison of reproductive and biological components of diseased and adjacent healthy tissue (n=36) and control tissues (n = 12). a) Presence/absence of ova detected, b) mean number of mesenteries, c) density of zooxanthellae per 1mm^2, d) mean number of polyps.

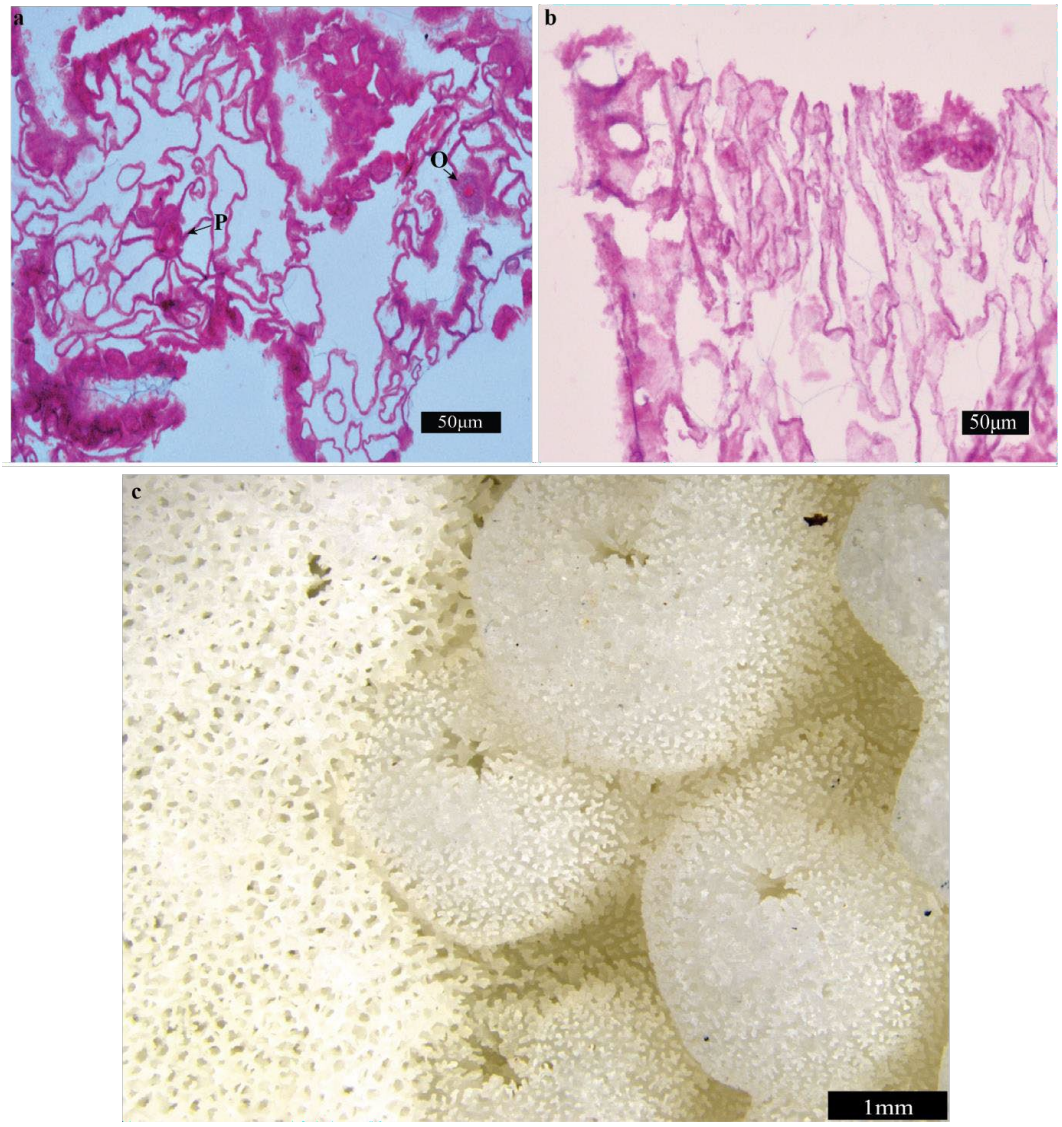

Figure 2-6: Microscope images of histological tissue sections and skeletal lesion boundary. a) healthy tissue samples with polyp (P) and primordial oocyte (O), note structure of gastrodermis and walls, b) diseased tissue sample with mesentery (arrow), and haphazard arrangement of tissues, c) intersection of growth anomaly (left) and normal skeleton (right), note absence of polypskeletal development in the diseased portion of the skeleton

2.7 Discussion

Disease outbreaks threaten the integrity, health, and productivity of coral communities across the globe, including at the remote oceanic atoll of CKI. Here, one third of the locally dominant *I. palifera* population is reported to be affected by GAs and is present at of 75% of sites surveyed. The finding of one third of the community affected, exceeds two published outbreak threshold levels; 5% of the population (Willis et al., 2004) and 30 cases per 750m^2 (Maynard et al., 2011). Furthermore, six sites reported greater than 5% of colonies were affected (Figure 2-4). Conversely only two sites recorded greater than 30 cases per 750m^2. Having established that this location is experiencing an outbreak, it is important to establish how GAs may influence the growth and future viability of the *I. palifera* population.

Growth anomalies are diagnosed by changes to skeletal growth processes, specifically rapid skeletal extension, low skeletal density, and changes to skeletal structures (Domart-Coulon et al., 2006; Gateño et al., 2003). Observation of coral skeletons, whilst not quantified in this study, indicated a loss of structure, and an increase in porosity (Figure 2-6), however, geochemical analysis of healthy and diseased skeletal subsamples provided further insight into the way the disease affects the skeleton. The investigation of trace element composition between healthy and diseased corals revealed changes indicative of a shift in crystal structure from aragonite (healthy samples) to the less dense calcite (diseased samples). More specifically, there was elevated barium and lower magnesium concentrations in diseased samples suggesting the reprecipitation of calcite. (Spies & Takabayashi, 2013; Webb et al., 2009). Furthermore, the reduction of uranium in diseased corals may be the result of the recrystallisation process to neogenic (newly-formed) calcite (Littlewood et al., 2017). The presence of calcite may be the result of the process of bacterial bioerosion where, within the GAs, the aragonite is being bioeroded by infecting bacteria and mobilised cations reprecipitating as an abiotic marine cement.

The process of bioerosion of carbonate structures and subsequent reprecipitation as alternate crystal forms has been reported in crustose coralline algae (Cox et al., 2017) and in fossil bones as the result of an aquatic biofilm (Pesquero & Fernández-Jalvo, 2014), and is an essential part of the marine diagenetic environment (Moore & Wade, 2013). Bioerosion in corals by intra-skeletal microborers, including algae, bacteria and fungi has been well documented (Glynn & Manzello, 2015; Tribollet & Golubic, 2011; Tribollet & Payri, 2001) and has been linked to disease epizootics (Gleason et al., 2017). Whilst further analysis by scanning electron microscopy (SEM) is required to investigate the story proposed here, the changes to the skeletal growth processes may be more complex than previously thought and include both abiotic and biotic factors.

Spatial differences in the geochemistry of samples are to be expected as trace elements are accumulated from surrounding seawater, however, unexpectedly, such differences were only apparent in diseased skeletons (Table 2-2). When investigating solely the difference between site, the composition of trace elements in samples collected from Kats Kable were dissimilar to the composition of those collected at other sites. This may be the reflection of the history of the site, which is a historic dump (Mccarthy, 2005), and scrap materials may be leaching into the sediment and water, however this requires further information. The finding of a significant difference in trace elements is evident in diseased skeletons, coupled with other GA characterisations (Domart-Coulon et al., 2006; Gateño et al., 2003; Squires, 1965), suggest that the effect of the disease on skeletal growth processes extends to a molecular level.

The division of energy away from reproductive and autotrophic activities, towards rapid skeletal growth, appears to have occurred at the detriment of the biological and reproductive functioning of the coral. GA-affected tissues presented with a reduction in the density of polyps, mesenterial filaments and zooxanthellae, and an almost complete absence of oocytes. These findings are consistent with the results of GA studies in other scleractinian coral species (Burns & Takabayashi, 2011; Domart-Coulon et al., 2006). More importantly, these findings suggest future declines in gamete availability are likely, however should be considered in the context of both the total proportion of colonies contributing to population fecundity (Palmer & Baird, 2018), and the proportion of individual colonies impacted, as healthy tissue adjacent to diseased tissue appeared to function normally. In this regard, reproductive potential of *I. palifera* at the Cocos (Keeling) Islands could be conservatively diminished by approximately 11%, based on GAs affecting a minimum of one-third of the surface area across one- third of the population. *Isopora* species encompassed, on average 6% of the total hard coral cover hence this outbreak threatens the future accretional potential of the CKI reefs. Furthermore, *I. palifera* is an important habitat forming species providing vertical complexity for associated taxa, however fish may also play a significant role in the mobility of GAs.

The outbreak of GAs appeared to be confined to *I. palifera* colonies only, and the mode of transmission of this disease has yet to be established. As seen in other disease studies, fish have been highlighted as possible vectors by carrying pathogens to susceptible hosts (Aeby & Santavy, 2006) or clean-up mechanisms through preferential feeding upon diseased tissue (Chong-Seng et al., 2011). In this case of GAs, it may be the latter, as fish scrapes were observed solely upon the seemingly softer lesions, and not the typically dense *I. palifera* skeletons. Whilst not quantified here, investigating the role of fish, in correlation with other transmission vectors may provide insight to the lifecycle of this disease. Previous studies have

highlighted host density (Aeby et al., 2011), direct contact (Kaczmarsky & Richardson, 2007) and coral cover (Williams et al., 2010) as key factors in this disease prevalence. Whilst this may be true for *I. palifera*, the abundance of *Acropora* and *Porites* species, normally disproportionally affected by GAs, was much greater than that of *I. palifera* (>18% each) and were observed to be in direct contact with diseased *I. palifera* colonies. Conflicting studies have discussed the role of direct contact in GA transmission and, by extension, the whether the disease is contagious (Irikawa et al., 2011; Kaczmarsky & Richardson, 2007). The results of this study call in to question whether GAs are indiscriminatory or display species specific modes of transmission. Previously, research has focussed on describing the effects of GAs in species specific cases (Burns & Takabayashi, 2011; Domart-Coulonet al., 2006; Work et al., 2008), and transmission only briefly discussed in the few long term monitoringcase studies (Irikawa et al., 2011).

The GA diagnosis provides sufficient detail to accurate identify the disease *in situ*, and understand theeffects on the host, however the causal mechanism of this disease are yet to be established. GAs have been linked to a variety of anthropogenic and environmental factors (Aeby et al., 2011; Work et al., 2015), and increasingly the bacterial communities within the tissues have been highlighted as possible refuges for pathogenic bacteria that may be implicated in the etiology of GAs (Chiu et al., 2012; Ng et al., 2015; Rajasabapathy et al., 2020). Similar coral disease studies are reporting a variety of microbialcausal agents, such as bacteria in *Montipora* white syndrome (Beurmann et al., 2017; Ushijima et al., 2014), terrestrial fungi linked with aspergillosis of sea fans (Kim & Harvell, 2002) and cyanobacterialmats of black band disease (Richardson, 2004). It is likely that an outbreak of GAs is the result of a combination of environmental, anthropogenic, and microbial factors, and the complexity of such interactions hinders the management of such a critical disease. Many questions still surround GAs, notjust regarding etiology, but long term progression of the disease, inter-species transmission, and the roleof corallivorous fish.

2.8 References

The owners of copyright material used in this thesis have been acknowledged with every fair and reasonable effort. I would be grateful to hear from any copyright owner should they feel they havebeen omitted or acknowledged incorrectly.

Aeby, G. S. (2006). Baseline levels of coral disease in the Northwestern Hawaiian Islands. *Atoll Research Bulletin*. https://repository.si.edu/bitstream/handle/10088/33856/Atoll_2006_Aeby.pdf?sequence=1&isAl lowed=y

Aeby, G. S., & Santavy, D. L. (2006). Factors affecting susceptibility of the coral Montastraea faveolata to black-band disease. *Marine Ecology Progress Series*, *318*, 103–110. https://doi.org/10.3354/meps318103

Aeby, G. S., Williams, G. J., Franklin, E. C., Haapkyla, J., Harvell, C., Neale, S., Page, C. A., Raymundo, L., Vargas-Ángel, B., Willis, B. L., Work, T. M., & Davy, S. K. (2011). Growth anomalies on the coral genera Acropora and Porites are strongly associated with host density andhuman population size across the Indo-Pacific. *PLoS ONE*, *6*(2), e16887. https://doi.org/10.1371/journal.pone.0016887

Andersson, E. R., Stewart, J. A., Work, T. M., Woodley, C. M., Schock, T. B., & Day, R. D. (2020). Morphological, elemental, and boron isotopic insights into pathophysiology of diseased coral growth anomalies. *Scientific Reports*, *10*(1), 8252. https://doi.org/10.1038/s41598-020-65118-6

Arenas-Islas, D., Huerta-Diaz, M. A., Norzagaray-López, C. O., Mejia-Piña, K. G., Valdivieso-Ojeda, J. A., Otero, X. L., & Arcega-Cabrera, F. (2019). Calibration of portable X-ray fluorescence equipment for the geochemical analysis of carbonate matrices. *Sedimentary Geology*, *391*, 105517. https://doi.org/10.1016/j.sedgeo.2019.105517

Beurmann, S., Ushijima, B., Videau, P., Svoboda, C. M., Smith, A. M., Rivers, O. S., Aeby, G. S., & Callahan, S. M. (2017). Pseudoalteromonas piratica strain OCN003 is a coral pathogen that causes a switch from chronic to acute Montipora white syndrome in Montipora capitata. *PLOS ONE*, *12*(11), e0188319. https://doi.org/10.1371/journal.pone.0188319

Bruckner, A. W. (2015). White syndromes of western Atlantic reef-building corals. In

Diseases of Coral (pp. 316–332). John Wiley & Sons. https://onlinelibrary.wiley.com/doi/pdf/10.1002/9781118828502#page=327

Burns, J. H. R., & Takabayashi, M. (2011). Histopathology of growth anomaly affecting the coral, Montipora capitata: implications on biological functions and population viability. *PLoS ONE*,*6*(12), e28854. https://doi.org/10.1371/journal.pone.0028854

Bythell, J., Pantos, O., & Richardson, L. L. (2004). White plague, white band, and other "white" diseases. In *Coral health and disease* (pp. 351–365). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-06414-6_20

Chiu, J. M. Y., Li, S., Li, A., Po, B., Zhang, R., Shin, P. K. S., & Qiu, J.-W. (2012). Bacteria associated with skeletal tissue growth anomalies in the coral Platygyra carnosus. *FEMS Microbiology Ecology*, *79*(2), 380–391. https://doi.org/10.1111/j.1574-6941.2011.01225.x

Chong-Seng, K. M., Cole, A. J., Pratchett, M. S., & Willis, B. L. (2011). Selective feeding by coral reef fishes on coral lesions associated with brown band and black band disease. *Coral Reefs*, *30*(2), 473–481. https://doi.org/10.1007/s00338-010-0707-1

Cox, T. E., Nash, M., Gazeau, F., Déniel, M., Legrand, E., Alliouane, S., Mahacek, P., Le Fur, A.,Gattuso, J. P., & Martin, S. (2017). Effects of in situ CO2 enrichment on Posidonia oceanica epiphytic community composition and mineralogy. *Marine Biology*, *164*(5), 103. https://doi.org/10.1007/s00227-017-3136-7

Domart-Coulon, I. J., Traylor-Knowles, N., Peters, E., Elbert, D., Downs, C. A., Price, K., Stubbs, J.,McLaughlin, S., Cox, E., Aeby, G. S., Brown, P. R., & Ostrander, G. K. (2006). Comprehensive characterization of skeletal tissue growth anomalies of the finger coral Porites compressa. *Coral Reefs*, *25*(4), 531–543. https://doi.org/10.1007/s00338-006-0133-6

Gateño, D., León, A., Barki, Y., Cortés, J., & Rinkevich, B. (2003). Skeletal tumor formations in the massive coral Pavona clavus. *Marine Ecology Progress Series*, *258*, 97–108. https://doi.org/10.3354/meps258097

Gilmour, J. P., Cook, K. L., Ryan, N. M., Puotinen, M. L., Green, R. H., Shedrawi, G., Hobbs, J.-P. A., Thomson, D. P., Babcock, R. C., Buckee, J., Foster, T., Richards, Z. T.,

Wilson, S. K., Barnes, P. B., Coutts, T. B., Radford, B. T., Piggott, C. H., Depczynski, M., Evans, S. N., … Oades, D. (2019). The state of Western Australia's coral reefs. *Coral Reefs*, 1–17. https://doi.org/10.1007/s00338-019-01795-8

Gleason, F. H., Gadd, G. M., Pitt, J. I., & Larkum, A. W. D. (2017). The roles of endolithic fungi inbioerosion and disease in marine ecosystems. I. General concepts. *Mycology*, *8*(3), 205–215. https://doi.org/10.1080/21501203.2017.1352049

Glynn, P. W., & Manzello, D. P. (2015). Bioerosion and coral reef growth: A dynamic balance. In *Coral Reefs in the Anthropocene* (pp. 67–97). Springer Netherlands. https://doi.org/10.1007/978-94-017-7249-5_4

Harvell, C. D., Kim, K., Burkholder, J. M., Colwell, R. R., Epstein, P. R., Grimes, D. J., Hofmann, E. E., Lipp, E. K., Osterhaus, A. D. M. E., Overstreet, R. M., Porter, J. W., Smith, G. W., & Vasta, G. R. (1999). Emerging marine diseases - climate links and anthropogenic factors. In *Science* (Vol. 285, Issue 5433, pp. 1505–1510). American Association for the Advancement of Science.https://doi.org/10.1126/science.285.5433.1505

Hobbs, J.-P. A., & Allen, G. R. (2014). Hybridisation among coral reef fishes at Christmas Island and the Cocos (Keeling) Islands. *Raffles Bulletin of Zoology*, *30*, 220–226. http://zoobank.org/urn:lsid:zoobank.org:pub:DF247BE6-A884-43C0-8EBA-6CBF9758C450

Hobbs, J.-P. A., Frisch, A. J., Allen, G. R., & Van Herwerden, L. (2009). Marine hybrid hotspot at Indo-Pacific biogeographic border. *Biology Letters*, *5*(2), 258–261. https://doi.org/10.1098/rsbl.2008.0561

Hoeksema, B. W., & Cairns, S. (2020). Madrepora kauaiensis Vaughan, 1907. In *World LIst ofScleractinia*. http://www.marinespecies.org/aphia.php?p=taxdetails&id=287093#sources

Irikawa, A., Casareto, B. E., Suzuki, Y., Agostini, S., Hidaka, M., & van Woesik, R. (2011). Growth anomalies on Acropora cytherea corals. *Marine Pollution Bulletin*, *62*(8), 1702–1707. https://doi.org/10.1016/J.MARPOLBUL.2011.05.033

Kaczmarsky, L., & Richardson, L. L. (2007). Transmission of growth anomalies between

Indo-Pacific Porites corals. *Journal of Invertebrate Pathology*, *94*(3), 218–221. https://doi.org/10.1016/J.JIP.2006.11.007

Kim, K., & Harvell, C. (2002). Aspergillosis of sea fan corals: disease dynamics in the Florida Keys. In J. Porter & K. Porter (Eds.), *The Everglades, Florida Bay, and coral reefs of the Florida Keys: an ecosystem sourcebook.* (1st ed., Vol. 1, pp. 813–824). CRC Press, Boca Raton, FL.http://www.reefbase.org/resource_center/publication/pub_13844.aspx

Lavers, J. L., Dicks, L., Dicks, M. R., & Finger, A. (2019). Significant plastic accumulation on the Cocos (Keeling) Islands, Australia. *Scientific Reports*, *9*(1), 7102. https://doi.org/10.1038/s41598-019-43375-4

Liao, S., Tao, C., Li, H., Zhang, G., Liang, J., & Yang, W. (2017). Use of portable X-ray fluorescencein the analysis of surficial sediments in the exploration of hydrothermal vents on the southwest Indian Ridge. *Acta Oceanologica Sinica*, *36*(7), 66–76. https://doi.org/10.1007/s13131-017- 1085-0

Littlewood, J. L., Shaw, S., Peacock, C. L., Bots, P., Trivedi, D., & Burke, I. T. (2017). Mechanism ofenhanced strontium uptake into calcite via an amorphous calcium carbonate crystallization pathway. *Crystal Growth and Design*, *17*(3), 1214–1223. https://doi.org/10.1021/acs.cgd.6b01599

Maynard, J. A., Anthony, K. R. N., Harvell, C., Burgman, M. A., Beeden, R., Sweatman, H., Heron, S. F., Lamb, J. B., & Willis, B. L. (2011). Predicting outbreaks of a climate-driven coral disease in the Great Barrier Reef. *Coral Reefs*, *30*(2), 485–495. https://doi.org/10.1007/s00338-010- 0708-0

Mccarthy, M. (2005). *Gerhard cannon site (Direction Island, Cocos Keeling Islands) inspection report*. http://museum.wa.gov.au/maritime-archaeology- db/sites/default/files /no._197_cannon_site_cocos.pdf

Moore, C. H., & Wade, W. J. (2013). Marine diagenetic environment. In *Developments in sedimentology* (Vol. 67, pp. 93–131). Elsevier B.V. https://doi.org/10.1016/B978-0-444-53831-4.00006-9

Ng, J. C. Y., Chan, Y., Tun, H. M., Leung, F. C. C., Shin, P. K. S., & Chiu, J. M. Y. (2015). Pyrosequencing of the bacteria associated with Platygyra carnosus corals with skeletal

growth anomalies reveals differences in bacterial community composition in apparently healthy and diseased tissues. *Frontiers in Microbiology*, *6*, 1142. https://doi.org/10.3389/fmicb.2015.01142

Palmer, C. V., & Baird, A. (2018). Coral tumor-like growth anomalies induce an immune response and reduce fecundity. *Diseases of Aquatic Organisms*, *130*(1), 77–81. https://doi.org/10.3354/dao03258

Pesquero, M. D., & Fernández-Jalvo, Y. (2014). Bioapatite to calcite, an unusual transformation seenin fossil bones affected by aquatic bioerosion. *Lethaia*, *47*(4), 533–546. https://doi.org/10.1111/let.12079

Peters, E., Halas, J. C., & McCarty, H. B. (1986). Calicoblastic neoplasms in Acropora palmata, witha review of reports on anomalies of growth and form in corals. *Journal of the National Cancer Institute*, *76*(5), 895–912.

Peters, E., Price, K. L., Horowitz, D. J. B., Price, K. L., & Horowitz, D. J. B. (2005). Histologicalpreparation of invertebrates for evaluating contaminant effects. In *Techniques in Aquatic Toxicology, Volume 2*. CRC Press.

Pollock, F. J., Morris, P. J., Willis, B. L., & Bourne, D. G. (2011). The urgent need for robust coral disease diagnostics. *PLoS Pathogens*, *7*(10), e1002183. https://doi.org/10.1371/journal.ppat.1002183

Preston, S., & Richards, Z. (2021). Biological consequences of an outbreak of growth anomalies onIsopora palifera at the Cocos (Keeling) Islands. *Coral Reefs*, *40*(1), 97–109. https://doi.org/10.1007/s00338-020-02019-0

Rajasabapathy, R., Ramasamy, K. P., Manikandan, B., Mohandass, C., & Arthur James, R. (2020).
Bacterial communities associated with healthy and diseased (skeletal growth anomaly) reef coral Acropora cytherea from Palk Bay, India. *Frontiers in Marine Science*, *7*, 92. https://doi.org/10.3389/fmars.2020.00092

Raymundo, LJ, Couch, C., & Harvell, C. (2008). *Coral disease handbook guidelines for assessment,monitoring & management*. http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.307.3231

Raymundo, LJ, Work, T. M., Bruckner, A. W., & Willis, B. L. (2008). A decision tree for describing coral lesions in the field. In Laurie Raymundo, C. S. Couch, & C. D. Harvell (Eds.), *Coral diseases handbook: guidelines for assessment, monitoring and management* (pp. 17–32). Coral reef targeted research and capacity building for management program. https://researchonline.jcu.edu.au/27750/

Richards, Z. T., & Newman, S. J. (2019). Outbreak of growth anomalies in Isopora palifera at Cocos (Keeling) Islands. *Marine Biodiversity*, 1–2. https://doi.org/10.1007/s12526-019-00967-7

Richardson, L. L. (2004). Black band disease. In *Coral health and disease* (pp. 325–336). SpringerBerlin Heidelberg. https://doi.org/10.1007/978-3-662-06414-6_18

Sato, Y., Bourne, D. G., & Willis, B. L. (2009). Dynamics of seasonal outbreaks of black band disease in an assemblage of Montipora species at Pelorus Island (Great Barrier Reef, Australia).*Proceedings of the Royal Society B: Biological Sciences*, *276*(1668), 2795–2803. https://doi.org/10.1098/rspb.2009.0481

Schatzlein, D. (2015). *XRF technology for non-scientists*. https://assets.thermofisher .com/TFS-Assets/CAD/brochures/TS-eBook-XRF-Technology-in-the-Field.pdf

Spies, N., & Takabayashi, M. (2013). Expression of galaxin and oncogene homologs in growth anomaly in the coral Montipora capitata. *Diseases of Aquatic Organisms*, *104*(3), 249–256.https://doi.org/10.3354/dao02603

Squires, D. F. (1965). Neoplasia in a coral? *Science*, *148*(3669), 503–505. https://doi.org/10.1126/science.148.3669.503

Tribollet, A., & Golubic, S. (2011). Reef bioerosion: agents and processes. In *Coral reefs: an ecosystem in transition* (pp. 435–449). Springer Netherlands. https://doi.org/10.1007/978-94-007-0114-4_25

Tribollet, A., & Payri, C. (2001). Bioerosion of the coralline alga Hydrolithon onkodes by microborers in the coral reefs of Moorea, French Polynesia. *Oceanologica Acta*, *24*(4), 329–342.https://doi.org/10.1016/S0399-1784(01)01150-1

Ushijima, B., Videau, P., Burger, A. H., Shore-Maggio, A., Runyon, C. M., Sudek, M., Aeby, G. S., & Callahan, S. M. (2014). Vibrio coralliilyticus strain OCN008 is an etiological agent of acute Montipora white syndrome. *Applied and Environmental Microbiology*, *80*(7), 2102–2109. https://doi.org/10.1128/AEM.03463-13

Webb, G. E., Nothdurft, L. D., Kamber, B. S., T., K. J., & Zhao, J.-X. (2009). Rare earth elementgeochemistry of scleractinian coral skeleton during meteoric diagenesis: a sequence through neomorphism of aragonite to calcite. *Sedimentology*, *56*(5), 1433–1463. https://doi.org/10.1111/j.1365-3091.2008.01041.x

Weil, E. (2004). Coral reef diseases in the wider Caribbean. In *Coral health and disease* (pp. 35–68). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-06414-6_2

Williams, G. J., Aeby, G. S., Cowie, R. O. M., & Davy, S. K. (2010). Predictive modeling of coraldisease distribution within a reef system. *PLoS ONE*, *5*(2), e9264. https://doi.org/10.1371/journal.pone.0009264

Williams, G. J., Work, T. M., Aeby, G. S., Knapp, I. S., & Davy, S. K. (2011). Gross and microscopic morphology of lesions in Cnidaria from Palmyra Atoll, Central Pacific. *Journal of Invertebrate Pathology*, *106*(2), 165–173. https://doi.org/10.1016/J.JIP.2010. 08.002

Willis, B. L., Page, C. A., & Dinsdale, E. A. (2004). Coral disease on the Great Barrier Reef. In *Coral health and disease* (pp. 69–104). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-06414-6_3

Work, T. M., Aeby, G. S., & Coles, S. (2008). Distribution and morphology of growth anomalies inAcropora from the Indo-Pacific. *Diseases of Aquatic Organisms*, *78*(3), 255–264. https://doi.org/10.3354/dao01881

Work, T. M., Kaczmarsky, L., & Peters, E. (2015). Skeletal growth anomalies in corals. In C. M. Woodley, C. Downs, A. W. Bruckner, J. W. Porter, & S. B. Galloway (Eds.), *Diseases of coral* (pp. 291–299). John Wiley & Sons, Inc. https://books.google.com.au/books?hl=en&lr=&id=bCqwCgAAQBAJ&oi=fnd&pg=PA291&dq=Skeletal+Growth+Anomalies+in+Corals+work+&ots=4k0c0i9L6E&sig=5Uv0zvlefvwUClK9- WKJmm7Arbo

Zhang, Y., Sun, J., Mu, H., Lun, J. C. Y., & Qiu, J.-W. (2017). Molecular pathology of skeletal growth anomalies in the brain coral Platygyra carnosa: A meta-transcriptomic analysis. *Marine Pollution Bulletin*, *124*(2), 660–667. https://doi.org/10.1016/J.MARPOLBUL.2017.03.047

2.9 Supplementary material

Table S2-1: Description of sites surveyed at the Cocos Keeling Islands including what survey method was conducted at each site.

Site	Acronym	Latitude	Longitude	Depth [m]	Ecological Survey	Sample
Direction Island Outside	DIO	S 12º05'01.9	E 096º52'40.3	10-12	Y	Y
Direction Island Inside Marker	DIIM	S 12º05'52.8	E 096º52'40.4	3-5	Y	-
Anchor Hole	AH	S 12º05'57.8	E 096º52'33.2	12-14	Y	Y
Blue Hole 5	BH5	S 12º10'39.7	E 096º52'52.8	1-2	Y	Y
Aquarium 1	A1	S 12º04'55.1	E 096º49.54.1	10-12	Y	-
Kats Kable	KK	S 12º05'20.9	E 096º52.21.0	10-12	Y	Y
Inside Rose Wall	IRW	S 12º05'37.3	E 096º50'35.5	3-5	Y	-
Rose Wall	RW	S 12º05'36.0	E 096º50'30.6	10-12	Y	-
Quarantine Station	QS	S 12º10'01.8	E 096º49'00.2	10-12	Y	-

Table S2-2: Ore Research & Exploration certified standards used in X-ray fluorescence analysis made available to this project.

CRM ID	Mg	Al	P	K	Ca	Sr	Ba	U
OREA S904	63000	320977.1	630	460	11.2	31.5	43.2	
OREA S022d	1320	462701.3		100	0.2	0.69	1.2	
OREA S062e	11730	55306	655	17475	43846	*349*	355	*0.79*

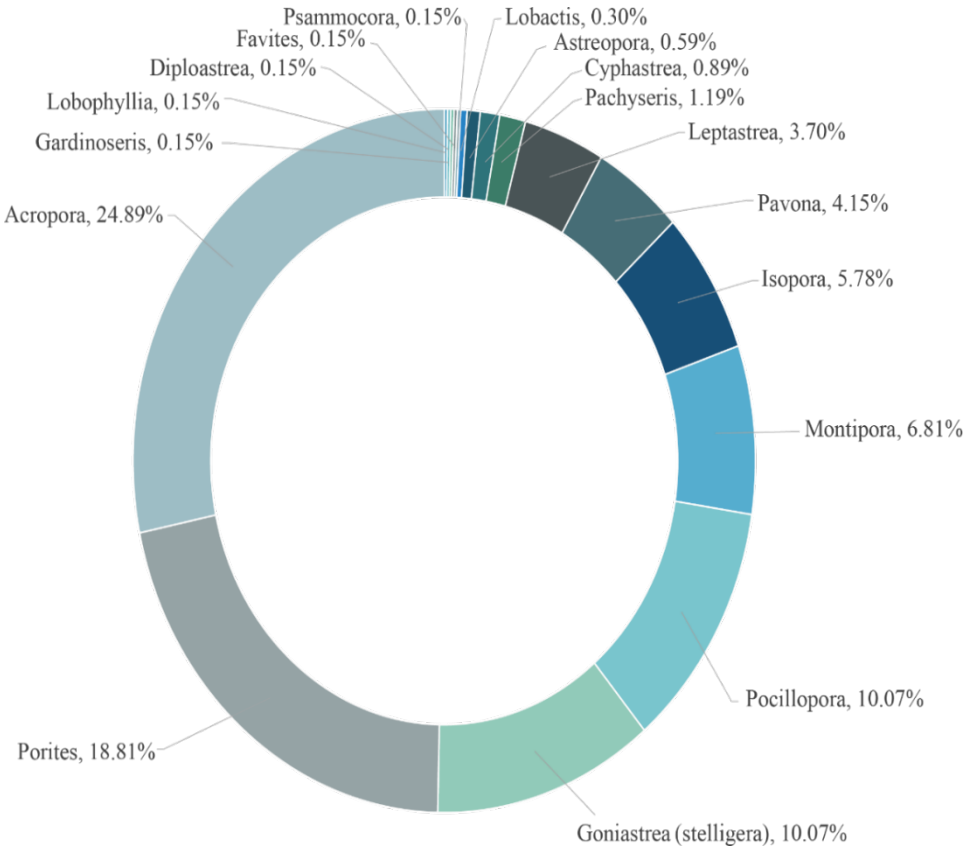

Figure S2-1: Coral cover of sites surveyed at Cocos Keeling Islands by genera. Percentage calculated as proportion of hard coral cover, accounting for 44% of total substrate. Coral cover surveyed by 3x point intersect transect method (50cm).

Table S2-3: Results of SIMPER analysis demonstrating contributions of each element analysed to dissimilarity in trace element composition between healthy and diseased skeletons. Average squared distance = 17.76

Variable	Group Healthy Av. Value	Group Diseased Av. Value	Av. Sq. Distance	Sq. Dist/SD	Contrib %	Cum. %
K	0.528	-0.528	2.54	0.82	14.95	14.95
Ba	-0.401	0.401	2.31	0.76	13.56	28.51
Ca	-0.228	0.228	2.09	0.70	12.28	40.79
Mg	0.189	-0.189	2.05	0.49	12.09	52.88
Al	-0.169	0.169	2.04	0.36	12.00	64.88
P	0.091	-0.091	2.00	0.13	11.76	76.65
Sr	0.0381	-0.0381	1.99	0.77	11.68	88.33
U	0.0126	-0.0126	1.98	0.16	11.67	100

Chapter 3

Bacterial associations of growth anomaly affected

Isopora palifera

3.1 Chapter Acknowledgements

This project was funded by ARC Linkage Project (LP160101508). Coral samples were collected underFisheries Exemption No 3080. For fieldwork I would like to thank Megs Weir and Dieter Gerhard fromCocosDive, and Taj Powell. For advice, support, and edits, I thank Dr. Nicola Browne, Dr. Stephen Newman and Dr. Michael Stat. Finally, I acknowledge and appreciate the extensive support of the TrEnD Laboratory, especially Dr. Katrina West, Matthew Heydenrych and the Coral CORE group.

3.2 Data Accessibility

We have provided the demultiplexed and unfiltered data for this project, along with the taxonomy and abundance data used for data analysis. These can be accessed at: https://osf.io/6zwbt/?view_only=56 b288156eca433ca0b5c3c6757ead6f or on request. The manuscript arising from this chapter is currently in preparation for submission to *Diseases of Aquatic Organisms*. As such, this chapter has been formatted for *Diseases of Aquatic Organisms.*

3.3 Abstract

Growth anomalies (GAs), a skeletal 'tumour', are prevalent across global coral reefs however the causalmechanism is not well understood, a common problem in many coral diseases. As instigation is often the result of complex interactions between abiotic and biotic factors, research has highlighted the importance of the associations of microorganisms, including bacteria, fungi, and viruses, in coral healthand disease. In GA affected corals, there is a lack of evidence for the role of a potential pathogen, and limited understanding of the structure and function of bacterial communities. The outbreak of GAs at the Cocos (Keeling) Islands, provides a unique opportunity to examine the bacterial associations of GA affected *Isopora palifera*. The community composition was established and compared to that found from water samples of the surrounding environment and potential point sourcesof pollution. There was no significant difference observed in bacterial communities of diseased and healthy *I. palifera* samples, and similar alpha diversity measures indicated homogeneity across all health states Whilst overall community composition was not different between samples, there were ASVs affiliated with potentially pathogenic bacteria present solely in diseased samples, which may be causal agents of GAs or the result of a compromised immune and defence system. Coral samples displayed a distinct microbiome than those found within the water column, and there was no apparent association with potential point sources of pollution from either coral or water samples. The bacterial community diversity patterns of broad similarity indicate dysbiosis of the coral microbiome may not be a factor in the onset of GAs, or the result of disease. The results of this study contradict the concepts of dysbiosis as a causative factor or the result of this coral disease. Further investigation should focus on non-bacterial agents of disease, underlying gene expression pathways and disease modelling and potential vectors to determine whether this disease is transmissible and its dynamics.

3.4 Introduction

The physiological impacts of growth anomalies have been well characterised, however the mechanisms behind instigation and transmission are not well understood. Growth anomaly outbreaks have been associated with thermal stress events and proximity to anthropogenic centres (Aeby et al., 2011), whilst research has shown the potential involvement of waterborne microbial transmission or direct contact between colonies (Kaczmarsky & Richardson, 2007; Work et al., 2015). The circumstances surrounding this disease are similar to that of other coral diseases, where extenuating factors and the complexity of the coral host itself hinder efforts to identify causal agents (Gignoux-Wolfsohn et al., 2017; Sussman et al., 2008). Despite this, investigation into the microbiome of affected corals has revealed potential pathogens for coral diseases (Pollock et al., 2016; Richardson et al., 2014; Sweet et al., 2014).

Corals and their symbionts are referred to as the 'holobiont', consisting of host, bacteria, fungi, dinoflagellates, and other associated microbiota (Knowlton & Rohwer, 2003; Rohwer et al., 2002). This holobiont is hosted in microhabitats (mucus, tissue, skeleton) across the complex structure, each displaying unique communities (Pollock et al., 2018; Sweet et al., 2011). The bacterial microbiome is a part of the holobiont, referring to the community of bacterium specific to the host, species, geographic location, environmental conditions, and health status (Bourne et al., 2016; Carlos et al., 2013; Chu & Vollmer, 2016; Thurber et al., 2009). This variability and transiency in community structure is a key trait of coral microbiomes (Hernandez-Agreda et al., 2018), and dynamic nature of the microbiome challenges classical methods of diagnosis. These techniques including the presence of unculturable- microbes (Richardson et al., 2015), the role of consortiums (Cooney et al., 2002) and the presence of potentially pathogenic bacterium in both enzootic and epizootic states (Lozada-Misa et al., 2015). Complementary traditional and modern methods of characterising coral diseases have been advocated to examine the complex interactions between host, symbiont, and environment in the context of coral diseases (Bourne et al., 2009; Burge et al., 2016). Current molecular methods have facilitated research into the structure and function of the bacterial microbiome from both stressed and healthy coral hosts (Egan & Gardiner, 2016; Glasl et al., 2019; Thurber et al., 2009) and identified potentially causative agents of disease (Morrow et al., 2012; Roder et al., 2014; Ushijima et al., 2012). Rethinking the structure and function of the microbiome under anomalous thermal stress has revealed adaptive dysbiosis (i.e., the imbalance in bacterial communities) associated with thermally tolerant coral species (Boilard et al., 2020; Pratte & Richardson, 2018). The same concept of imbalance in microbial communities has been applied to

investigate the pathogens behind coral diseases and shifts in associated bacterial communities (Pollock et al., 2016; Rajasabapathy et al., 2020), particularly where an outbreak occurs without obvious environmental triggers (Egan & Gardiner, 2016).

The bacterial associations of growth anomaly affected scleractinian corals remains largely unknown. The microbial communities of GAs have reported a higher growth rate than those of healthy colonies (Breitbart et al., 2005) and two studies found community composition to be affected by health status (Ng et al., 2015; Rajasabapathy et al., 2020). Conversely, a broader study found that changes to bacterial composition were more related to environmental variables, rather than health status, after sampling across seasons and locations (Chiu et al., 2012). This study aims to establish the bacterial associations of healthy and diseased *Isopora palifera* to characterise community composition and account for any spatial distribution. Secondly, this study aims to investigate the bacterial associations of the surrounding water column, and from potential sources of aquatic pollution and compare to those found within the coral samples to identify any indicator taxa. The findings of this study provide new information regarding the bacterial associations of GA-affected corals, particularly in a unique environment where anthropogenic pressure is minimal, and the reef has experienced no known, recent environmental stress event (Preston & Richards, 2021).

3.5 Methods

3.5.1 Ecological surveys and specimen collection

Whole branch specimens were collected during May 2019 at four sites at CKI (Figure 3-1) from affectedand asymptomatic healthy *Isopora palifera* (Figure 3-2). GA-affected colonies were identified by the presence of pale, tumorous masses. Three replicate branches were collected from three diseased colonies, and one branch from three control healthy colonies per site (n = 48). Samples were stored in seawater in separate labelled bags to prevent contamination until processed at field laboratory. Specimens were divided into 3-5cm subsamples and stored in ethanol for transport to the Trace and Environmental DNA (TrEnD) Laboratory, Curtin University, Western Australia. Branches from GA- affected colonies were subsampled into diseased and adjacent 'healthy' samples to account to intra-colonyvariation.

Water samples were also collected at the same four sites, in addition to two possible point sources of aquatic pollution (West Island Outfall and Dump, Figure 3-1). At each site six 1L samples were collected utilising either a Niskin bottle where depth exceeded 2m, or by hand (n=24). Access and coordinates of the West Island Outfall were arranged with the local Water Corporation office, to coincide with a manual override of the outflow. Using two Sentino peristaltic microbiology pumps (Pall Life Sciences, New York, USA), samples were filtered through sterile 47mm cellulose filter membraneswith a pore size of 0.22um (Pall Life Sciences, New York, USA). Filtering equipment was soaked in 10% sodium hypochlorite solution for a minimum of 15 minutes prior to use and between processing each sample, after which, equipment was rinsed with deionised water. Sodium hypochlorite controls (1L) were filtered daily post processing, as well as a one-off 1L sample of tap water from the West Island supply. Filter membranes were stored in individual labelled sterile zip lock bags and frozen at - 20ºC until DNA extraction in the TrEnD Laboratory. Samples were transported on ice back to Perth, Western Australia, under Permit to Import Conditionally Non Prohibited Goods (Permit No: 0002817498) issued to Dr. Michael Bunce at the TrEnD Laboratory, Curtin University. No animal ethics approvals were required for this project.

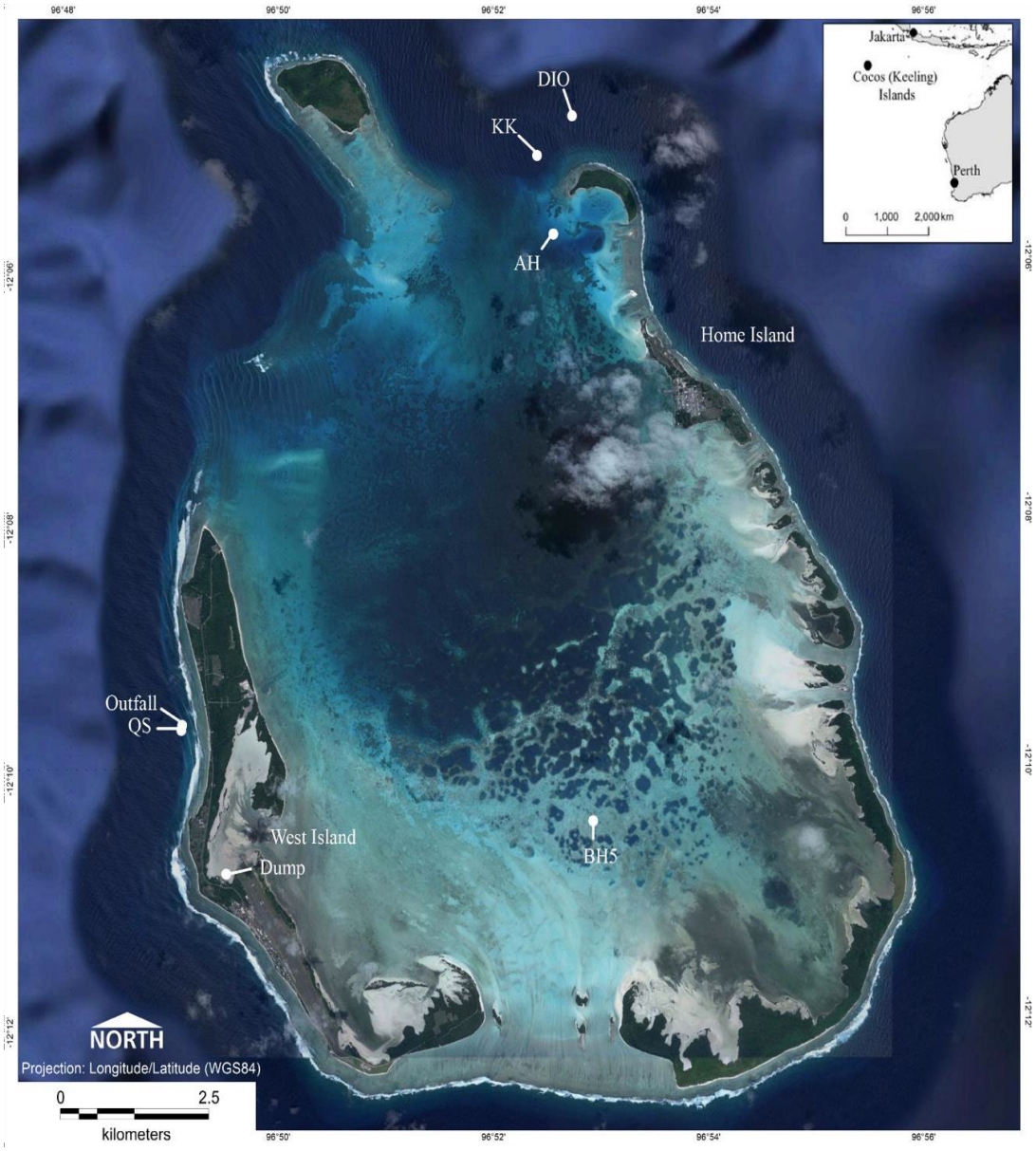

Figure 3-1: Map of sample sites at Cocos (Keeling) Islands. For detailed site information and specimen collection, see Table S3-1.

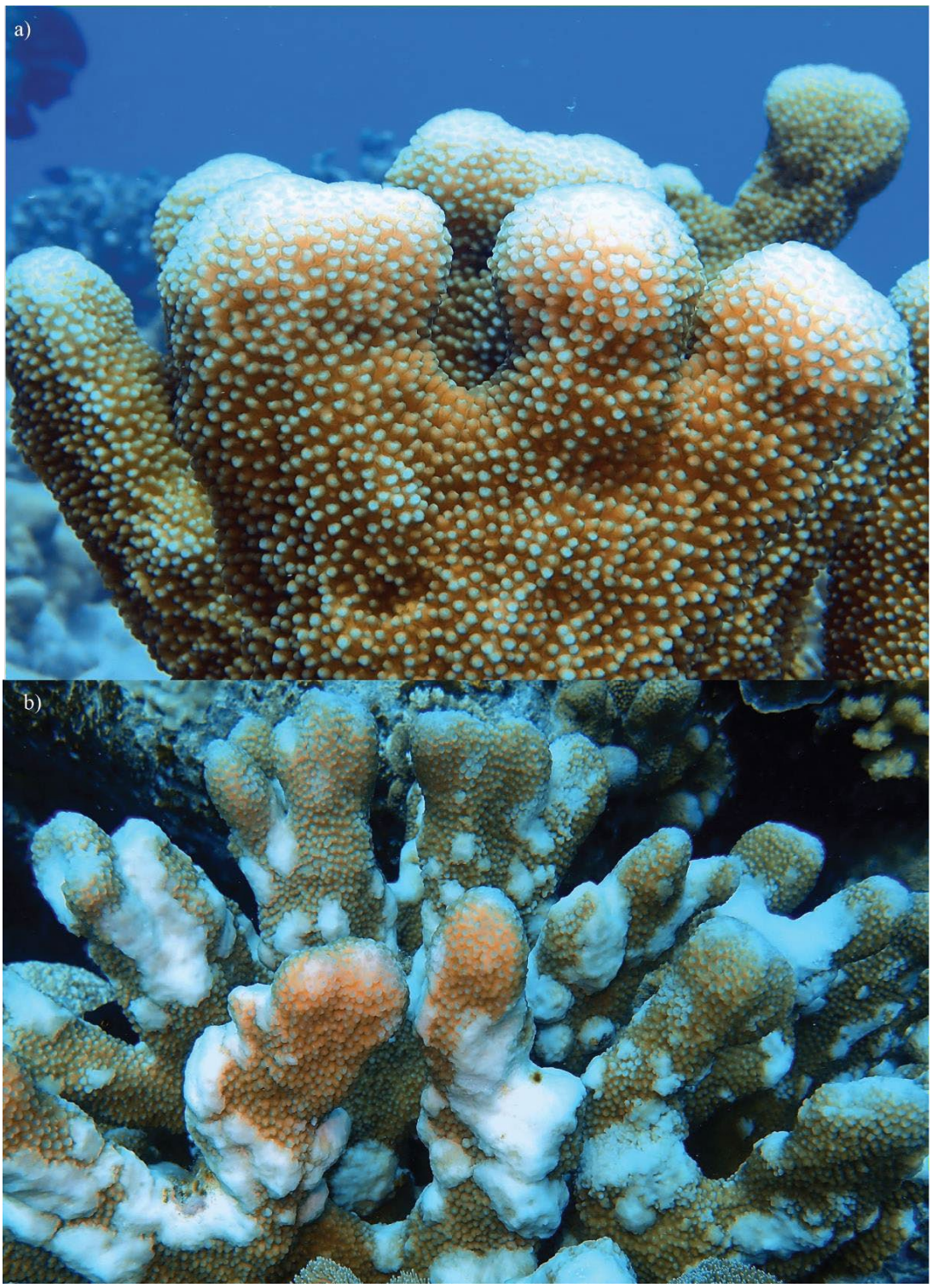

Figure 3-2: Field photo of a) healthy *I. palifera* colony and b) GA-affected *I. palifera*

3.5.2 DNA extraction and sequencing

A small piece (3-5 mm) of whole coral sample (n = 64), and half the water filter papers (n=77) were used for DNA extraction using DNeasy Blood and Tissue Kit (Qiagen; Venlo, Netherlands), following manufacturer protocol with the following modifications: 360 ul ATL Buffer and 40 ul proteinase K. A whole sample of coral was included in the extraction to capture the bacterial communities of each microhabitat of the coral. The remaining water filter paper were stored for future use. The samples and field controls, in additionto extraction controls (n = 6) were digested overnight at 56ºC and 400ul of digested supernatant was transferred to a clean 2 ml tube for extraction, using a QIAcube automated sample preparation platform(Qiagen; Venlo, Netherlands). DNA extracts were then stored at -20ºC until further analyses

Quantitative PCR (qPCR; Applied Biosystems StepOnePlus, Massachusetts, USA) was used to assess the primer binding efficiency, quality, and quantity of DNA in the extracts (n = 71) at three dilution points (Neat, 1/10, 1/100). Extracts were carried through at the most optimal dilution point for low inhibition, as determined by initial qPCR screen, and assigned a fusion tagged primer, consisting of anIllumina sequencing adaptor, unique multiplex identifier tag (MID-tag, 6-8 bp), and Bact16S primers 341 (5'-CCTACGGGNGGCWGCAG-3') and Bact16S 785 (5'-GACTACHVGGGTATCTAATCC- 3') targeting the hypervariable V3-V4 region (Klindworth et al., 2013). All qPCR reactions were prepared in a dedicated ultra clean laboratory and reaction volumes consisting of: 1 x PCR Gold Buffer(Applied Biosystems), 2 mM $MgCl_2$ (Applied Biosystems), 0.4 µM dNTPs (Astral Scientific, Australia), 0.1 mg/ml bovine serum albumin (BSA; Fisher Biotec, Australia), 0.4 µM forward and reverse primers, SYBR-Green dye (Life Technologies, USA), 0.2 µL AmpliTaq Gold (Applied Biosystems) and 2 uL of DNA extract solution, made up to volume with Ultrapure™ Distilled Water (Life Technologies; Table S3-2). The qPCR reaction was performed under the following conditions: denaturation for 5 minutes at 95ºC, 40 cycles of 95ºC for 30 seconds, 55ºC for 30 seconds then 72ºC for 30 seconds, followed by 60ºC for 1 min, 95ºC for 15 seconds and a final 10 minutes at 72ºC (Alexander et al., 2018).

Fusion tagged PCR reactions were conducted in duplicate for each extract including lab, field and PCR reaction controls, and qPCR cycling conditions were as above with the exception of 45 cycles. Post fusion tag PCR amplification, approximately five fusion tagged samples were pooled (minipooled).together in equimolar ratios and amplicon size distribution and concentration of each minipool was assessed using QIAxcel Advanced platform (Qiagen) prior to equimolar blending into a final library forDNA sequencing. The final library was size

selected (160-600 bp) using Pippin Prep (Sage Sciences, USA) to remove any primer-dimer products (<160 bp) that may have formed during fusion tag PCR reactions, and the size selected library was purified on silicate columns (Qiaquick Purification Kit (Qiagen). The final library concentration was determined using a QuBit™ 4 Fluorometer (ThermoFisher, Australia) and sequencing by synthesis on a MiSeq platform (Illumina, USA) was performed as per Illumina protocols for amplicon sequencing, using a paired-end 600 cycle V3 kit.

3.5.3 Bioinformatic analyses

Raw paired end sequence data were downloaded as separate Read 1 (R1) and Read 2 (R2) fastq files from the Illumina BaseSpace platform (Illumina, USA). Before demultiplexing, run integrity was checked via flow cell clustering density and overall sequence quality visualised using the FastQC tool (Andrews, 2018). One water sample (AWFS680) was removed for poor sequence quality (<Q30). Raw R1 and R2 fastq files were demultiplexed into individual samples according toits unique MID-tag combination, using shi7 (Al-Ghalith et al., 2018). Once sequences were binned intotheir corresponding samples, all variants of the forward and reverse primer sequences were selected and removed using the cutadapt tool (v2.5) (Martin, 2011) retaining only biological target sequences.

Demultiplexed reads were filtered using a DADA2 pipeline (Callahan et al., 2016), with the followingparameters: maximum of 2 errors allowed per read and R1 length truncated to 270 bp and R2 to 230 bp,a minimum quality score of 30, no ambiguous bases (N) allowed, and removal of reads aligning to phix(bacterial phage). Quality scores of trimmed and filtered reads were checked, error rates estimated, andsequences were dereplicated and merged, with a minimum overlap of 20 bp. Sample amplicon sequence variants (ASVs) (Callahan et al., 2017) were inferred and cleaned according to the estimated ampliconlength (399 – 460 bp), chimeric sequences removed, after which the amplicon length was refined oncemore (400-430bp). Taxonomy of the unique ASVs was assigned using DADA2 (AssignTaxonomy command) and a combined reference database (Alishum, 2019) utilising both the Genome Taxonomy Database (GTDB v89) (Parks et al., 2018) and a combined NCBI RefSeq and RDB Database (Cole et al., 2014; O'Leary et al., 2016). The AssignTaxonomy command followed the RDP naïve bays classifier(Wang et al., 2007) at a bootstrap confidence of 50%.

To confirm the accuracy of taxonomy assignment, ASVs were blasted using blastn tool (v2.9.0) (NCBI)against all blast converted DADA2 databases mentioned above to detect any additional species hits in the database using the following parameters: word size: 30, percentage id: 99%, query cover: 100%, evalue: 1e-10. ASVs with multiple taxonomic hits at 100% were checked for origin close to sample type, and those not originating from an

environmental source were removed as contamination. If multiple annotations of marine origin were returned, then ASV annotations were collapsed to last common phylogenetic rank. ASV tables from both databases were combined, all sequences found in field and laboratory controls were removed as contamination, and a cut-off of 10 reads total across all samples was applied (Camp et al., 2020). Two water samples (AWFS739 and AWFS741) were removed post filtering for poor sequence recovery.

All bioinformatic analysis were performed on RStudio (v1.1.452).

3.5.4 Data analysis

ASVs were imported into presence/absence and relative abundance matrices to assess potential differences of bacterial communities between health status (diseased, adjacent asymptomatic, control healthy) and between sites. Abundance data from diseased and adjacent asymptomatic coral samples from the same colony (n = 34), and control healthy samples (n = 12), were converted into a Bray-Curtis similarityresemblance matrix. A two way permutational analysis of variance (PERMANOVA) main test (health status, site) was performed based on 9999 permutations and unrestricted permutations and significant results were further analysed using a pairwise PERMANOVA applying the same parameters. This analysis was repeated for both presence/absence and relative abundance matrices.

Alpha diversity of coral samples was first analysed using the DIVERSE function in Primer 7, and thenany significant differences were identified using a two-way PERMANOVA, for site and health status. The core bacterial microbiome at genera level was described where core is defined as the genera beingpresent in ≥80% of samples (Hernandez-Agreda et al., 2018), for all coral samples, and separate health statuses. Known bacterial pathogens of other coral diseases and unique genera to each health status were also identified in samples.

The bacterial associations of healthy and GA-affected samples were further examined in both a SIMPER analysis, examining Site effects and based on a presence/absence Bray Curtis resemblance matrix in PRIMER 7 and by using an mvabund(v4.1.6) approach (Wang & Wright, 2012) on raw ASVs. A generalised linear model was created considering negative binomial distribution and tested using an ANOVA for both health status and site. The results were visualised using the pheatmap package (v1.0.12) (Kolde, 2012).

To establish whether there was a distinct microbiome or shared taxa between the water column and coral samples, a two-way PERMANOVA was performed on ASVs between type (water, coral) and sites. The parameters used were as above and followed with a pairwise PERMANOVA to examine differences between point sources of pollution and the water and

coral samples. Alpha diversity was also assessed here using the DIVERSE function, and. All analyses were conducted in RStudio (v1.2.500) and R (v3.6.1) or Primer 7 (v7.0.17).

3.6 Results

A total of 6919 good quality amplicon sequence variants (ASVs) were obtained from water samples and samples collected from diseased and healthy *I. palifera* colonies. Post filtering, this resulted in 3092 ASVs belonging to the Bacteria (96.98%) and Chromista (3.02%) kingdoms; 1092 from coral samples and 2326 from water samples. There was an average of 10486 ± 1602.27 reads per sample across all (n = 135), or 4421.63 ± 616.29 from coral samples (n=64) and 15952.5 ± 2852.34 from water samples (n=71).

Within coral samples, there were unique ASVs assigned to control, adjacent heathy and diseased coral samples(39, 23 and 62 respectively; Table 3-1). Control healthy samples were dominated (>5%) by sequences belonging to phyla Proteobacteria (40.61%), Cyanobacteria (37.82%), Chlorobi (9.08%) and Bacteroidetes (8.81%). Asymptomatic samples from diseased colonies were dominated by sequences associated with phyla Proteobacteria (63.41%), Cyanobacteria (21.65%) and Bacteroidetes (11.47%). Growth anomalies affected samples were dominated by sequences associated with phyla Actinobacteria (54.46%), Bacillariophyta (14.87%), Proteobacteria (14.67%), Bacteroidetes (6.40%).

Alpha diversity measures were comparable between samples indicating similar bacterial richness and diversity between apparently healthy colonies, and the healthy and diseased samples of affected *I. palifera* colonies. The core microbiome for all *I. palifera* samples (represented in 80% of samples) were *Endozoicomonas* associated ASVs and unclassified ASVs. SIMPER analysis revealed *Endozoicomonas* associated ASVs accounted for 12.01%, 12.88% and 17.09% of similarity within control, healthy and diseased samples, respectively (Table S3-3).

Table 3-1: Summary of assigned bacterial diversity, at ASV level, of diseased, adjacent asymptomatic and control samples of *Isopora palifera* colonies

	Control	**Adjacent asymptomatic**	**Diseased**
Kingdom	2	2	2
Phyla	8	7	8
Classes	14	15	15
Orders	25	24	27
Families	53	48	61
Genera	108	86	127
Number ASVs exclusive to group	332	142	344
Species evenness (Pielou's) ± SE	0.64 ± 0.03	0.65 ± 0.04	0.64 ± 0.03
Species richness indices (Margalef) ± SE	5.04 ± 0.64	5.34 ± 0.85	5.51 ± 1.01
Shannon-Weaver estimate ± SE	2.25 ± 0.15	2.30 ± 0.19	2.34 ± 0.20

A multivariate PERMANOVA based on a presence/absence Bray-Curtis similarity matrix revealed a significant interaction between site and health status (control, adjacent asymptomatic, diseased), and for site main effects (Table 3-2). The same PERMANOVA based on a relative abundance matrix founda significant difference in Site only (Table 3-3). The significant effect of Site on community composition was confirmed in a MVABUND analysis, driven by 38 genera (Figure S3-1). Further pairwise PERMANOVA's on both matrices revealed sites to be the only significantly different factor (Figure 3-3). There was no significant difference in bacterial communities driven by health status in either presence/absence or relative abundance matrices.

Table 3-2: Two way PERMANOVA based on a presence/absence Bray-Curtis similarity matrix. * <0.05, **<0.005

Source	**Df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Perms**
Health Status	2	6667.2	3333.6	1.2219	0.1297	9848
Site	3	41353	13784	5.0527	0.0001**	9828
Health Status x Site	6	21340	3556.6	1.3037	0.0109*	9740
Res	52	$1.4186e^{05}$	2728.1			
Total	63	$2.1207e^{05}$				

Table 3-3: Two way PERMANOVA based on a relative abundance Bray-Curtis similarity matrix.

Source	Df	SS	MS	Pseudo-F	P(perm)	Perms
Health Status	2	6335.8	3167.9	1.0545	0.3414	9842
Site	3	45836	15279	5.0856	0.0001**	9794
Health Status x Site	6	18693	3115.4	1.037	0.3555	9725
Res	52	$1.5622e^{05}$	3004.3			
Total	63	$2.2842e^{05}$				

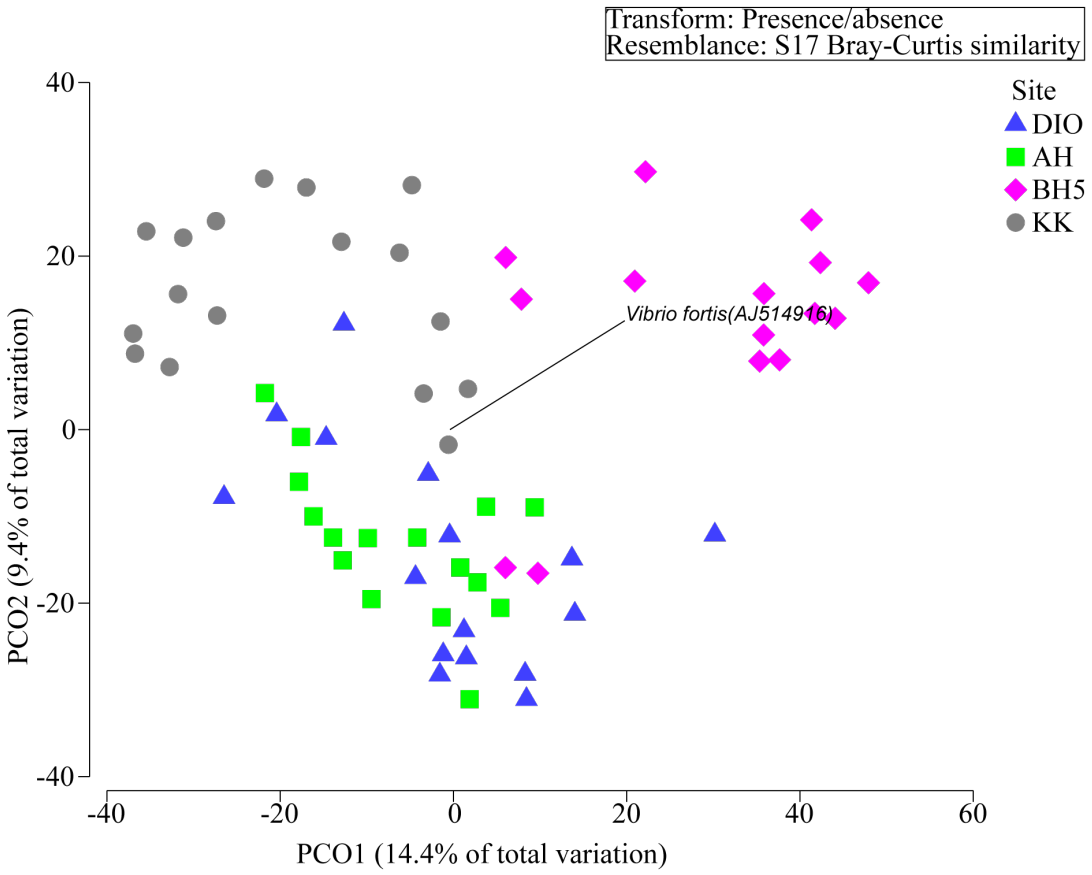

Figure 3-3:PCO of bacterial communities of *I. palifera* reflecting site distribution. Based on presence/absence Bray-Curtis similarity matrix. Pearson correlations to 0.8.

3.6.1 Bacterioplankton and coral associated microbial communities

Bacterioplankton communities were significantly different to that found within the samples of *I. palifera* (p = 0.0001; Table 3-4; Figure 3-4). Water samples displayed a higher alpha diversity than that of coral samples, with a species richness of 23.34 ± 1.90 compared to 5.50 ± 0.51 in coral samples (Table 3-5).A pairwise PERMANOVA revealed there was also a

significant difference between water samples taken from each site, and coral samples (Table S3-4). In particular, they appeared to present in three distinct clusters (Figure 3-4) with those taken from Blue Hole 5 (BH5) and the West Island Dump (WID) driven by ASVs associated with *Litoricola* and *Phaeocystidibacter.* Water samples retrieved from the north-eastern side of the atoll, as well as from the West Island Outfall, were distributed between two groups. There was however a significant difference in the bacterioplankton communities found within *I. palifera* than those in the water column ($p = 0.0001$; Table 3-4), predominantly driven by ASVs affiliated with the common endosymbiont *Endozoicomonas* (Figure 3-4). Furthermore, water samples displayed a higher alpha diversity than that of the coral samples, with a species richness of 23.34 ± 1.90 compared to 5.50 ± 0.51 in coral samples (Table 3-5). Across all water samples, 126 ASVs were identified as potentially pathogenic (Lamb et al., 2017), including ASVs associated with members of *Vibrio, Clostridium* and *Arcobacter*, however their presence across the atoll could not be directly linked to potential point sources of pollution (Figure S3-2).

Table 3-4: One-way PERMANOVA based on a presence/absence Bray-Curtis similarity matrix.

Source	Df	SS	MS	Pseudo-F	P(perm)	Perms
Type	1	83813	83813	22.823	0.0001***	9797
Res	125	$4.5904e^{05}$	2457.4			
Total	126	$5.4285e^{05}$				

Table 3-5: Alpha diversity measures of bacterial communities of coral samples and the surrounding water column.

	Coral	Water
Species evenness (Pielou's) ± SE	0.65 ± 0.02	0.61 ± 0.02
Species richness indices (Margalef) ± SE	5.50 ± 0.51	23.34 ± 1.90
Shannon-Weaver estimate ± SE	2.33 ± 0.11	2.94 ± 0.14

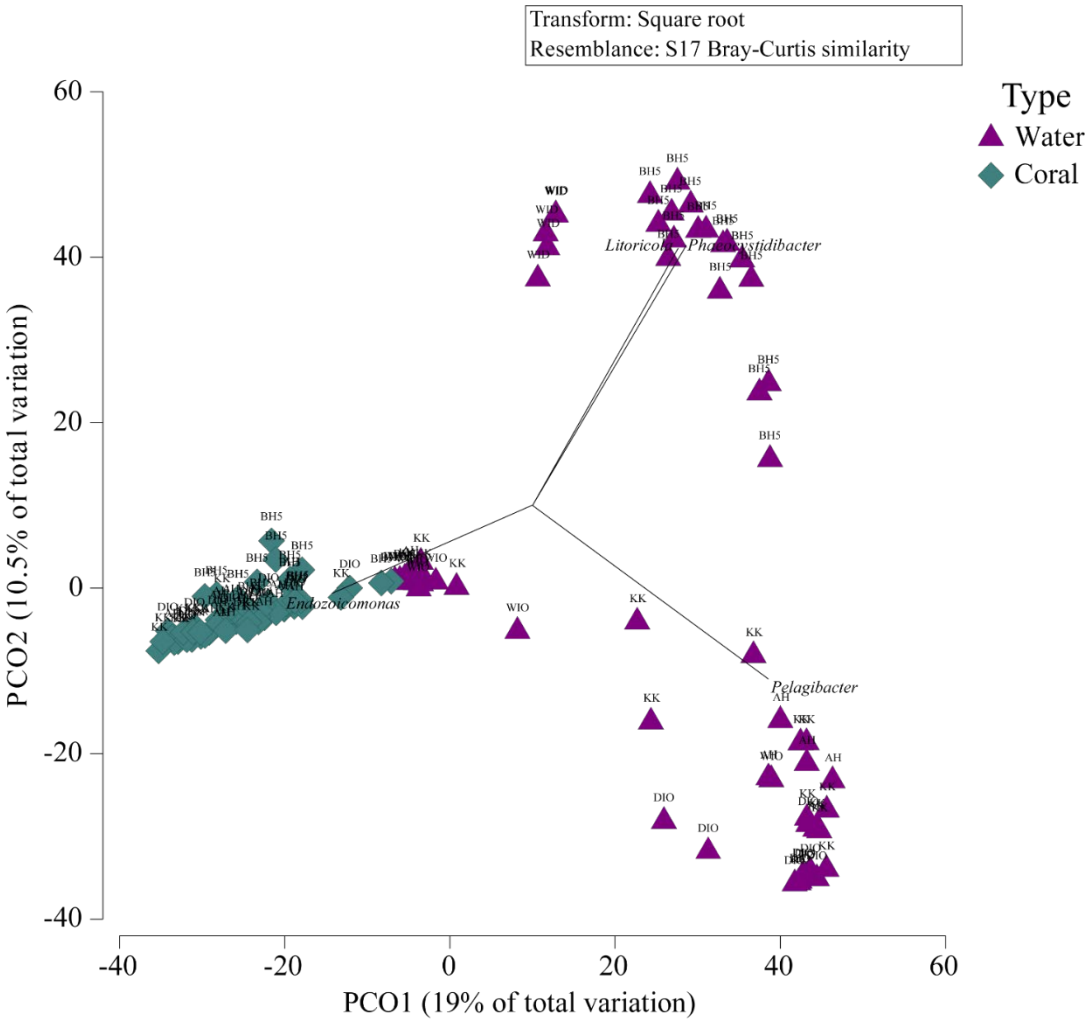

Figure 3-4: PCO of bacterial communities of *I. palifera* and the surrounding water column. Based on presence/absence Bray-Curtis similarity matrix. Pearson correlations to 0.8.

3.7 Discussion

Characterising the complex and dynamic bacterial associations of reef building corals in various health states is an important foundation step towards identifying causal agents or mechanisms of coral diseases. Here, the bacterial communities associated with healthy and diseased samples of *I. palifera* were found to be broadly overlapping with no clear evidence that GA-affected samples possessed unique bacterial communities. Genera that were differentially abundant between control, apparently healthy and diseased samples of *I. palifera* were predominantly from the phylum Proteobacteria (52.63%) followed by Bacteroides (18.42%), Acidobacteria (10.53%), Cyanobacteria/Chloroplast and Firmicutes (7.89% each), and Chlorobi (2.63%). These genera however were not found to be associated with a particular health state. Similarly, alpha diversity measures were also found to be comparable across samples, despite a higher number of genera reported solely in diseased samples. The findings of this study are consistent with other reports of bacterial associations of GA affected corals (Ng et al., 2015; Rajasabapathy et al., 2020) whereby no significant difference in bacterial diversity were detected between samples. Furthermore, Chiu et al (2012), reported alpha diversity to be affected by environmental variables (seasonal variations), rather than the effects of growth anomalies.

Several genera associated with disease and infection in the marine environment were identified within GA-affected samples. Particularly, Cyanobacteria were in relatively high abundance across all health states (Table S3-3). Whilst this may be the result of endolithic communities typically found in *I. palifera* (Yang et al., 2016, 2019), the relationship between endolithic communities and potentially parasitic communities warrants their inclusion in future microbiome studies (Ainsworth et al., 2017). Furthermore, several ASVS affiliated with genera associated with Black Band disease were found, including members of the *Clostridium* consortium (Cooney et al., 2002; Frias-Lopez et al., 2002), and *Adhaeribacter* (Sekar et al., 2006). An ASV assigned to the genus *Arenicella* was detected in GA- affected samples, similar to another study on GA bacterial associations (Ng et al., 2015). Of note is the presence of *Bacteroides* associated ASV, a member of the Cytophaga-Flexibacter-Bacteroides group associated with the destructive white band disease and white plague (Garcia et al., 2013; Pantos & Bythell, 2006), and the presence of an ASV associated with *Bdellovibrio*, a genus of predatory bacteria that feed on pathogenic bacteria such as *Vibrio* (Welsh et al., 2016). Whilst these potential pathogens of coral disease were found solely within diseased samples, given that GAs are known to compromise the immune systems of corals (Kelly et al., 2016; Palmer & Baird, 2018; Zhang et al., 2017), their proliferation is likely that of an opportunistic microbe.

Bacterial communities differed significantly by site, a reflection of the dynamic nature of the coral microbiome and the influence of spatio-temporal variation (Zhang et al., 2015; Ziegler et al., 2019). Environmental variables, such as waterflow, temperature, salinity, and nutrients have all reported to affect the structure and function of the coral microbiome (Carlos et al., 2013; McKew et al., 2012; Pantos et al., 2015; Zhang et al., 2015). Furthermore, corals have demonstrated both intra and inter colony variation, adapting their symbionts to the local environment (Chu & Vollmer, 2016; Morrow et al., 2012). The difference in bacterial communities found at sites across the Cocos (Keeling) Islands may be reflective of their largely transient and environmentally responsive communities (Hernandez- Agreda et al., 2018; Morrow et al., 2015) and future studies should increase sample effort over time totrack the response of these communities to fluctuations in environmental conditions. Despite these factors, both diseased and apparently healthy colonies displayed distinct common microbiomes when compared to that found in the surrounding water column, and in particular, no apparent associations with potential sources of aquatic pollution (Table 3-3). This finding is particularly important given growth anomalies have been associated with sewerage (Yoshioka et al., 2016) and anthropogenic pressure (Aeby et al., 2011). Whilst there was a significant difference between coral associated bacterial sequences, and those found within the water column, Figure 3-4 demonstrated that several water samples displayed similarities to coral samples. This is potentially the result of the transient communities of the coral microbiome (Hernandez- Agreda et al., 2018; Morrow et al., 2015), and further investigation is warranted into the interactions between bacterioplankton and the coral microbiome. The bacterial communities of corals are comprised of sequences associated with endosymbionts, such as *Endozoicomonas*, nutrient cycling including *Alteromonas, Pseudomonas and Vibrio* (Raina et al., 2009), nitrogen cycling (Chimetto et al., 2008) and antipathogenic activity (Sa'adah et al., 2018). Water samples were dominated by sequences associated with microbial plankton and algae, including *Pelagibacter* and *Phaeocystidibacter* (Figure 3-4) and bacteria associated with biogeochemical and atmospheric processes (Louca et al., 2016; Moran, 2015). The fine scale spatial variation between water samples is likely to be the result of water movement around the atoll, particularly the distinct separation of the West Island Dump, and Blue Hole 5 samples located at the southern end of the atoll.

Given that no common indicator taxa could be attributed to the core microbiome of diseased samples, with the exception of the common endosymbiont *Endozoicomonas*, it is unlikely that this disease is either the result of a single pathogen as seen in other studies (Luna et al., 2009; Ushijima et al., 2012, 2014), or that the causal agent is found within the unclassified consortium. Furthermore, there was no indication of a shift in diversity measures between

healthy and diseased samples indicating stability in the bacterial communities. Measuring microbial diversity is commonly used to monitor coral health (Zaneveld et al., 2017), and dysbiosis (the imbalance in communities) has been associated with both the onset (Egan & Gardiner, 2016; Moriarty et al., 2020; Thurber et al., 2020), and result (Boilard et al., 2020; Silva-Lima et al., 2020) of coral disease. Instead, the stability found here supports the idea of compositional homogeneity (Sweet et al., 2019; Sweet & Bulling, 2017), however alpha diversity measures were comparable between all coral sample types indicating that the structure of the bacterial microbiome may not be as influential in regard to GAs.

The unique conditions of this outbreak (low anthropogenic presence and no apparent environmental anomaly) (Preston & Richards, 2021), allow for a rare opportunity to study the bacterial associations of growth anomaly affected corals without a significant contributing factor, a situation not often afforded when characterising coral diseases. The complexity of the coral microbiome has proved a significant challenge to establishing the causation of many coral diseases (Glasl et al., 2019; Schellenberg & Clarke, 2020). Abiotic environmental factors aside, the coral microbiome is also influence by interstitial fauna (Ainsworth et al., 2020), anthropogenic pressure (Kelly et al., 2014; Ziegler et al., 2016) and predation (van Oppen & Blackall, 2016; Welsh et al., 2016). This study was conducted using the 16S rRNA gene as a genetic marker for both species' identification and diversity. Whilst useful in its approach, it is limited in its ability to discriminate strains that carry specific pathogenicity genes and mutations in pathogenicity-associated genes that would render the microbe more pathogenic of improve evasion of host immune response. For GAs, understanding the dynamics of functional niches filled by bacteria may be more useful than community composition, as well as the interactions between bacterium and other microbes (Rypien et al., 2010; Thurber et al., 2017).

3.8 Conclusion

This study has provided the first characterisation of the microbiome of both the CKI population of *I. palifera*, and the bacterial associations of growth anomaly affected corals, free of anomalous environmental influence and experiencing low anthropogenic influences from sewerage outfalls and population density. The homogeneity identified across both control, adjacent asymptomatic, and diseased samples indicates broad similarities in bacterial composition, and future research should focus on the functional role of bacterial communities, temporal inconsistencies in microbiome community composition, potential acquired virulence and alternative microbial agents to establish the causation of growth anomalies. Furthermore, the distinct microbiomes of coral samples and the water column

indicated no association with the potential point sources of pollution on West Island. Whilst this outbreak provided a unique opportunity to study the microbiome of growth anomaly affected corals, itraises the question of how this remote atoll came to be affected by a disease previously thought to be associated with environmental or anthropogenic stressors.

3.9 References

The owners of copyright material used in this thesis have been acknowledged with every fair and reasonable effort. I would be grateful to hear from any copyright owner should they feel they have been omitted or acknowledged incorrectly.

Aeby, G. S., Williams, G. J., Franklin, E. C., Haapkyla, J., Harvell, C., Neale, S., Page, C. A., Raymundo, L., Vargas-Ángel, B., Willis, B. L., Work, T. M., & Davy, S. K. (2011). Growth anomalies on the coral genera Acropora and Porites are strongly associated with host density andhuman population size across the Indo-Pacific. *PLoS ONE*, *6*(2), e16887. https://doi.org/10.1371/journal.pone.0016887

Ainsworth, T. D., Fordyce, A. J., & Camp, E. F. (2017). The Other Microeukaryotes of the Coral Reef Microbiome. Trends in Microbiology, 25(12), 980–991. https://doi.org/10.1016/j.tim.2017.06.007

Ainsworth, T. D., Renzi, J. J., & Silliman, B. R. (2020). Positive Interactions in the Coral Macro and Microbiome. *Trends in Microbiology*, *28*(8), 602–604. https://doi.org/10.1016/j.tim.2020.02.009

Al-Ghalith, G. A., Hillmann, B., Ang, K., Shields-Cutler, R., & Knights, D. (2018). SHI7 Is a Self-Learning Pipeline for Multipurpose Short-Read DNA Quality Control. *MSystems*, *3*(3). https://doi.org/10.1128/msystems.00202-17

Alexander, J., Bunce, M., White, N., Wilkinson, S., Stat, M., Berry, T., Newman, S., & Richards, Z. T. (2018). Evaluating environmental DNA (eDNA) metabarcoding as a viable method of detecting diversity in reef-building corals of the Cocos (Keeling) Islands. *Unpublished*, 8.

Alishum, A. (2019). *DADA2 formatted 16S rRNA gene sequences for both bacteria & archaea(Version 2) [Data set]*. Zenodo. https://doi.org/10.5281/zenodo.3266798

Andrews, S. (2018). *FastQC A Quality Control tool for High Throughput Sequence Data* (0.11.8).Babraham Bioinformatics. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Boilard, A., Dubé, C. E., Gruet, C., Mercière, A., Hernandez-Agreda, A., & Derome, N. (2020). Defining coral bleaching as a microbial dysbiosis within the coral holobiont. *Microorganisms*,*8*(11), 1–26. https://doi.org/10.3390/microorganisms8111682

Bourne, D. G., Garren, M., Work, T. M., Rosenberg, E., Smith, G. W., & Harvell, C. (2009). Microbial disease and the coral holobiont. In *Trends in Microbiology* (Vol. 17, Issue 12,

pp.554–562). https://doi.org/10.1016/j.tim.2009.09.004

Bourne, D. G., Morrow, K. M., & Webster, N. S. (2016). Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. *Annual Review of Microbiology*,*70*(1), 317–340. https://doi.org/10.1146/annurev-micro-102215-095440

Breitbart, M., Bhagooli, R., Griffin, S., Johnston, I., & Rohwer, F. (2005). Microbial communities associated with skeletal tumors on Porites compressa. *FEMS Microbiology Letters*, *243*(2), 431–436. https://doi.org/10.1016/j.femsle.2005.01.004

Burge, C. A., Friedman, C. S., Getchell, R., House, M., Lafferty, K. D., Mydlarz, L. D., Prager, K. C., Sutherland, K., Renault, T., Kiryu, I., & Thurber, R. V. (2016). Complementary approaches to diagnosing marine diseases: A union of the modern and the classic. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1689). https://doi.org/10.1098/rstb.2015.0207

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal*, *11*(12), 2639–2643.https://doi.org/10.1038/ismej.2017.119

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*,*13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Camp, E. F., Suggett, D. J., Pogoreutz, C., Nitschke, M. R., Houlbreque, F., Hume, B. C. C., Gardner, S. G., Zampighi, M., Rodolfo-Metalpa, R., & Voolstra, C. R. (2020). Corals exhibit distinct patterns of microbial reorganisation to thrive in an extreme inshore environment. *Coral Reefs*,1–16. https://doi.org/10.1007/s00338-019-01889-3

Carlos, C., Torres, T. T., & Ottoboni, L. M. M. (2013). Bacterial communities and species-specificassociations with the mucus of Brazilian coral species. *Scientific Reports*, *3*(1), 1–7. https://doi.org/10.1038/srep01624

Chimetto, L. A., Brocchi, M., Thompson, C. C., Martins, R. C. R., Ramos, H. R., & Thompson, F. L. (2008). Vibrios dominate as culturable nitrogen-fixing bacteria of the Brazilian coral Mussismilia hispida. *Systematic and Applied Microbiology*, *31*(4), 312–319. https://doi.org/10.1016/j.syapm.2008.06.001

Chiu, J. M. Y., Li, S., Li, A., Po, B., Zhang, R., Shin, P. K. S., & Qiu, J.-W. (2012). Bacteria associated with skeletal tissue growth anomalies in the coral Platygyra carnosus. *FEMS Microbiology Ecology*, *79*(2), 380–391. https://doi.org/10.1111/j.1574-6941.2011.01225.x

Chu, N. D., & Vollmer, S. V. (2016). Caribbean corals house shared and host-specific microbialsymbionts over time and space. *Environmental Microbiology Reports*, *8*(4), 493–500. https://doi.org/10.1111/1758-2229.12412

Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. M., Sun, Y., Brown, C. T., Porras-Alfaro, A., Kuske, C. R., & Tiedje, J. M. (2014). Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Research*, *42*(D1), D633–D642. https://doi.org/10.1093/nar/gkt1244

Cooney, R. P., Pantos, O., Tissier, M. D. A. Le, Barer, M. R., O'Donnell, A. G., & Bythell, J. (2002). Characterization of the bacterial consortium associated with black band disease in coral usingmolecular microbiological techniques. *Environmental Microbiology*, *4*(7), 401–413. https://doi.org/10.1046/j.1462-2920.2002.00308.x

Egan, S., & Gardiner, M. (2016). Microbial dysbiosis: Rethinking disease in marine ecosystems. *Frontiers in Microbiology*, *7*(JUN), 991. https://doi.org/10.3389/fmicb.2016.00991

Frias-Lopez, J., Zerkle, A. L., Bonheyo, G. T., & Fouke, B. W. (2002). Partitioning of bacterialcommunities between seawater and healthy, black band diseased, and dead coral surfaces. *Applied and Environmental Microbiology*, *68*(5), 2214–2228. https://doi.org/10.1128/AEM.68.5.2214-2228.2002

Garcia, G. D., Gregoracci, G. B., Santos, E. de O., Meirelles, P. M., Silva, G. G. Z., Edwards, R., Sawabe, T., Gotoh, K., Nakamura, S., Iida, T., de Moura, R. L., & Thompson, F. L. (2013). Metagenomic Analysis of Healthy and White Plague-Affected Mussismilia braziliensis Corals. *Microbial Ecology*, *65*(4), 1076–1086. https://doi.org/10.1007/s00248-012-0161-4

Gignoux-Wolfsohn, S. A., Aronson, F. M., & Vollmer, S. V. (2017). Complex interactions betweenpotentially pathogenic, opportunistic, and resident bacteria emerge during infection on a reef- building coral. *FEMS Microbiology Ecology*, *93*(7). https://doi.org/10.1093/femsec/fix080

Glasl, B., Smith, C. E., Bourne, D. G., & Webster, N. S. (2019). Disentangling the effect of host- genotype and environment on the microbiome of the coral Acropora tenuis. *PeerJ*, *7*, e6377.https://doi.org/10.7717/peerj.6377

Hernandez-Agreda, A., Leggat, W., Bongaerts, P., Herrera, C., & Ainsworth, T. D. (2018). Rethinking the coral microbiome: Simplicity exists within a diverse microbial biosphere. *MBio*,*9*(5). https://doi.org/10.1128/mBio.00812-18

Kaczmarsky, L., & Richardson, L. L. (2007). Transmission of growth anomalies between Indo-Pacific Porites corals. *Journal of Invertebrate Pathology*, *94*(3), 218–221. https://doi.org/10.1016/J.JIP.2006.11.007

Kelly, L. A., Heintz, T., Lamb, J. B., Kelly, L. A., Heintz, T., Lamb, J. B., Ainsworth, T. D., & Willis, B. L. (2016). Ecology and Pathology of Novel Plaque-Like Growth Anomalies Affecting a Reef-Building Coral on the Great Barrier Reef. *Frontiers in Marine Science*, *3*, 151. https://doi.org/10.3389/fmars.2016.00151

Kelly, L. W., Williams, G. J., Barott, K. L., Carlson, C. A., Dinsdale, E. A., Edwards, R. A., Haas, A. F., Haynes, M., Lim, Y. W., McDole, T., Nelson, C. E., Sala, E., Sandin, S. A., Smith, J. E.,Vermeij, M. J. A., Youle, M., & Rohwer, F. (2014). Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(28),10227–10232. https://doi.org/10.1073/pnas.1403319111

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013).Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1). https://doi.org/10.1093/nar/gks808

Knowlton, N., & Rohwer, F. (2003). Multispecies Microbial Mutualisms on Coral Reefs: The Host as a Habitat. *The American Naturalist*, *162*(S4), S51–S62. https://doi.org/10.1086/378684

Kolde, R. (2012). *Pheatmap: pretty heatmaps* (R package version 1.2). https://scholar.google.com/scholar?hl=en&as_sdt=0,5&cluster=14492249782051319874#d=gs_cit&u=%2Fscholar%3Fq%3Dinfo%3AQrBmv2TQHskJ%3Ascholar.google.com%2F%26output %3Dcite%26scirp%3D0%26scfhb%3D1%26hl%3Den

Louca, S., Parfrey, L. W., & Doebeli, M. (2016). Decoupling function and taxonomy in the global ocean microbiome. *Science*, *353*(6305), 1272–1277. https://doi.org/10.1126/science.aaf4507

Lozada-Misa, P., Kerr, A., & Raymundo, L. (2015). Contrasting lesion dynamics of white syndrome among the scleractinian corals Porites spp. *PLOS ONE*, *10*(6), e0129841. https://doi.org/10.1371/journal.pone.0129841

Luna, G. M., Bongiorni, L., Gili, C., Biavasco, F., & Danovaro, R. (2009). Vibrio harveyi as a causative agent of the White Syndrome in tropical stony corals. *Environmental MicrobiologyReports*, *2*(1), 120–127. https://doi.org/10.1111/j.1758-2229.2009.00114.x

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10. https://doi.org/10.14806/ej.17.1.200

McKew, B. A., Dumbrell, A. J., Daud, S. D., Hepburn, L., Thorpe, E., Mogensen, L., & Whitby, C. (2012). Characterization of geographically distinct bacterial communities associated with coral mucus produced by Acropora spp. and Porites spp. *Applied and Environmental Microbiology*, *78*(15), 5229–5237. https://doi.org/10.1128/AEM.07764-11

Moran, M. A. (2015). The global ocean microbiome. *Science*, *350*(6266), aac8455–aac8455. https://doi.org/10.1126/science.aac8455

Moriarty, T., Leggat, W., Huggett, M. J., & Ainsworth, T. D. (2020). Coral Disease Causes, Consequences, and Risk within Coral Restoration. *Trends in Microbiology*, *0*(0). https://doi.org/10.1016/j.tim.2020.06.002

Morrow, K. M., Bourne, D. G., Humphrey, C., Botté, E. S., Laffy, P., Zaneveld, J., Uthicke, S., Fabricius, K. E., & Webster, N. S. (2015). Natural volcanic CO2 seeps reveal future trajectories for host-microbial associations in corals and sponges. *ISME Journal*, *9*, 894–908. https://doi.org/10.1038/ismej.2014.188

Morrow, K. M., Moss, A. G., Chadwick, N. E., & Liles, M. R. (2012). Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. *Applied and Environmental Microbiology*, *78*(18), 6438–6449. https://doi.org/10.1128/AEM.01162-12

Ng, J. C. Y., Chan, Y., Tun, H. M., Leung, F. C. C., Shin, P. K. S., & Chiu, J. M. Y. (2015). Pyrosequencing of the bacteria associated with Platygyra carnosus corals with skeletal growth anomalies reveals differences in bacterial community composition in apparently healthy and diseased tissues. *Frontiers in Microbiology*, *6*, 1142. https://doi.org/10.3389/fmicb.2015.01142

O'Leary, N. A., Wright, M. W., Brister, J. R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretdin, A., Bao, Y., Blinkova,O., Brover, V., Chetvernin, V., Choi, J., Cox, E., Ermolaeva, O., … Pruitt, K. D. (2016).

Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Research*, *44*(D1), D733–D745. https://doi.org/10.1093/nar/gkv1189

Palmer, C. V., & Baird, A. (2018). Coral tumor-like growth anomalies induce an immune response and reduce fecundity. *Diseases of Aquatic Organisms*, *130*(1), 77–81.

https://doi.org/10.3354/dao03258

Pantos, O., Bongaerts, P., Dennis, P. G., Tyson, G. W., & Hoegh-Guldberg, O. (2015). Habitat- specific environmental conditions primarily control the microbiomes of the coral Seriatoporahystrix. *ISME Journal*, *9*(9), 1916–1927. https://doi.org/10.1038/ismej.2015.3

Pantos, O., & Bythell, J. (2006). Bacterial community structure associated with white band disease in the elkhorn coral Acropora palmata determined using culture-independent 16S rRNA techniques. *Diseases of Aquatic Organisms*, *69*(1), 79–88. https://doi.org/10.3354/dao069079

Parks, D. H., Chuvochina, M., Waite, D. W., Rinke, C., Skarshewski, A., Chaumeil, P. A., & Hugenholtz, P. (2018). A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nature Biotechnology*, *36*(10), 996. https://doi.org/10.1038/nbt.4229

Pollock, F. J., McMinds, R., Smith, S., Bourne, D. G., Willis, B. L., Medina, M., Thurber, R. V., & Zaneveld, J. R. (2018). Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nature Communications*, *9*(1), 4921. https://doi.org/10.1038/s41467-018-07275-x

Pollock, F. J., Wada, N., Torda, G., Willis, B. L., & Bourne, D. G. (2016). White Syndrome-Affected Corals Have a Distinct Microbiome at Disease Lesion Fronts. *Applied and Environmental Microbiology*, *83*(2). https://doi.org/10.1128/AEM.02799-16

Pratte, Z., & Richardson, L. (2018). Microbiome dynamics of two differentially resilient corals. *Diseases of Aquatic Organisms*, *131*(3), 213–226. https://doi.org/10.3354/dao03289

Preston, S., & Richards, Z. (2021). Biological consequences of an outbreak of growth anomalies on Isopora palifera at the Cocos (Keeling) Islands. *Coral Reefs*, *40*(1), 97–109. https://doi.org/10.1007/s00338-020-02019-0

Raina, J. B., Tapiolas, D., Willis, B. L., & Bourne, D. G. (2009). Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. *Applied and Environmental Microbiology*, *75*(11),3492–3501. https://doi.org/10.1128/AEM.02567-08

Rajasabapathy, R., Ramasamy, K. P., Manikandan, B., Mohandass, C., & Arthur James, R. (2020). Bacterial communities associated with healthy and diseased (skeletal growth anomaly) reef coral Acropora cytherea from Palk Bay, India. *Frontiers in Marine Science*, *7*, 92. https://doi.org/10.3389/fmars.2020.00092

Richardson, L. L., Miller, A. W., Blackwelder, P. L., & Al-Sayegh, H. (2015). Cyanobacterial- Associated Colored-Band Diseases of the Atlantic/Caribbean. In C. M.

Woodley, C. A. Downs, A. W. Bruckner, J. W. Porter, & S. B. Galloway (Eds.), *Diseases of Coral* (pp. 345–353). John Wiley & Sons, Inc. https://doi.org/10.1002/9781118828502

Richardson, L. L., Stanić, D., May, A., Brownell, A., Gantar, M., & Campagna, S. R. (2014). Ecologyand physiology of the pathogenic cyanobacterium Roseofilum reptotaenium. *Life*, *4*(4), 968– 987. https://doi.org/10.3390/life4040968

Roder, C., Arif, C., Bayer, T., Aranda, M., Daniels, C., Shibl, A., Chavanich, S., & Voolstra, C. R. (2014). Bacterial profiling of White Plague Disease in a comparative coral species framework.*ISME Journal*, *8*(1), 31–39. https://doi.org/10.1038/ismej.2013.127

Rohwer, F., Seguritan, V., Azam, F., & Knowlton, N. (2002). Diversity and distribution of coral- associated bacteria. *Marine Ecology Progress Series*, *243*, 1–10. https://doi.org/10.3354/meps243001

Rypien, K. L., Ward, J. R., & Azam, F. (2010). Antagonistic interactions among coral-associated bacteria. *Environmental Microbiology*, *12*(1), 28–39. https://doi.org/10.1111/j.1462- 2920.2009.02027.x

Sa'adah, N., Sabdono, A., & and Diah Permata, W. (2018). Identification of Antipathogenic Bacterial Coral Symbionts Against Porites Ulcerative White Spots Disease. *IOP Conference Series: Earth and Environmental Science*, 116. https://doi.org/10.1088/1755-1315/116/1/012054

Schellenberg, L., & Clarke, L. J. (2020). Spatial Structure of Marine Host-Associated Microbiomes: Effect of Taxonomy, Species Traits, and Study Design. In *Frontiers in Marine Science* (Vol. 7).Frontiers Media S.A. https://doi.org/10.3389/fmars.2020.00146

Sekar, R., Mills, D. E. K., Remily, E. R., Voss, J. D., & Richardson, L. L. (2006). Microbial communities in the surface mucopolysaccharide layer and the black band microbial mat of black band-diseased Siderastrea siderea. *Applied and Environmental Microbiology*, *72*(9), 5963–5973.https://doi.org/10.1128/AEM.00843-06

Silva-Lima, A. W., Froes, A. M., Garcia, G. D., Tonon, L. A. C., Swings, J., Cosenza, C. A. N.,Medina, M., Penn, K., Thompson, J. R., Thompson, C. C., & Thompson, F. L. (2020).

Mussismilia braziliensis White Plague Disease Is Characterized by an Affected Coral Immune System and Dysbiosis. *Microbial Ecology*, 1–12. https://doi.org/10.1007/s00248-020-01588-5

Sussman, M., Willis, B. L., Victor, S., & Bourne, D. G. (2008). Coral pathogens identified for white syndrome (WS) epizootics in the Indo-Pacific. *PLoS ONE*, *3*(6), e2393. https://doi.org/10.1371/journal.pone.0002393

Sweet, M. J., & Bulling, M. T. (2017). On the importance of the microbiome and pathobiome in coral health and disease. In *Frontiers in Marine Science* (Vol. 4). Frontiers Media S. A. https://doi.org/10.3389/fmars.2017.00009

Sweet, M. J., Burian, A., Fifer, J., Bulling, M., Elliott, D., & Raymundo, L. (2019). Compositional homogeneity in the pathobiome of a new, slow-spreading coral disease. *Microbiome*, *7*(1), 1–14.https://doi.org/10.1186/s40168-019-0759-6

Sweet, M. J., Croquer, A., & Bythell, J. (2014). Experimental antibiotic treatment identifies potential pathogens of white band disease in the endangered Caribbean coral Acropora cervicornis.

Proceedings of the Royal Society B: Biological Sciences, *281*(1788), 20140094–20140094. https://doi.org/10.1098/rspb.2014.0094

Sweet, M. J., Croquer, A., & Bythell, J. C. (2011). Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs*, *30*(1), 39–52. https://doi.org/10.1007/s00338-010-0695-1

Thurber, R. V., Mydlarz, L. D., Brandt, M., Harvell, D., Weil, E., Raymundo6, L., Willis, B. L., Langevin, S., Tracy, A. M., Littman, R., Kemp, K. M., Dawkins, P., Prager, K. C., Garren, M., & Lamb, J. (2020). Deciphering Coral Disease Dynamics: Integrating Host, Microbiome, and the Changing Environment. *Frontiers in Ecology and Evolution*, *8*(575927). https://doi.org/10.3389/fevo.2020.575927

Thurber, R. V., Payet, J. P., Thurber, A. R., & Correa, A. M. S. (2017). Virus-host interactions and their roles in coral reef health and disease. In *Nature Reviews Microbiology* (Vol. 15, Issue 4, pp. 205–216). Nature Publishing Group. https://doi.org/10.1038/nrmicro.2016.176

Thurber, R. V., Willner-Hall, D., Rodriguez-Mueller, B., Desnues, C., Edwards, R. A., Angly, F., Dinsdale, E., Kelly, L. W., & Rohwer, F. (2009). Metagenomic analysis of stressed coral holobionts. *Environmental Microbiology*, *11*(8), 2148–2163. https://doi.org/10.1111/j.1462-2920.2009.01935.x

Ushijima, B., Smith, A., Aeby, G. S., & Callahan, S. M. (2012). Vibrio owensii induces the tissue loss disease Montipora white syndrome in the Hawaiian reef coral Montipora capitata. *PLoS ONE*, *7*(10), e46717. https://doi.org/10.1371/journal.pone.0046717

Ushijima, B., Videau, P., Burger, A. H., Shore-Maggio, A., Runyon, C. M., Sudek, M., Aeby, G. S., & Callahan, S. M. (2014). Vibrio coralliilyticus strain OCN008 is an etiological agent of acute Montipora white syndrome. *Applied and Environmental*

Microbiology, *80*(7), 2102–2109. https://doi.org/10.1128/AEM.03463-13

van Oppen, M., & Blackall, L. L. (2016). Coral microbiome dynamics, functions and design in a changing world. *The ISME Journal*, *10*(6), 1540–1544. https://doi.org/10.1038/s41579-019-0223-4

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and EnvironmentalMicrobiology*, *73*(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wang, Y. A., & Wright, S. T. (2012). mvabund-an R package for model-based analysis of multivariate abundance data. *Methods in Ecology and Evolution*, *3*(3), 471–474. https://doi.org/10.1111/j.2041-210X.2012.00190.x

Welsh, R. M., Zaneveld, J. R., Rosales, S. M., Payet, J. P., Burkepile, D. E., & Thurber, R. V. (2016). Bacterial predation in a marine host-associated microbiome. *ISME Journal*, *10*(6), 1540–1544. https://doi.org/10.1038/ismej.2015.219

Work, T. M., Kaczmarsky, L., & Peters, E. (2015). Skeletal growth anomalies in corals. In C. M., Woodley, C. Downs, A. W. Bruckner, J. W. Porter, & S. B. Galloway (Eds.), *Diseases of coral* (pp. 291–299). John Wiley & Sons, Inc. https://books.google.com.au/books ?hl=en&lr=&id=bCqwCgAAQBAJ&oi=fnd&pg=PA291&dq=Skeletal+Growth+Anomalies +in+Corals+work+&ots=4k0c0i9L6E&sig=5Uv0zvlefvwUClK9- WKJmm7Arbo

Yang, S. H., Lee, S. T. M., Huang, C. R., Tseng, C. H., Chiang, P. W., Chen, C. P., Chen, H. J., & Tang, S. L. (2016). Prevalence of potential nitrogen-fixing, green sulfur bacteria in the skeleton of reef-building coral Isopora palifera. Limnology and Oceanography, 61(3), 1078–1086. https://doi.org/10.1002/lno.10277

Yang, S. H., Tandon, K., Lu, C. Y., Wada, N., Shih, C. J., Hsiao, S. S. Y., Jane, W. N., Lee, T. C., Yang, C. M., Liu, C. Te, Denis, V., Wu, Y. T., Wang, L. T., Huang, L., Lee, D. C., Wu, Y. W., Yamashiro, H., & Tang, S. L. (2019). Metagenomic, phylogenetic, and functional characterization of predominant endolithic green sulfur bacteria in the coral Isopora palifera. Microbiome, 7(1), 1–13. https://doi.org/10.1186/s40168-018-0616-z

Yoshioka, R. M., Kim, C. J. S., Tracy, A. M., Most, R., & Harvell, C. D. (2016). Linking sewagepollution and water quality to spatial patterns of Porites lobata growth anomalies in Puako, Hawaii. *Marine Pollution Bulletin*, *104*(1–2), 313–321. https://doi.org/10.1016/j.marpolbul.2016.01.002

Zaneveld, J. R., McMinds, R., & Thurber, Rebecca VegaThurber, R. V. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nature*

Microbiology, *2*(9), 17121. https://doi.org/10.1038/nmicrobiol.2017.121

Zhang, Yanying, Ling, J., Yang, Q. S., Wang, Y. S., Sun, C. C., Sun, H. Y., Feng, J. Bin, Jiang, Y. F., Zhang, Y. Z., Wu, M. L., & Dong, J. De. (2015). The diversity of coral associated bacteria and the environmental factors affect their community variation. *Ecotoxicology*, *24*(7–8), 1467–1477.https://doi.org/10.1007/s10646-015-1454-4

Zhang, Yu, Sun, J., Mu, H., Lun, J. C. Y., & Qiu, J.-W. (2017). Molecular pathology of skeletal growth anomalies in the brain coral Platygyra carnosa: A meta-transcriptomic analysis. *Marine Pollution Bulletin*, *124*(2), 660–667. https://doi.org/10.1016/J.MARPOLBUL.2017.03.047

Ziegler, M., Grupstra, C. G. B., Barreto, M. M., Eaton, M., BaOmar, J., Zubier, K., Al-Sofyani, A.,Turki, A. J., Ormond, R., & Voolstra, C. R. (2019). Coral bacterial community structure responds to environmental change in a host-specific manner. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-019-10969-5

Ziegler, M., Roik, A., Porter, A., Zubier, K., Mudarris, M. S., Ormond, R., & Voolstra, C. R. (2016). Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea. *Marine Pollution Bulletin*, *105*(2), 629–640. https://doi.org/10.1016/j.marpolbul.2015.12.045

3.10 Supplementary Information

Table S3-1: Description of sites and samples taken at each. * denotes where water samples taken and stored for future analysis.

Site	Acronym	Latitude	Longitude	Depth (m)
Direction Island Outside	DIO	S 12º05'01.9	E 096º52'40.3	10-12
Anchor Hole	AH	S 12º05'57.8	E 096º52'33.2	12-14
Blue Hole 5	BH5	S 12º10'39.7	E 096º52'52.8	1-2
Kats Kable	KK	S 12º05'20.9	E 096º52.21.0	10-12
Outfall	-	S 12º09'57.9	E 096º49'01.6	10
Dump	-	S 12º10'51.1	E 096º49.26.1	<1

Table S3-2: PCR master mix volumes for 1 reaction.

Reagent	Volume (ul)
H_2O	16.45
10x Gold Buffer	2.5
$MgCl_2$	2
dNTPs	0.25
BSA	1
Bact16S_341F	1
Bact16S_785R	1
SYBR-Green dye	0.6
Taq polymerase	0.2

Table S3-3: Results of a SIMPER analysis assessing percentage contribution of ASVs to within group similarity between Health Status only. Cut off of 80%. Based on a presence/absence Bray-Curtis resemblance matrix.

Group Diseased					
Average similarity: 14.07					
Species	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Endozoicomonas sp.	0.88	2.4	0.95	17.09	17.09
Chloroplast (Unclassified)	0.82	2.04	0.81	14.48	31.57
Chlorophyta sp.	0.59	1.18	0.51	8.41	39.98
Gammaproteobacteria (Unclassified)	0.71	1.15	0.68	8.15	48.13
Alteromonas sp.(AB920393)	0.41	0.6	0.36	4.3	52.43
Myxococcales (Unclassified)	0.41	0.42	0.33	2.97	55.4
Bacteroidetes (Unclassified)	0.47	0.38	0.37	2.73	58.13
Alphaproteobacteria (Unclassified)	0.29	0.38	0.21	2.68	60.81
Chloroplast (Unclassified)	0.47	0.37	0.38	2.64	63.45
Thalassotalea sp.	0.47	0.3	0.42	2.16	65.61
Pseudoalteromonas sp.	0.47	0.3	0.43	2.15	67.76
Halomonas lutea(EF674852)	0.41	0.29	0.35	2.07	69.84
Chlorophyta sp.	0.41	0.27	0.34	1.94	71.78
Pseudoalteromonas arabiensis(AB576636)	0.29	0.24	0.27	1.73	73.51
Chloroplast (Unclassified)	0.41	0.23	0.34	1.64	75.15
Pseudoalteromonas phenolica(NR113299.1)	0.29	0.23	0.27	1.63	76.78
Bacteroidetes (Unclassified)	0.24	0.22	0.21	1.58	78.36
Bacteroidetes (Unclassified)	0.24	0.2	0.2	1.42	79.79
Streptococcus infantis(AY485603)	0.41	0.19	0.37	1.38	81.17
Group Healthy					
Average similarity: 20.38					
Species	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Chloroplast (Unclassified)	0.94	2.62	1.6	12.86	12.86
Endozoicomonas sp.	0.88	2.37	1.29	11.62	24.48
Chloroplast (Unclassified)	0.71	1.61	0.85	7.92	32.39
Chlorophyta sp.	0.59	1.43	0.66	7.03	39.43
Gammaproteobacteria (Unclassified)	0.65	1.21	0.69	5.92	45.35
Bacteroidetes (Unclassified)	0.59	1.06	0.59	5.23	50.57

Alphaproteobacteria (Unclassified)	0.41	0.66	0.41	3.26	53.83
Pseudoalteromonas sp.	0.53	0.64	0.54	3.16	56.99
Chloroplast (Unclassified)	0.41	0.61	0.41	2.97	59.96
Chloroplast (Unclassified)	0.53	0.59	0.56	2.92	62.88
Thalassotalea sp.	0.47	0.47	0.45	2.32	65.2
Halomonas lutea(EF674852)	0.41	0.35	0.39	1.72	66.92
Vibrio fortis(AJ514916)	0.41	0.32	0.37	1.56	68.48
Acinetobacter lwoffii(X81665)	0.41	0.32	0.39	1.55	70.03
Rhodospillales (Unclassified)	0.35	0.31	0.3	1.51	71.54
Myxococcales (Unclassified)	0.35	0.3	0.32	1.49	73.04
Chlorophyta sp.	0.35	0.3	0.32	1.47	74.5
Bacteroidetes (Unclassified)	0.29	0.29	0.25	1.42	75.92
Chloroplast (Unclassified)	0.35	0.29	0.31	1.42	77.34
Pseudoalteromonas arabiensis(AB576636)	0.35	0.28	0.31	1.38	78.71
Streptococcus infantis(AY485603)	0.35	0.28	0.32	1.37	80.09
Group Control					
Average similarity: 20.24					
Species	**Av.Abund**	**Av.Sim**	**Sim/SD**	**Contrib%**	**Cum.%**
Endozoicomonas sp.	0.93	2.43	1.49	12.01	12.01
Chloroplast (Unclassified)	0.87	1.8	1.36	8.89	20.9
Pseudoalteromonas sp.	0.7	1.11	0.85	5.49	26.39
Gammaproteobacteria (Unclassified)	0.63	0.97	0.71	4.77	31.16
Bacteroidetes (Unclassified)	0.57	0.86	0.57	4.23	35.39
Rhodospirillaceae (Unclassified)	0.6	0.82	0.64	4.07	39.46
Alteromonas sp.(AB920393)	0.57	0.79	0.51	3.89	43.35
Chlorophyta sp.	0.53	0.76	0.6	3.74	47.09
Chlorophyta sp.	0.5	0.76	0.52	3.74	50.82
Thalassomonas ganghwensis(AY194066)	0.6	0.73	0.68	3.62	54.44
Bacteroidetes (Unclassified)	0.47	0.7	0.45	3.48	57.92
Chloroplast (Unclassified)	0.5	0.7	0.54	3.47	61.39
Thalassotalea sp.	0.57	0.63	0.63	3.1	64.49
Shimia isoporae(FJ976449)	0.47	0.44	0.48	2.15	66.65
Fulvivirga sp.	0.43	0.41	0.43	2.05	68.69
Thalassomonas	0.43	0.37	0.45	1.84	70.53

ganghwensis(AY194066)					
Vibrio fortis(AJ514916)	0.43	0.32	0.44	1.61	72.14
Pseudoalteromonas arabiensis(AB576636)	0.4	0.28	0.38	1.38	73.52
Alphaproteobacteria (Unclassified)	0.3	0.25	0.28	1.22	74.73
Thalassomonas ganghwensis(AY194066)	0.37	0.22	0.36	1.1	75.83
Chloroplast (Unclassified)	0.3	0.22	0.28	1.07	76.9
Bacteroidetes (Unclassified)	0.27	0.21	0.25	1.06	77.96
Streptococcus infantis(AY485603)	0.27	0.19	0.26	0.93	78.9
Chloroplast (Unclassified)	0.3	0.17	0.29	0.83	79.73
Pseudoalteromonas arabiensis(AB576636)	0.33	0.17	0.32	0.83	80.56

Table S3-4: Results of a two-way pairwise PERMANOVA exploring the differences between tissue and water samples. Analysis based on a presence/absence Bray-Curtis similarity matrix. * denotes a potential point source of pollution into the atoll.

SitexType for pairs of levels of factor 'Type'					
Water, Tissue					
	t	**P(Perm)**	**Unique perms**	**Denominator**	**Den.df**
DIO	3.5635	0.0001	9837	1*Res	23
AH	2.2243	0.0001	9308	1*Res	20
BH5	4.2555	0.0001	9919	1*Res	31
KK	3.6405	0.0001	9930	1*Res	31
TypexSite for pairs of levels of factor 'Site'					
Water					
	t	**P(Perm)**	**Unique perms**	**Denominator**	**Den.df**
DIO, AH	1.6895	0.0016	4299	1*Res	13
DIO, BH5	5.0746	0.0001	9919	1*Res	25
DIO, WID	4.3867	0.0003	4330	1*Res	13
DIO, KK	1.9564	0.0012	9903	1*Res	23
DIO, WIO	2.8537	0.0001	8150	1*Res	15
AH, BH5	2.8196	0.0001	9365	1*Res	22
AH, WID	2.1642	0.0038	336	1*Res	10
AH, KK	1.2562	0.0913	9267	1*Res	20
AH, WIO	1.5666	0.0109	2891	1*Res	12
BH5, WID	2.8544	0.0001	9569	1*Res	22
BH5, KK	3.6583	0.0001	9909	1*Res	32

BH5, WIO	3.3014	0.0001	9866	1*Res	24
WID, KK	2.9417	0.0001	9224	1*Res	20
WID, WIO	2.1685	0.001	2887	1*Res	12
KK, WIO	2.059	0.0005	9854	1*Res	22

TypexLocation for pairs of levels of factor 'Location'

Tissue

	t	**P(Perm)**	**Unique perms**	**Denominator**	**Den.df**
DIO, AH	1.456	0.0002	9860	1*Res	30
DIO, BH5	2.0584	0.0001	9855	1*Res	29
DIO, KK	1.8522	0.0001	9853	1*Res	31
AH, BH5	2.2938	0.0001	9874	1*Res	29
AH, KK	2.191	0.0001	9862	1*Res	31
BH5, KK	2.5216	0.0001	9887	1*Res	30

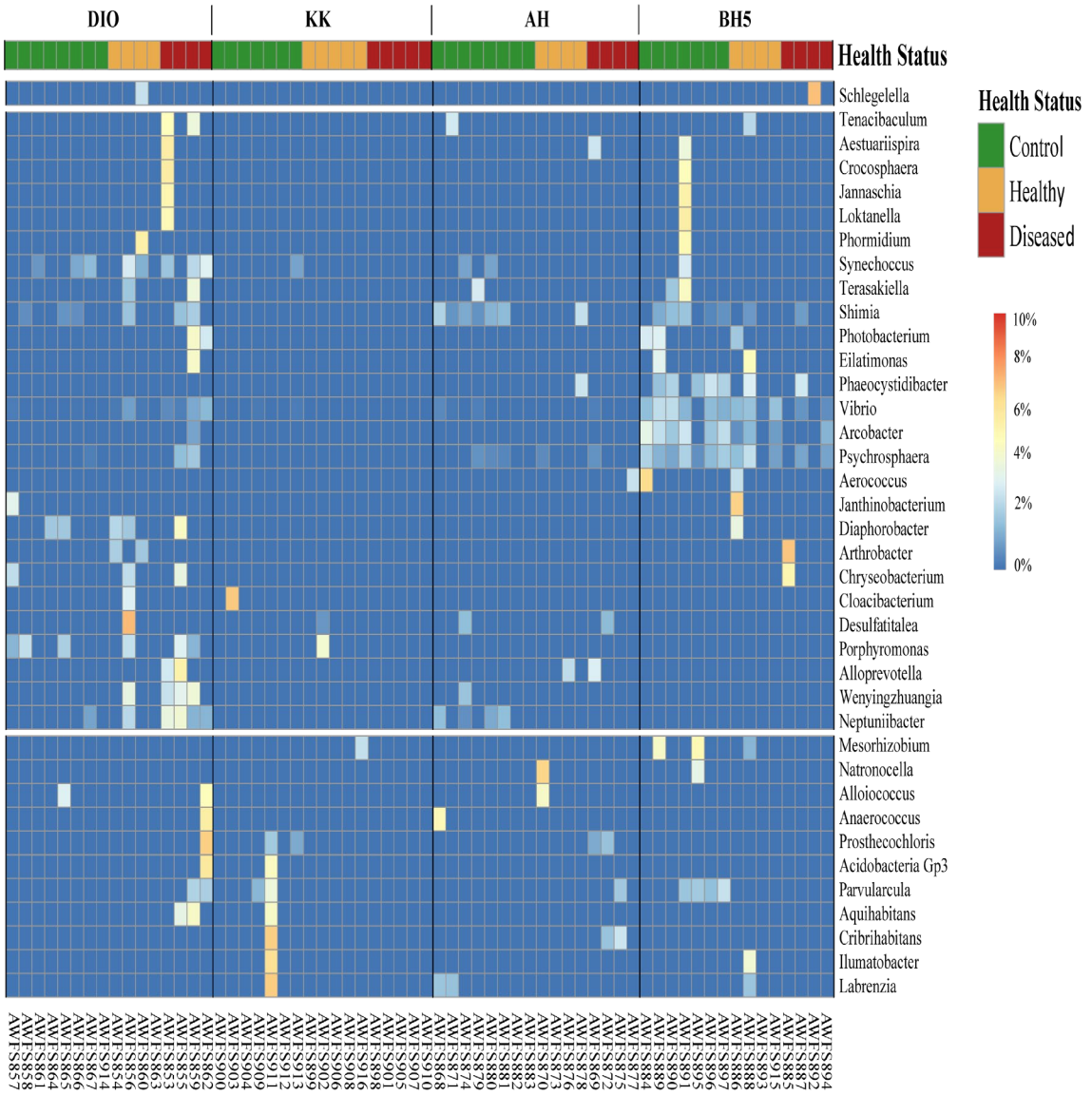

Figure S3-1: Heatmap of the relative abundance of 38 genera that differed significantly and contributed to an interaction effect between Site and Health Status. Abundances scaled within each genus to show relative range of each genera across samples. Significance level ($p<0.05$). Division of rows based on ability to assign genera to Site (4 levels). DIO – Direction Island Outside, KK – Kats Kable, AH – Anchor Hole, BH5 – Blue Hole 5.

Figure S3-2: Heatmap of the relative abundance of potentially pathogenic bacteria found within the water samples and identified from literature (Lamb et al., 2017). Abundances scaled within each genus to show relative range of each ASV across samples. Significance level ($p<0.05$). DIO – Direction Island Outside, KK – Kats Kable, AH – Anchor Hole, BH5 – Blue Hole 5, WIO – West Island Outfall, WID – West Island Dump.

Chapter 4

Discussion

4.1 Pre word

The manuscript arising from a combination of this chapter, and Chapter 1, is currently in preparation for submission to *Environmental DNA,* as a minireview titled "Integrating eDNA into the genomics age toolkit for characterising scleractinian coral diseases". As such, this chapter has been formatted for *Environmental DNA*.

4.2 Acknowledgements

I would like to thank the advice and guidance of Dr. Tracy Ainsworth in shaping this chapter. I would also like to thank the members of the Trace and Environmental DNA Laboratory for their advice and support, particularly Jackie Jones, Claus Christophersen and Ali Alishum.

4.3 Summary of Findings

This thesis applies a multidisciplinary approach, combining classical (i.e., histology, skeletal measurements, and ecological surveys) and molecular techniques (i.e., DNA metabarcoding) to characterize the novel occurrence of growth anomalies (GAs) on *I. palifera* at the CKI, the first reported occurrence of this coral disease on this species globally. The results highlight the importance for a united approach to understanding coral diseases as well as inconsistencies within the field. This final discussion chapter synthesizes the main questions and results of each data chapter and examines the limitations and significance of this thesis along with the potential for an integrated approach to be used in coral disease characterization. Finally, future research directions are discussed.

The occurrence of this disease at CKI is unique in that there were no apparent environmental triggers or significant local anthropogenic pressures to account for this outbreak. The results of this research confirm the *I. palifera* population is suffering an outbreak of growth anomalies, and this disease posesa serious threat to the current and future population through altered skeletal growth processes and reduced biological and reproductive functioning. It also confirmed that there were no significant bacterial causal agents found, and that the outbreak could not be definitively linked to the potential point sources of aquatic pollution at the atoll. This thesis contributes to the global understanding of growth anomalies and the impact it has on reef-building coral species. Importantly, it also highlights the potential for alternative microbial causative agents, such as fungal or viral agents, and questions the concept of 'one disease – one pathogen'. Several key findings from this thesis are summarized below:

4.3.1 Research finding #1. An outbreak of growth anomalies is currently affecting the Cocos (Keeling) Islands.

Diagnosing and characterising a coral disease outbreak is typically challenging, and the frameworks that exist have not been universally adopted. In particular, there is no standardised approach for quantifying an outbreak, particularly where no baseline data exists. In chapter 2, two published outbreak thresholds were used to assess the proportion of *I. palifera* affected by GAs being either >5% of the population (Willis et al., 2004) or 60 cases per 1500m^2 (Maynard et al., 2011). The results of this studyfound that the occurrence of GAs surpasses at least one outbreak threshold at regional scale, satisfying

the conditions to be classed as an outbreak. Surveys reported one-third of the population to be displayingsigns of GAs, with six of nine sites recording over the 5% threshold. Alternatively, the average numberof cases across the atoll was reported at 16.26 per 750m^2 (33.24/1500m^2) and only two sites recorded greater than 30 cases per 750m^2.

4.3.2 Research finding #2. Growth anomalies affect the biological and reproductive functioning of both the current and future population of *Isopora palifera.*

A multidisciplinary traditional approach revealed reduced capacity of the host for biological functioning and reproduction within lesions. In Chapter 2, key biological components including mesenteries, polyps, oocytes, and symbiotic zooxanthellae were found to be significantly reduced within diseased tissues. The effects of this disease however, appeared to be confined to the lesions, with adjacent healthy tissues containing seemingly normal biologically functioning components. This histological approach revealed results consistent with other characterizations of growth anomalies from the Indo-Pacific region (Burns & Takabayashi, 2011; Domart-Coulon et al., 2006; Irikawa et al., 2011), and confirmed the diagnosis.

The disease appeared to compromise the biological and reproductive functioning by directing energy towards rapid skeletal development. Conservative estimates indicate that the future reproductive potential of the population could be diminished by 11% based on GAs affecting a minimum of one- third of the surface area across one-third of the population. This division of resources may have seriousconsequences for the future gamete availability of the *I. palifera* population, and further implications for the accretion potential of the CKI reefs given a significant number of *I. palifera* colonies were affected with GAs.

4.3.3 Research finding #3. Geochemical analysis of healthy and diseased *I. palifera* skeletons revealed evidence of bacterial bioerosion within lesions.

In Chapter 2, novel trace element analysis techniques revealed significant changes to the geochemical composition of lesions indicative of a shift from aragonite to calcite. This shift may be the result of bacterial bioerosion. In this process, the rapidly developing porous aragonite structure may be bioeroded by bacteria present in the microbiome, and reprecipitated as a marine abiotic cement. Scanning electronmicroscopy (SEM) is required to confirm this process.

4.3.4 Research finding #4. There were broad similarities between the bacterial associations of diseased and healthy *I. palifera* samples.

Chapter 3 described the bacterial associations found within coral samples across numerous sites. 16S sequencing revealed no significant difference between the bacterial associations of control and diseasedcolonies. Previous studies investigating the bacterial associations of GA-affected corals have returned similar results, attributing shifts in the microbiome to be the result of environmental variables rather than health status (Chiu et al., 2012). Two studies have however, reported significant differences in bacterial associations due to health status, but the broader significance of the results were hampered by small sample sizes (Ng et al., 2015; Rajasabapathy et al., 2020).

Alpha diversity measures revealed similarities between control, diseased and healthy samples, indicatingcompositional homogeneity in the microbiome and contrasting the Anna-Karenina principle. This concept proposes that chaotic disturbances in microbial communities, and increased beta diversity are triggered by disruptions such as disease (Zaneveld et al., 2017). Whilst dysbiosis (imbalance in communities) is an important tool in characterising a disease (Egan & Gardiner, 2016; Silva-Lima et al., 2020), the results of this chapter supports the shift away from 'one-disease one-pathogen' concept (Sweet et al., 2019), and provides support for other research that has suggested bacterial agents are notthe sole causative factor in GA instigation (Wong et al., 2021).

4.3.5 Research finding #5. *I. palifera* displayed a distinct microbiome from those found in the water column or from point sources of pollution.

In Chapter 3, the bacterial associations appeared to differ significantly between site, a result of the environmentally responsive microbiome, however, were significantly different to those found within the water column. This distinctiveness of the coral microbiome was driven primarily by the endosymbionts in the coral tissue. In addition, no taxa were shared taxa between water samples collected from point sources of pollution or *I. palifera* samples (diseased or healthy), indicating no causal link between the GA outbreak and local pollution.

4.4 Limitations of Thesis

This thesis demonstrates the application of an integrated approach to characterising coral diseases, utilising both traditional and modern methods, however several limitations still apply to this research. Firstly, 16S ribosomal RNA (rRNA) gene region selection remains a significant problem in microbiology, where choice of hypervariable region can affect the resolution of metabarcoding results (Bukin et al., 2019). The 16S rRNA gene is commonly used for the identification of mixed microbial communities, consisting of nine hypervariable regions (V1-V9). This approach has been commonly adopted by researchers globally for its culture-free methods, evident in the creation of the Earth Microbiome Project and available information is both in-depth and extensive (Thompson et al., 2017).Nevertheless, the quality and quantity of information obtained is dependent on the resolution (and conservation) of the targeted region (Zhang et al., 2018). Highly conservative regions are utilised for determining higher-ranking taxa, whereas more quickly evolving regions are needed to distinguish to a genus or species level. In this thesis, I utilised the V3-V4 region, commonly used in bacterial communitystudies as the V3 region contains maximum nucleotide heterogeneity (mutations) and displays maximum discriminatory power between taxa (Chakravorty et al., 2007) and V4 is endorsed for large scale environmental microbiota assessments (Thompson et al., 2017). Utilising the 341F/785R primer set (Klindworth et al., 2013) ensured maximum coverage of both Bacteria and Archaea domains whilst ensuring sequences matched major publicly available databases (Almeida et al., 2018; Kim et al., 2011). However, previous studies have demonstrated the taxonomic assignment is highly sensitive to the length, and region, of 16S rRNA sequenced (Bukin et al., 2019; Liu et al., 2008). An alternative approach could be to utilise full length 16S rRNA sequences for superior taxonomic resolution at species level, however this method of long read sequencing is hindered by higher costs and error rates and a less developed bioinformatics systems (Callahan et al., 2019). It is likely that short read sequencing techniques will dominate bacterial community profiling until the development of long read technology is more widely adopted. Another option could be the use of alternate single copy genes suchas *rpoB* to complement 16S rRNA (Ogier et al., 2019; Vos et al., 2012), however bioinformatic systems and databases are also limited for this gene (Ki et al., 2009).

The development of next generation sequencing methodologies has seen an increase in large volumes of data, however the lack of effectual annotation and analysis programs, coupled with increasing complexity of sequences, means bacterial genetic reference databases remain incomplete. It is likely that as bioinformatic pipelines are developed, more comprehensive reference databases will allow for higher resolution taxonomic assignments, particularly for

the under studied marine microbiome (discussed further in future directions). To account for incomplete reference databases, I assigned taxonomy using two DADA2 formatted bacterial and archaeal 16S rRNA databases (RefSeq/RDP, GTDB), and verified against the NCBI database. Analyses were conducted at ASV, and if needed, genus level, and a strict approach ensured maximum confidence in taxonomic assignment, particularly where comparative studies typically analyse higher ranking taxa.

4.5 Significance of thesis

This thesis demonstrates the application of classical and modern methods for the characterisation of GAs and propose a number of avenues for further investigation within the disease's life cycle. The main findings of this thesis contribute to the global understanding of growth anomalies and questions the current understanding of the onset of this coral disease. Notably, this is the first occurrence of GAs without an obvious environmental trigger or anthropogenic stressor allowing for the characterisation of this disease under the influence of extenuating factors. The geochemical and histological results indicate GAs led to significant changes to the internal functioning of the host. The loss of biological functioning coupled with changes to skeletal growth processes indicates that the effects upon the host may extend to a molecular level. This is also the first study to utilise non-destructive XRF technologies to examine the trace element composition of diseased coral skeletons. Overall, the data presented in this study suggests that there is a significant threat to future population viability of *I. palifera* at this isolated location and this may have flow-on effects for the reef-accretion because *I. palifera* is a dominant reef-building species at this location.

This highlights the need for standardised and universally adopted toolkits for coral disease diagnoses to allow for comparison between cases. Chapter 3 investigates the bacterial associations of GA affected coral based on one of the largest sample sets ever examined (to date). The broad similarities in bacterial associations across multiple health states indicates there is no obvious sign of a bacterial causal agent for the outbreak. The findings validate the shift away from a 'one – disease one – pathogen' concept and support the findings from prior research that coral GAs may not be caused by bacteria (Chiu et al.,2012). Lastly, the results of this study support the premise of compositional homogeneity within the microbiome (Sweet et al., 2019).

4.6 Future research questions arising from this thesis.

When applying an integrated approach to characterising coral diseases, a wide range of tests and experiments required to characterise GAs and to unequivocally identify causal agents and transmission mechanisms. Whilst not an exhaustive list, I have identified possible research questions that can be examined using either ecological, molecular, or statistical methodologies to further understand the outbreak of growth anomalies at the Cocos (Keeling) Islands.

4.6.1 Research question #1: The role of corallivorous fish within the life cycle of GAs.

Corallivores and the role they play in the lifecycle of coral diseases is a source of constant debate, in particular the effect of corallivorous reef fish (Casey et al., 2014; Nicolet et al., 2018). These fish have demonstrated selected consumption of diseased coral samples, highlighting their important role in coraldisease dynamics (Chong-Seng et al., 2011). Several case studies have linked butterflyfish and damselfish to the proliferation of coral disease through feeding habits (Aeby & Santavy, 2006; Raymundo et al., 2009) and farming behaviour (Casey et al., 2014). Conversely, feeding of damselfish and wrasse on black band diseased *Acropora muricata* appeared to slow disease progression (Cole et al., 2009). Whilst not quantified here, feeding scars were observed upon the softer and more porous lesions (Chapter 2), suggesting that the reef fishes of CKI may play an important role in either GA removal, or in the transfer of potential pathogens onto healthy samples. Further temporal and spatial observations and disease modelling would be required to examine this relationship, and the feeding habits of the local population of corallivorous fish.

4.6.2 Research question #2: Non-bacterial agents of disease.

Genomic techniques have been applied for the identification of other non-bacterial pathogens, such as fungi and marine viruses and protists. Marine viruses have been identified as key determinants in coralhealth and diseases (Brussaard et al., 2016; Marhaver et al., 2008; Thurber et al., 2017), and can induce virulence in pathogens (Weynberg et al., 2015). Next generation sequencing approaches are allowing researchers to more broadly profile virus-bacteria-host interactions and communities associated with the coral holobiont (Brussaard et al., 2016; Thurber et al., 2017). Research to date has shown that some bacteriophages have been associated with both herpes-like infections (Correa et al., 2016; Marhaver et al., 2008)

and have potential in controlling the effects of the coral pathogen *Vibrio coralliilyticus* (Jacquemot et al., 2018). Marine protozoans, in particular the ciliate *Halofolliculina corallasia* has been established as the pathogen of skeletal eroding band through classical approaches and was linked to the Caribbean ciliate infection by utilising morpho-molecular analysis (Montano et al., 2020). Furthermore,some diseases are the result of marine fungal pathogens, such as aspergillosis, potentially caused by *Aspergillus sydowii* which affects sea fans across the Caribbean and Mediterranean (Alker et al., 2001; Soler-Hurtado et al., 2016), and dark spot disease (Sweet et al., 2013). Given that this study, and others have found significant evidence against the implication of bacterial agents in the causation of GAs, further investigation should consider the role of viral agents or potential fungal pathogens in the onsetof this disease.

4.6.3 Research question #3: Network analyses.

Quantifying the microbes found in the coral microbiome, surrounding water column, interstitial vectors, and anthropogenic outflows generates an ever-increasing volume of sequencing data. A significant avenue moving forward is to investigate the complex interactions between microbiomes, abiotic and biotic factors and over spatio-temporal scales using network analyses. This technique has been applied to a number of coral microbiome studies (Leite et al., 2018; Soffer et al., 2015; Sweet et al., 2019), andallows for the behaviour of individual taxa to be examined under the influence of a variety of factors (Barberán et al., 2012).

4.7 Future directions in coral disease research.

The multidisciplinary nature of coral disease research means technology has rapidly advanced across anumber of fields. Here I identify future directions and research questions to contribute to the advancement of coral disease research and management.

4.7.1 Future direction #1: Innate immune system

Of increasing interest in applied coral health research is the role of the innate immune system (Mydlarzet al., 2010). Coral immunity is a consistent life history trait displaying interspecies variation, and is reflected in the susceptibility of the species of both coral diseases and overall stressor events (Palmer et al., 2010, 2011). The innate immune system of corals comprises of physical barriers (mucus secretion), and a widely reviewed classical invertebrate system comprising of sensing and effector arms(Miller et al., 2007; Palmer, 2018; Palmer & Traylor-Knowles, 2012; Toledo-Hernández & Ruiz-Diaz,2014). Immune systems respond to environmental variables (Palmer, 2018), anthropogenic stressors (L.W. Kelly et al., 2020) and coral diseases (Silva-Lima et al., 2020; Takagi et al., 2020). In the case of coral diseases, the immune response may occur in association with direct pathogen contact, whereby hosts direct resources away from disease induced lesions through the translocation of photosynthetic products (Roff et al., 2006). Efforts to characterise the innate immunity of corals through genome and transcriptome analysis, have revealed elements of stress management and immune response pathways (Shinzato et al., 2011; Wright et al., 2015). For example, bacterial challenge experiments have demonstrated malignant bacteria suppress gene expression (Takagi et al., 2020). Understanding such processes will aid in the development and application of diagnostic tools for coral disease.

4.7.2 Future direction #2: Gene expression and regulation

Molecular tools have been utilised in assessing the sublethal effects of coral diseases through changes to gene expression and regulation, particularly for the widespread GA disease. For example, molecularpathology of skeletal GAs on *Platygyra carnosa* revealed the expression of genes associated with osteogenesis (fragile skeletons), oncogenesis (tumour development), and the downregulation of genes related to maintaining bone homeostasis indicating viral and bacterial presences (Zhang et al., 2017). A study investigating gene expression in GAs on *Montipora capitata* revealed reduced expression of the galaxin gene (associated with the calcifying matrix) in affected tissues indicating a possible reduction in

the calcification mechanism (Spies & Takabayashi, 2013). Several investigations into the immune response of *Acropora hyacinthus* and *A. muricata* to growth anomalies revealed higher prophenoloxidase activity commonly associated with an immune response within the tumour (Palmer & Baird, 2018). Whereas after a bleaching event, primed immune responses within the prophenoloxidase-activating cascade were inferred to be related to either suppressed host response or the down-regulation of calcification pathway genes (Kelly et al., 2016). Investigating the changes to gene expression and regulation will expand the coral disease diagnosis, and further inform both effortsto find causal agents, and management and recovery plans.

4.7.3 Future direction #3: Environmental DNA

Environmental DNA metabarcoding is a molecular tool for profiling multi-species genetic material in water, soil, sediment, faecal and gut samples (Bohmann et al., 2014; Shaw et al., 2016; Thomsen et al., 2012). The use of eDNA in the detection or management of coral disease is not yet widely utilised butmay offer potential in the detection of already identified causal agents. For example, *Porites* trematodiasis has been reported in over 60% of Hawaiian reefs, with a demonstrated range extension toNew Caledonia (Aeby et al., 2016; Work et al., 2014). The causal agent, the diagenetic trematode *Polypipapiliotrema stenometra*, has been partially sequenced (Martin et al., 2018), and the developmentof targeted assays could help to scale up the effort to identify the presence of this pathogen on reefs. Similar techniques have already been applied to the identification of trematodes in water samples fromsoutheast Asia (Hashizume et al., 2017) and in pasture lands (Jones et al., 2018). Furthermore, eDNA could be used to assess interstitial fauna on coral reefs and identify the nature of their influence on the coral microbiome (Ainsworth et al., 2020). A significant limitation to this method is that presence doesnot necessarily equate to disease, and other causative factors may be involved (Stat et al., 2017). Nevertheless, eDNA approach offers a valuable tool for species detection, be it vector based or causal agent.

4.7.4 Future direction #4: Rapid vector detection

Rapid testing for coral pathogens or vectors could be achieved with the development of targeted ‘dipstick’ tests, similar to the recently developed tool for crown-of-thorns starfish (CoTS) assessmentson the Great Barrier Reef (Doyle & Uthicke, 2020). Dipstick tests utilise lateral flow assays, similar tothat of a human pregnancy test, to rapidly detect target analyte, without the need for expensive equipment and lengthy laboratory procedures (Koczula &

Gallotta, 2016; Sajid et al., 2015). Whilst having widespread potential for early detection of coral diseases and subsequent management decisions transmission vectors and causative agents of diseases must be identified to enable the potential of this technology to be reached.

4.7.5 Future direction #5: Global reference databases

A major challenge in understanding the structure and function of the coral holobiont is the lack of a baseline understanding of what is considered 'healthy' (Egan & Gardiner, 2016). The variability of the coral microbiome makes it difficult to determine from a taxonomic standpoint what drives dysbiosis in stressed corals. Perhaps approaching the question by addressing the functionality of bacteria within themicrobiome may reveal more answers. This sentiment is applied in coral probiotics, with the hypothesis that corals can alter their holobionts to promote host persistence under changing conditions (Dunphy et al., 2019; Reshef et al., 2006). Such analyses are however reliant on a comprehensive understanding of host-microbe interactions, long-term and seasonal fluctuations in composition, and an understanding of the functionality of bacteria under varying conditions. These large scale datasets are the result of global microbiome projects, following in the footsteps of the Earth Microbiome Project (Dunphy et al., 2019; Gilbert et al., 2014) and the NIH Human Microbiome Project (Peterson et al., 2009). Comparatively theCoral Microbiome Database is still very much under early development (Huggett & Apprill, 2019), and requires widescale adoption and contribution to identify global patterns in the coral holobiont. By developing a database of diseased and healthy bacterial community states, especially those linked with disease, both the determination of potential causal agents and the eventual use as tool for disease characterisation may be possible, particularly as the field shifts from a one disease – one pathogen to amore multi-faceted paradigm (Sweet et al., 2019).

4.8 Thesis conclusion

Coral diseases are both a natural part of reef ecosystems and a direct and indirect result of the collective effects of climate change and anthropogenic disturbances. Understanding their causes and consequencesis challenged by a lack of universally adopted diagnosis framework, and the use of complementary traditional techniques, with modern genomic advances for a holistic characterisation of disease. Whilstgrowth anomalies have been described across the Indo-Pacific and Caribbean regions, a significant proportion of the disease process remains unknown. An effective approach for characterising coral diseases is to conduct a multidisciplinary assessment, including environmental assessment through to molecular level. The overarching theme of this thesis is to utilise a combination of traditional diagnostic techniques and modern methods to describe a novel outbreak of growth anomalies at a remote coral reef atoll. This thesis examines growth anomalies at various levels of biological complexity. It establishes the extent of the *I. palifera* population affected, suggests that GAs result in a localised reduction in reproductive and biological functioning of the host, and questions the future reproductive output and accretion potential of the CKI reefs. This thesis also utilises the largest sample set to date, to characterisethe bacteria associations of *I. palifera* in both healthy, asymptomatic, and diseased health states, and contributes to the shift away from dysbiosis as an indicator of disease. The results of this research support the integration of classical and modern techniques and support the adoption of a standardised toolkit universally, for the characterisation of diseases and dynamics. In this regard, early warning systems and effective mitigation plans can be established to enable more proactive management of coralreef ecosystems.

4.9 References

The owners of copyright material used in this thesis have been acknowledged with every fair and reasonable effort. I would be grateful to hear from any copyright owner should they feel they havebeen omitted or acknowledged incorrectly.

Aeby, G. S., Callahan, S., Cox, E., Runyon, C., Smith, A., Stanton, F., Ushijima, B., & Work, T. M.(2016). Emerging coral diseases in Kāne‘ohe Bay, O‘ahu, Hawai‘i (USA): two major disease outbreaks of acute Montipora white syndrome. *Diseases of Aquatic Organisms*, *119*(3), 189– 198. https://doi.org/10.3354/dao02996

Aeby, G. S., & Santavy, D. L. (2006). Factors affecting susceptibility of the coral Montastraea faveolata to black-band disease. *Marine Ecology Progress Series*, *318*, 103–110. https://doi.org/10.3354/meps318103

Ainsworth, T. D., Renzi, J. J., & Silliman, B. R. (2020). Positive Interactions in the Coral Macro and Microbiome. *Trends in Microbiology*, *28*(8), 602–604. https://doi.org/10.1016/j.tim.2020.02.009

Alker, A. P., Smith, G. W., & Kim, K. (2001). Characterization of Aspergillus sydowii (Thom et Church), a fungal pathogen of Caribbean sea fan corals. *Hydrobiologia*, *460*(1), 105–111. https://doi.org/10.1023/A:1013145524136

Almeida, A., Mitchell, A. L., Tarkowska, A., & Finn, R. D. (2018). Benchmarking taxonomic assignments based on 16S rRNA gene profiling of the microbiota from commonly sampled environments. *GigaScience*, *7*(5), 1–10. https://doi.org/10.1093/gigascience/giy054

Barberán, A., Bates, S. T., Casamayor, E. O., & Fierer, N. (2012). Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME Journal*, *6*(2), 343–351. https://doi.org/10.1038/ismej.2011.119

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. In *Trends in Ecology and Evolution* (Vol. 29, Issue 6, pp. 358–367). Elsevier Ltd. https://doi.org/10.1016/j.tree.2014.04.003

Brussaard, C. P. D., Baudoux, A. C., & Rodríguez-Valera, F. (2016). Marine viruses. In L. Stal & M.Cretoiu (Eds.), *The marine microbiome: an untapped source of biodiversity and biotechnological potential* (pp. 155–183). Springer. https://doi.org/10.1007/978-3-319-33000- 6_5

Bukin, Y. S., Galachyants, Y. P., Morozov, I. V., Bukin, S. V., Zakharenko, A. S., & Zemskaya, T. I. (2019). The effect of 16s rRNA region choice on bacterial community metabarcoding results. *Scientific Data*, *6*(1), 1–14. https://doi.org/10.1038/sdata.2019.7

Burns, J. H. R., & Takabayashi, M. (2011). Histopathology of growth anomaly affecting the coral, Montipora capitata: implications on biological functions and population viability. *PLoS ONE*,*6*(12), e28854. https://doi.org/10.1371/journal.pone.0028854

Callahan, B. J., Wong, J., Heiner, C., Oh, S., Theriot, C. M., Gulati, A. S., McGill, S. K., & Dougherty, M. K. (2019). High-throughput amplicon sequencing of the full-length 16S rRNA gene with single-nucleotide resolution. *Nucleic Acids Research*, *47*(18), e103. https://doi.org/10.1093/nar/gkz569

Casey, J. M., Ainsworth, T. D., Choat, J. H., & Connolly, S. R. (2014). Farming behaviour of reeffishes increases the prevalence of coral disease associated microbes and black band disease. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1788), 20141032. https://doi.org/10.1098/rspb.2014.1032

Chakravorty, S., Helb, D., Burday, M., Connell, N., & Alland, D. (2007). A detailed analysis of 16Sribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *Journal of Microbiological Methods*, *69*(2), 330–339. https://doi.org/10.1016/j.mimet.2007.02.005

Chiu, J. M. Y., Li, S., Li, A., Po, B., Zhang, R., Shin, P. K. S., & Qiu, J.-W. (2012). Bacteria associated with skeletal tissue growth anomalies in the coral Platygyra carnosus. *FEMS Microbiology Ecology*, *79*(2), 380–391. https://doi.org/10.1111/j.1574-6941.2011.01225.x

Chong-Seng, K. M., Cole, A. J., Pratchett, M. S., & Willis, B. L. (2011). Selective feeding by coral reef fishes on coral lesions associated with brown band and black band disease. *Coral Reefs*, *30*(2), 473–481. https://doi.org/10.1007/s00338-010-0707-1

Cole, A. J., Seng, K. M. C., Pratchett, M. S., & Jones, G. P. (2009). Coral-feeding fishes slow progression of black-band disease. *Coral Reefs*, *28*(4), 965–965. https://doi.org/10.1007/s00338-009-0519-3

Correa, A. M. S., Ainsworth, T. D., Rosales, S. M., Thurber, A. R., Butler, C. R., & Thurber, R. V. (2016). Viral outbreak in corals associated with an in situ bleaching event: Atypical herpes-like viruses and a new megavirus infecting symbiodinium. *Frontiers in Microbiology*, *7*(FEB), 127.https://doi.org/10.3389/fmicb.2016.00127

Domart-Coulon, I. J., Traylor-Knowles, N., Peters, E., Elbert, D., Downs, C. A., Price, K., Stubbs, J.,McLaughlin, S., Cox, E., Aeby, G. S., Brown, P. R., & Ostrander, G. K. (2006). Comprehensive characterization of skeletal tissue growth anomalies of the finger coral

Porites compressa. *Coral Reefs*, *25*(4), 531–543. https://doi.org/10.1007/s00338-006-0133-6

Doyle, J., & Uthicke, S. (2020). Sensitive environmental DNA detection via lateral flow assay (dipstick)—A case study on corallivorous crown-of-thorns sea star (*Acanthaster cf. solaris*)detection. *Environmental DNA*, edn3.123. https://doi.org/10.1002/edn3.123

Dunphy, C. M., Gouhier, T. C., Chu, N. D., & Vollmer, S. V. (2019). Structure and stability of the coral microbiome in space and time. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-43268-6

Egan, S., & Gardiner, M. (2016). Microbial dysbiosis: Rethinking disease in marine ecosystems. *Frontiers in Microbiology*, *7*(JUN), 991. https://doi.org/10.3389/fmicb.2016.00991

Gilbert, J. A., Jansson, J. K., & Knight, R. (2014). The Earth Microbiome project: Successes and aspirations. In *BMC Biology* (Vol. 12, Issue 1, pp. 1–4). BioMed Central Ltd. https://doi.org/10.1186/s12915-014-0069-1

Hashizume, H., Sato, M., Sato, M. O., Ikeda, S., Yoonuan, T., Sanguankiat, S., Pongvongsa, T., Moji,K., & Minamoto, T. (2017). Application of environmental DNA analysis for the detection of Opisthorchis viverrini DNA in water samples. *Acta Tropica*, *169*, 1–7. https://doi.org/10.1016/j.actatropica.2017.01.008

Huggett, M. J., & Apprill, A. (2019). Coral microbiome database: Integration of sequences reveals high diversity and relatedness of coral-associated microbes. *Environmental Microbiology Reports*, *11*(3), 372–385. https://doi.org/10.1111/1758-2229.12686

Irikawa, A., Casareto, B. E., Suzuki, Y., Agostini, S., Hidaka, M., & van Woesik, R. (2011). Growth anomalies on Acropora cytherea corals. *Marine Pollution Bulletin*, *62*(8), 1702–1707. https://doi.org/10.1016/J.MARPOLBUL.2011.05.033

Jacquemot, L., Bettarel, Y., Monjol, J., Corre, E., Halary, S., Desnues, C., Bouvier, T., Ferrier-Pagès,C., & Baudoux, A.-C. (2018). Therapeutic Potential of a New Jumbo Phage That Infects Vibrio coralliilyticus, a Widespread Coral Pathogen. *Frontiers in Microbiology*, *9*(OCT), 2501. https://doi.org/10.3389/fmicb.2018.02501

Jones, R. A., Brophy, P. M., Davis, C. N., Davies, T. E., Emberson, H., Rees Stevens, P., & Williams, H. W. (2018). Detection of Galba truncatula, Fasciola hepatica and Calicophoron daubneyi environmental DNA within water sources on pasture land, a future tool for fluke control? *Parasites and Vectors*, *11*(1), 342. https://doi.org/10.1186/s13071-018-2928-z

Kelly, L. A., Heintz, T., Lamb, J. B., Kelly, L. A., Heintz, T., Lamb, J. B., Ainsworth, T. D.,

& Willis, B. L. (2016). Ecology and Pathology of Novel Plaque-Like Growth Anomalies Affecting a Reef-Building Coral on the Great Barrier Reef. *Frontiers in Marine Science*, *3*, 151. https://doi.org/10.3389/fmars.2016.00151

Kelly, L. W., Richmond, R. H., Pogoreutz, C., Rodriguez-Lanetty, M., Dougan, K. E., Ladd, M. C., Fuchs, C., Thurber, R. V., & Burkepile, D. E. (2020). Nutrient Pollution and Predation Differentially Affect Innate Immune Pathways in the Coral Porites porites. *Frontiers in MarineScience*, *7*(563565). https://doi.org/10.3389/fmars.2020.563865

Ki, J. S., Zhang, W., & Qian, P. Y. (2009). Discovery of marine Bacillus species by 16S rRNA and rpoB comparisons and their usefulness for species identification. *Journal of Microbiological Methods*, *77*(1), 48–57. https://doi.org/10.1016/j.mimet.2009.01.003

Kim, M., Morrison, M., & Yu, Z. (2011). Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes. *Journal of Microbiological Methods*, *84*(1),81–87. https://doi.org/10.1016/j.mimet.2010.10.020

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013).Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1). https://doi.org/10.1093/nar/gks808

Koczula, K. M., & Gallotta, A. (2016). Lateral flow assays. *Essays in Biochemistry*, *60*(1), 111–120.https://doi.org/10.1042/EBC20150012

Leite, D. C. A., Salles, J. F., Calderon, E. N., Castro, C. B., Bianchini, A., Marques, J. A., van Elsas, J. D., & Peixoto, R. S. (2018). Coral Bacterial-Core Abundance and Network Complexity as Proxies for Anthropogenic Pollution. *Frontiers in Microbiology*, *9*(APR), 833. https://doi.org/10.3389/fmicb.2018.00833

Liu, Z., Desantis, T. Z., Andersen, G. L., & Knight, R. (2008). Accurate taxonomy assignments from 16S rRNA sequences produced by highly parallel pyrosequencers. *Nucleic Acids Research*, *36*(18). https://doi.org/10.1093/nar/gkn491

Marhaver, K. L., Edwards, R. A., & Rohwer, F. (2008). Viral communities associated with healthy and bleaching corals. *Environmental Microbiology*, *10*(9), 2277–2286. https://doi.org/10.1111/j.1462-2920.2008.01652.x

Martin, S. B., Sasal, P., Cutmore, S. C., Ward, S., Aeby, G. S., & Cribb, T. H. (2018). Intermediate host switches drive diversification among the largest trematode family: evidence from the Polypipapiliotrematinae n. subf. (Opecoelidae), parasites transmitted to butterflyfishes via predation of coral polyps. *International Journal for Parasitology*,

48(14), 1107–1126. https://doi.org/10.1016/j.ijpara.2018.09.003

Maynard, J. A., Anthony, K. R. N., Harvell, C., Burgman, M. A., Beeden, R., Sweatman, H., Heron, S. F., Lamb, J. B., & Willis, B. L. (2011). Predicting outbreaks of a climate-driven coral disease in the Great Barrier Reef. *Coral Reefs*, *30*(2), 485–495. https://doi.org/10.1007/s00338-010-0708-0

Miller, D. J., Hemmrich, G., Ball, E. E., Hayward, D. C., Khalturin, K., Funayama, N., Agata, K., &Bosch, T. C. G. (2007). The innate immune repertoire in Cnidaria - Ancestral complexity and stochastic gene loss. *Genome Biology*, *8*(4), 1–13. https://doi.org/10.1186/gb-2007-8-4-r59

Montano, S., Maggioni, D., Liguori, G., Arrigoni, R., Berumen, M. L., Seveso, D., Galli, P., & Hoeksema, B. W. (2020). Morpho-molecular traits of Indo-Pacific and Caribbean Halofolliculina ciliate infections. *Coral Reefs*, *39*, 375–386. https://doi.org/10.1007/s00338-020-01899-6

Mydlarz, L. D., McGinty, E. S., & Drew Harvell, C. (2010). What are the physiological and immunological responses of coral to climate warming and disease? *Journal of ExperimentalBiology*, *213*(6), 934–945. https://doi.org/10.1242/jeb.037580

Ng, J. C. Y., Chan, Y., Tun, H. M., Leung, F. C. C., Shin, P. K. S., & Chiu, J. M. Y. (2015). Pyrosequencing of the bacteria associated with Platygyra carnosus corals with skeletal growth anomalies reveals differences in bacterial community composition in apparently healthy and diseased tissues. *Frontiers in Microbiology*, *6*, 1142. https://doi.org/10.3389/fmicb.2015.01142

Nicolet, K. J., Chong-Seng, K. M., Pratchett, M. S., Willis, B. L., & Hoogenboom, M. O. (2018). Predation scars may influence host susceptibility to pathogens: Evaluating the role of corallivores as vectors of coral disease. *Scientific Reports*, *8*(1), 5258. https://doi.org/10.1038/s41598-018-23361-y

Ogier, J. C., Pagès, S., Galan, M., Barret, M., & Gaudriault, S. (2019). RpoB, a promising marker foranalyzing the diversity of bacterial communities by amplicon sequencing. *BMC Microbiology*, *19*(1), 171. https://doi.org/10.1186/s12866-019-1546-z

Palmer, C. V. (2018). Immunity and the coral crisis. *Communications Biology*, *1*(91). https://doi.org/10.1038/s42003-018-0097-4

Palmer, C. V., & Baird, A. (2018). Coral tumor-like growth anomalies induce an immune response and reduce fecundity. *Diseases of Aquatic Organisms*, *130*(1), 77–81. https://doi.org/10.3354/dao03258

Palmer, C. V., Bythell, J. C., & Willis, B. L. (2010). Levels of immunity parameters underpin bleaching and disease susceptibility of reef corals. *The FASEB Journal*, *24*(6), 1935–1946.https://doi.org/10.1096/fj.09-152447

Palmer, C. V., McGinty, E. S., Cummings, D. J., Smith, S. M., Bartels, E., & Mydlarz, L. D. (2011). Patterns of coral ecological immunology: Variation in the responses of Caribbean corals to elevated temperature and a pathogen elicitor. *Journal of Experimental Biology*, *214*(24), 4240–4249. https://doi.org/10.1242/jeb.061267

Palmer, C. V., & Traylor-Knowles, N. (2012). Towards an integrated network of coral immune mechanisms. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1745), 4106–4114.https://doi.org/10.1098/rspb.2012.1477

Peterson, J., Garges, S., Giovanni, M., McInnes, P., Wang, L., Schloss, J. A., Bonazzi, V., McEwen, J. E., Wetterstrand, K. A., Deal, C., Baker, C. C., Di Francesco, V., Howcroft, T. K., Karp, R.W., Lunsford, R. D., Wellington, C. R., Belachew, T., Wright, M., Giblin, C., … Guyer, M. (2009). The NIH Human Microbiome Project. *Genome Research*, *19*(12), 2317–2323. https://doi.org/10.1101/gr.096651.109

Rajasabapathy, R., Ramasamy, K. P., Manikandan, B., Mohandass, C., & Arthur James, R. (2020). Bacterial communities associated with healthy and diseased (skeletal growth anomaly) reef coralAcropora cytherea from Palk Bay, India. *Frontiers in Marine Science*, *7*, 92. https://doi.org/10.3389/fmars.2020.00092

Raymundo, L., Halford, A. R., Maypa, A. P., & Kerr, A. M. (2009). Functionally diverse reef-fish communities ameliorate coral disease. *Proceedings of the National Academy of Sciences of theUnited States of America*, *106*(40), 17067–10070. https://doi.org/10.1073

Reshef, L., Koren, O., Loya, Y., Zilber-Rosenberg, I., & Rosenberg, E. (2006). The Coral Probiotic Hypothesis. *Environmental Microbiology*, *8*(12), 2068–2073. https://doi.org/10.1111/j.1462- 2920.2006.01148.x

Roff, G., Hoegh-Guldberg, O., & Fine, M. (2006). Intra-colonial response to Acroporid "whitesyndrome" lesions in tabular Acropora spp. (Scleractinia). *Coral Reefs*, *25*(2), 255–264. https://doi.org/10.1007/s00338-006-0099-4

Sajid, M., Kawde, A. N., & Daud, M. (2015). Designs, formats and applications of lateral flow assay: A literature review. *Journal of Saudi Chemical Society*, *19*(6), 689–705. https://doi.org/10.1016/j.jscs.2014.09.001

Shaw, J. L. A., Clarke, L. J., Wedderburn, S. D., Barnes, T. C., Weyrich, L. S., & Cooper, A. (2016). Comparison of environmental DNA metabarcoding and conventional fish survey

methods in a river system. *Biological Conservation*, *197*, 131–138. https://doi.org/10.1016/j.biocon.2016.03.010

Shinzato, C., Shoguchi, E., Kawashima, T., Hamada, M., Hisata, K., Tanaka, M., Fujie, M., Fujiwara, M., Koyanagi, R., Ikuta, T., Fujiyama, A., Miller, D. J., & Satoh, N. (2011). Using the Acropora digitifera genome to understand coral responses to environmental change. *Nature*, *476*(7360),320–323. https://doi.org/10.1038/nature10249

Silva-Lima, A. W., Froes, A. M., Garcia, G. D., Tonon, L. A. C., Swings, J., Cosenza, C. A. N.,Medina, M., Penn, K., Thompson, J. R., Thompson, C. C., & Thompson, F. L. (2020). Mussismilia braziliensis White Plague Disease Is Characterized by an Affected Coral Immune System and Dysbiosis. *Microbial Ecology*, 1–12. https://doi.org/10.1007/s00248-020-01588-5

Soffer, N., Zaneveld, J., & Thurber, R. V. (2015). Phage-bacteria network analysis and its implication for the understanding of coral disease. *Environmental Microbiology*, *17*(4), 1203–1218. https://doi.org/10.1111/1462-2920.12553

Soler-Hurtado, M. M., Sandoval-Sierra, J. V., Machordom, A., & Diéguez-Uribeondo, J. (2016).Aspergillus sydowii and Other Potential Fungal Pathogens in Gorgonian Octocorals of the Ecuadorian Pacific. *PLOS ONE*, *11*(11), e0165992. https://doi.org/10.1371/journal.pone.0165992

Spies, N., & Takabayashi, M. (2013). Expression of galaxin and oncogene homologs in growth anomaly in the coral Montipora capitata. *Diseases of Aquatic Organisms*, *104*(3), 249–256.https://doi.org/10.3354/dao02603

Stat, M., Huggett, M. J., Bernasconi, R., Dibattista, J. D., Berry, T. E., Newman, S. J., Harvey, E. S.,& Bunce, M. (2017). Ecosystem biomonitoring with eDNA: Metabarcoding across the tree of life in a tropical marine environment. *Scientific Reports*, *7*(1), 1–11. https://doi.org/10.1038/s41598-017-12501-5

Sweet, M. J., Burian, A., Fifer, J., Bulling, M., Elliott, D., & Raymundo, L. (2019). Compositional homogeneity in the pathobiome of a new, slow-spreading coral disease. *Microbiome*, *7*(1), 1–14.https://doi.org/10.1186/s40168-019-0759-6

Sweet, M. J., Burn, D., Croquer, A., & Leary, P. (2013). Characterisation of the bacterial and fungal communities associated with different lesion sizes of dark spot syndrome occurring in the coral Stephanocoenia intersepta. *PLoS ONE*, *8*(4), e62580. https://doi.org/10.1371/journal.pone.0062580

Takagi, T., Yoshioka, Y., Zayasu, Y., Satoh, N., & Shinzato, C. (2020). Transcriptome

Analyses of Immune System Behaviors in Primary Polyp of Coral Acropora digitifera Exposed to the Bacterial Pathogen Vibrio coralliilyticus under Thermal Loading. *Marine Biotechnology*, 1–12.https://doi.org/10.1007/s10126-020-09984-1

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., Prill, R. J., Tripathi, A., Gibbons, S. M., Ackermann, G., Navas-Molina, J. A., Janssen, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J. T., Mirarab, S., Xu, Z. Z., Jiang, L., … Zhao, H.(2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, *551*(7681), 457–463. https://doi.org/10.1038/nature24621

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples.*PLoS ONE*, *7*(8), e41732. https://doi.org/10.1371/journal.pone.0041732

Thurber, R. V., Payet, J. P., Thurber, A. R., & Correa, A. M. S. (2017). Virus-host interactions andtheir roles in coral reef health and disease. In *Nature Reviews Microbiology* (Vol. 15, Issue 4, pp. 205–216). Nature Publishing Group. https://doi.org/10.1038/nrmicro.2016.176

Toledo-Hernández, C., & Ruiz-Diaz, C. P. (2014). The immune responses of the coral | Invertebrate Survival Journal. *Invertebrate Survival Journal*, *11*(1), 319–328. http://www.isj.unimore.it/index.php/ISJ/article/view/334

Vos, M., Quince, C., Pijl, A. S., de Hollander, M., & Kowalchuk, G. A. (2012). A Comparison of rpoB and 16S rRNA as Markers in Pyrosequencing Studies of Bacterial Diversity. *PLoS ONE*,*7*(2), e30600. https://doi.org/10.1371/journal.pone.0030600

Weynberg, K. D., Voolstra, C. R., Neave, M. J., Buerger, P., & van Oppen, M. (2015). From cholera to corals: Viruses as drivers of virulence in a major coral bacterial pathogen. *Scientific Reports*,*5*. https://doi.org/10.1038/srep17889

Willis, B. L., Page, C. A., & Dinsdale, E. A. (2004). Coral disease on the Great Barrier Reef. In *Coral health and disease* (pp. 69–104). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-06414-6_3

Wong, Y. H., Zhang, Y., Lun, J. C. Y., & Qiu, J. W. (2021). A proteomic analysis of skeletal tissue anomaly in the brain coral Platygyra carnosa. *Marine Pollution Bulletin*, *164*, 111982. https://doi.org/10.1016/j.marpolbul.2021.111982

Work, T. M., Aeby, G. S., Lasne, G., & Tribollet, A. (2014). Gross and microscopic pathology of hard and soft corals in New Caledonia. *Journal of Invertebrate Pathology*, *120*, 50–58. https://doi.org/10.1016/J.JIP.2014.05.007

Wright, R. M., Aglyamova, G. V., Meyer, E., & Matz, M. V. (2015). Gene expression associated with white syndromes in a reef building coral, Acropora hyacinthus. *BMC Genomics*, *16*(1), 1–12. https://doi.org/10.1186/s12864-015-1540-2

Zaneveld, J. R., McMinds, R., & Thurber, Rebecca VegaThurber, R. V. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nature Microbiology*, *2*(9),17121. https://doi.org/10.1038/nmicrobiol.2017.121

Zhang, J., Ding, X., Guan, R., Zhu, C., Xu, C., Zhu, B., Zhang, H., Xiong, Z., Xue, Y., Tu, J., & Lu, Z. (2018). Evaluation of different 16S rRNA gene V regions for exploring bacterial diversity in a eutrophic freshwater lake. *Science of the Total Environment*, *618*, 1254–1267. https://doi.org/10.1016/j.scitotenv.2017.09.228

Zhang, Y., Sun, J., Mu, H., Lun, J. C. Y., & Qiu, J.-W. (2017). Molecular pathology of skeletal growth anomalies in the brain coral Platygyra carnosa: A meta-transcriptomic analysis. *Marine Pollution Bulletin*, *124*(2), 660–667. https://doi.org/10.1016/J.MARPOLBUL.2017.03.047

Appendices

Appendix I

I.I Copyright statements

Chapter 2: Published in journal Coral Reefs. As first author, permission is automatically granted to reproduce this copyright material if it constitutes less than half of the total material in such a publication.

To whom it may concern, I, Sophie Preston, contributed to the conceptualisation of the study, collectedand processed the samples, analysed the data, and co-wrote and edited the resulting manuscript.

Preston, S., Richards, Z. Biological consequences of an outbreak of growth anomalies on *Isopora palifera* at the Cocos (Keeling) Islands. *Coral Reefs* (2020). https://doi.org/10.1007/s00338-020- 02019-0

I, as a co-author, endorse that this level of contribution by the candidate indicated above is appropriate.

Zoe Richards _____

Chapter 3: In preparation for publication

To whom it may concern, I, Sophie Preston, contributed to the conceptualisation of the study, collected,and processed the samples, analysed the data, and co-wrote and edited the resulting manuscript.

Preston, S., Adam, A., Huggett, M.J., White, N. E., Christopherson, C., Tan, K.C., Richards, Z. Bacterial associations of growth anomaly affected *Isopora palifera*. *[in preparation]*

I, as a co-author, endorse that this level of contribution by the candidate indicated above is appropriate.

Arne A. S. Adam _____

Megan J. Huggett _____

Nicole E. White _____

Claus Christopherson _____

Kar-Chun Tan _____

Zoe Richards _____

Chapter 1&4: In preparation for publication

To whom it may concern, I, Sophie Preston, contributed to the conceptualisation of the study andwrote and edited the resulting manuscript.

Preston, S., Heydenrych, M., West, K., Huggett, M.J., Tan, K.C., Richards, Z. Integrating eDNA into the genomics age toolkit for characterising scleractinian coral diseases. *[in preparation]*

I, as a co-author, endorse that this level of contribution by the candidate indicated above is appropriate.

Matthew Heydenrych _____

Katrina West _____

Megan J. Huggett _____

Kar-Chun Tan _____

Zoe Richards _____

Appendix II: Research output

II.I Publications

Preston, S., Richards, Z. Biological consequences of an outbreak of growth anomalies on *Isopora palifera* at the Cocos (Keeling) Islands. *Coral Reefs* (2021). https://doi.org/10.1007/s00338-020-02019-0

II.II Awards, Grants and Conference Presentations

2020

Western Australian Naturalist Society Serventy Memorial Grant

Australian Coral Reef Society Student Writing Retreat Scholarship

Linnean Society of New South Wales William Macleay Microbiology Grant (Chapter 2)

Biological consequences of an outbreak of growth anomalies on Isopora palifera at the Cocos (Keeling) Islands. [poster presentation] Combined Biological Sciences Meeting, Perth

Best Image Prize

Combined Biological Sciences Meeting, Perth

Coral Reefs
https://doi.org/10.1007/s00338-020-02019-0

REPORT

Biological consequences of an outbreak of growth anomalies on *Isopora palifera* at the Cocos (Keeling) Islands

Sophie Preston[1] · **Zoe Richards**[1,2]

Received: 14 July 2020 / Accepted: 14 October 2020

Abstract Growth anomalies (GAs), a tumour-like disease affecting scleractinian corals, have been reported across the major reef systems of the Indo-Pacific and wider Atlantic regions, predominantly affecting *Acropora* and *Porites* species. In 2018, GAs were observed for the first time on *Isopora palifera*, an important observation from the isolated Cocos (Keeling) Islands in the East Indian Ocean, as the species is a key reef building coral at the atoll. In this study, the local distribution and abundance of GAs was quantified to determine if this occurrence could be classified as an outbreak, and the effects of this disease on *I. palifera* on reproductive potential and growth was described using histological and geochemical analysis. Growth anomalies were documented at 75% of sites and affected approximately one third of the *I. palifera* colonies examined. This disease compromises the biological and reproductive functioning of the host, as evidenced by a significant reduction in the density of oocytes, mesenteries, polyps, and zooxanthellae in infected tissues in comparison to healthy tissue. Furthermore, geochemical analysis indicates changes to key trace elements may be the result of bioerosion processes by infecting bacteria and the reprecipitation of calcite. The results of this study indicate the division of energy to the rapid skeletal development that characterises the disease, may have occurred at the detriment of the future reproductive potential of the population.

Keywords Atoll · Biology · Coral · Coral reef · Disease · Ecology · Histology · Geochemistry

Topic Editor Steve Vollmer

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00338-020-02019-0) contains supplementary material, which is available to authorised users.

✉ Sophie Preston
sophie.preston@postgrad.curtin.edu.au

[1] Trace Environmental DNA Laboratory, Coral Conservation and Research Group, School of Molecular and Life Science, Curtin University, Bentley, WA 6102, Australia

[2] Department of Aquatic Zoology, Western Australian Museum, Kew Street, Welshpool, WA 6106, Australia

Introduction

Growth anomalies (GAs) are pale, tumour-like disease that commonly affect important reef building species across the Indo-Pacific and Atlantic coral reefs (Weil 2004; Aeby et al. 2011). First described on *Madrepora kauaiensis* (revised *Madrepora oculata* (Hoeksema and Cairns 2020)) in 1965 (Squires 1965), they have been reported to affect over 14 genera; however species belonging to *Acroporidae* and *Poritidae* are disproportionately affected (17 and 7 species, respectively; Aeby et al. 2011). GAs have been defined as tumours (Gateño et al. 2003), calicoblastic neoplasms (Peters et al. 1986), neoplasia (Squires 1965) or skeletal tissue anomalies (Domart-Coulon et al. 2006) resulting in ambiguity in the literature. Despite this, GAs are generally characterised using histological methods, by reduced fecundity, zooxanthellae density and fewer polyps (Burns and Takabayashi 2011; Work et al. 2015). Recent analyses on the skeletons of growth anomalies have demonstrated a reduced density and abnormal development of structures (Domart-Coulon et al. 2006). Molecular work has identified changes to gene expression (Zhang et al. 2017), immune response (Palmer and Baird 2018) and growth processes (Andersson et al. 2020). The global presence of GAs has led to a framework for diagnosis

Published online: 27 October 2020

(Raymundo et al. 2008b; Pollock et al. 2011); however there is much still unknown about this disease.

The consequences of GAs on the functioning of the host has been described consistently through the literature (Williams et al. 2011; Burns and Takabayashi 2011), and the effect on skeletal structures is of increasing interest (Domart-Coulon et al. 2006; Andersson et al. 2020); however transmission and causal mechanism of the disease is yet to be established. The instigation of GAs has been linked to everything from environmental and anthropogenic factors (Aeby et al. 2011), to microbial agents (Domart-Coulon et al. 2006; Work et al. 2015), and transmission of the disease to host density, coral cover and direct contact (Kaczmarsky and Richardson 2007). It is likely that the etiology and progression of GAs is the result of a combination of factors and establishing the mechanisms of this disease is challenging without understanding the natural prevalence of GAs on coral reefs (Harvell et al. 1999; Aeby 2006).

To understand the level of threat that a disease may pose to an ecosystem, it is necessary to understand what constitutes as an outbreak. A disease outbreak is technically defined as an R_0 value greater than one, where R_0 is 'the average number of secondary infections produced when one infected individual is introduced into a population of susceptible hosts' and is measured over time (Raymundo et al. 2008a). However, coral disease outbreaks are often seasonal (Sato et al. 2009), or baseline levels of coral disease upon the reef are unknown (Harvell et al. 1999; Aeby 2006), so alternative methods of determining the threshold to define an outbreak have also been used. Natural prevalence of coral diseases on reefs is approximately $< 1\%$, become of concern at 3% prevalence, and qualified as an outbreak at $> 5\%$ (Willis et al. 2004). An alternative threshold used is the abundance of cases, where 60 cases/1500 m^2 qualifies as an outbreak, and 100 cases/1500 m^2 as severe (Maynard et al. 2011). The conflicting methods inhibit the ability to compare between regions and highlights the need for standardised thresholds as to what constitutes as an outbreak. Furthermore, coral disease observations are typically made over a single survey, leading to ambiguous descriptions of similar diseases (Bythell et al. 2004; Bruckner 2015). Often defining characteristics, such as rate and pattern of progression, require temporal studies or microscope analyses to correctly identify the disease.

In 2018, a putative outbreak of growth anomalies was reported on *Isopora palifera* (Richards and Newman 2019), a key reef building species on the Cocos (Keeling) Islands. The occurrence of GAs on *I. palifera* has only been mentioned once in the literature (Irikawa et al. 2011); however no details were provided about the extent, causality or consequences of the disease on this species. Whilst CKI has experienced anoxic events and extreme weather events, the reefs have escaped significant stress events in recent history (Gilmour et al. 2019), and the potential triggers of this unique occurrence of GAs are yet to be established. This study aims to investigate the local distribution and abundance of *I. palifera* and to quantify the proportion of colonies infected with GAs to establish whether this occurrence constitutes an outbreak. Secondly, this study aims to establish the biological consequences of growth anomalies on *Isopora palifera* by comparing changes to the skeletal, biological, and reproductive status of diseased and healthy colonies. The results of this study establish the local extent and significance of the disease and contribute to the global understanding of growth anomalies.

Methods

Specimen collection, ecological surveys, and analysis

The Cocos (Keeling) Islands (CKI) is a remote atoll of approximately 600 inhabitants, situated in the Indian Ocean 2,100 km north west of Exmouth, Western Australia (Lavers et al. 2019). CKI is a key marine hybrid zone (Hobbs et al. 2009), located at the junction between the Indian and Pacific Oceans, and has been reported to host marine species typically affiliated with both bioregions (Hobbs and Allen 2014). The corals of this region escaped the severe bleaching events that impacted reefs across the north west of Australia during the 2016/2017 marine heatwave, and coral cover was either preserved or increased (Gilmour et al. 2019).

Ecological surveys and specimen collections were undertaken on 17th–24th May 2019, at 9 sites to document the distribution and abundance of infected and healthy *I. palifera* colonies (Fig. 1). Growth anomalies were identified by pale, tumourous masses protruding from the colony (Fig. 2). Whilst the causal mechanism of the GA disease has yet to be established, for the purposes of this study, GA affected tissues will be referred to as 'infected'. Sites ranged in depth from 1 to 14 m, and chosen based on sites where GAs were observed in a previous study (Richards and Newman 2019), prevailing weather conditions, boat accessibility and time constraints. Strong south-easterly trade winds rendered the east to south-east side of the atoll inaccessible, and the majority of southern lagoon (blue holes area) was too shallow to access safely. At each site, the abundance of healthy and infected *I. palifera* colonies was measured along three 25 m long by 10 m wide belt transects. At four of these sites, healthy and infected specimens were collected (Table 1). Infected and adjacent healthy samples were collected from three replicate branches on three infected colonies, in addition to three healthy

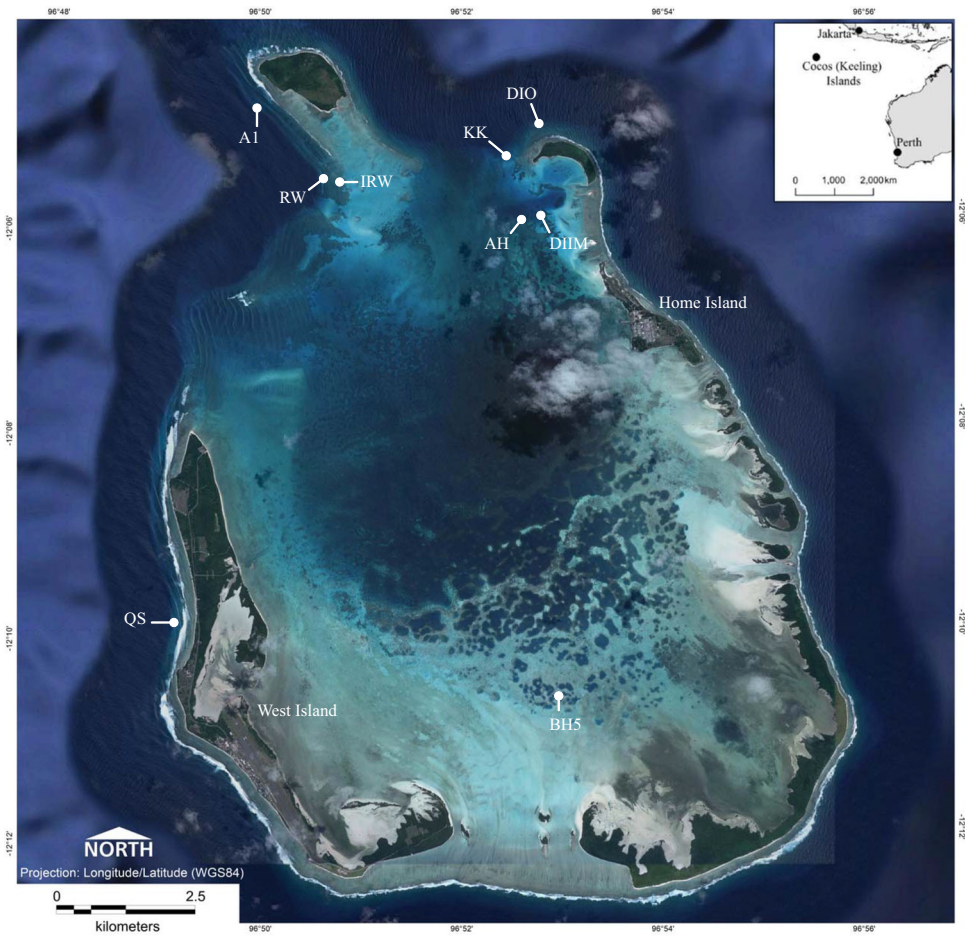

Fig. 1 Sample sites at Cocos (Keeling) Islands (CKI) as well as main settlements (Home and West Islands). For detailed site information and specimen collection, see Table 1

samples from three healthy control colonies at each site (n = 12 per site). Samples were split into three subsamples which were preserved in the following ways—100% ethanol (for subsequent genetic analysis), 10% formalin for histological analysis and for geochemical analysis, subsamples were bleached in 30% sodium hypochlorite solution, rinsed in freshwater water and air-dried.

Prior to subsampling, the proportion of surface area covered in GAs on individual colonies was calculated using 10cmx10cm cards, premade with 6 randomly punched holes (0.5 cm diameter). A card was randomly chosen and applied to 3 random branches and the presence/absence of GAs was counted under each point. To estimate the percentage contribution that *Isopora* makes to the overall benthic community, coral cover was surveyed across the 9 sites using three 25 m point intersect transects that were randomly deployed at each site. Substrate was recorded every 50 cm and hard corals were identified to a genus level.

To examine the abundance and distribution of growth anomalies, data were analysed by a 2-way analysis of variance (ANOVA) assessing site and health status as factors, after meeting assumptions of normality and variance. Tukeys HSD post hoc analysis was used to identify any significance between levels (Health Status, Site). All analysis was completed in R (v3.6.1) and RStudio (v1.2.500). Presence/absence of GAs was calculated per colony and averaged to establish proportion of surface area of individuals infected across sites. The percentage mean and SE of overall hard coral cover and scleractinian genera cover was calculated at a site level.

Geochemical methodology and analysis

To test differences in trace element composition of infected *I. palifera* skeletons, 10 replicate branches were analysed at 6 infected points, and 6 adjacent healthy points per skeleton (n = 120) (Andersson et al. 2020). Branches were 8–12 cm in length, and featured multiple tumours of over 2 cm in diameter, to ensure independence between samples. Samples were subject to X-ray fluorescence analysis using a ThermoScientific Niton™ XL3t Ultra Analyser (Thermo Fisher), an increasingly used technique for accurate and rapid results in marine, environmental and geological contexts (Liao et al. 2017; Arenas-Islas et al. 2019). The percentage composition of 8 trace elements was

Fig. 2 *Isopora palifera* field photographs. **a** healthy colony, **b** growth anomaly (GA) infected colony, **c** close up of GA-infected colony, **d** fish scrape on growth anomaly. Green arrow denotes healthy *I. palifera* skeleton, red denotes GA tumour

quantified (Ca, Sr, Ba, Mg, K, P, U and Al) (Domart-Coulon et al. 2006; Andersson et al. 2020). The instrument used an Ag anode (6-50 kV, 0–200 μA max) with a count time of 240 s per sample to deliver analytical precision of $< 1\%$ and 3 reference materials (Table 2) were used between samples to standardise results, as per manufacturers protocol (Schatzlein 2015).

To describe changes in chemical composition of skeletons, normalised data were converted into a Euclidean distance resemblance matrix. A 2 way PERMANOVA main test was performed to examine the null hypothesis that there is a significant difference in chemical composition between healthy and infected skeletons and between sites, using unrestricted permutation of raw data, 9999 permutations and type III sum of squares. A pairwise test using the same parameters was performed to test to address the interaction effect between health status and site. Separate permutational analyses of multivariate dispersion (PERMDISPs) were performed on the same matrices, with 9999 permutations, for both Health Status and Site to ascertain the significance of variation. The percentage contribution of each element was assessed using similarity

Table 1 Description of sites surveyed at the Cocos Keeling Islands including what survey method was conducted at each site

Site	Acronym	Latitude	Longitude	Depth (m)	Ecological survey	Tissue sample
Direction Island outside	DIO	S 12°05′01.9	E 096°52′40.3	10–12	Y	Y
Direction Island inside marker	DIIM	S 12°05′52.8	E 096°52′40.4	3–5	Y	–
Anchor hole	AH	S 12°05′57.8	E 096°52′33.2	12–14	Y	Y
Blue hole 5	BH5	S 12°10′39.7	E 096°52′52.8	1–2	Y	Y
Aquarium 1	A1	S 12°04′55.1	E 096°49.54.1	10–12	Y	–
Kats Kable	KK	S 12°05′20.9	E 096°52.21.0	10–12	Y	Y
Inside rose wall	IRW	S 12°05′37.3	E 096°50′35.5	3–5	Y	–
Rose wall	RW	S 12°05′36.0	E 096°50′30.6	10–12	Y	–
quarantine station	QS	S 12°10′01.8	E 096°49′00.2	10–12	Y	–

Table 2 Ore Research and Exploration certified standards used in X-ray fluorescence analysis made available to this project

CRM ID	Analytical method	Host lithology	Matrix	Mineralisation Style	Mg	Al	P	K	Ca	Sr	Ba	U
OREAS 904	4-Acid Digestion	Argillaceous Sandstone	Sediment	Sediment hosted	63,000	320,977.1	630	460	11.2	31.5	43.2	
OREAS 022d	4-Acid Digestion	Quartz	SiO_2	Barren	1320	462,701.3		100	0.2	0.69	1.2	
OREAS 062e	Pb Fire Assay	Meta-andesite	Intermediate	Low sulphidation epithermal	11,730	55,306	655	17,475	43,846	349	355	0.79

percentage analysis (SIMPER), with a cut-off of 100%. Data analysis was carried out in Primer 7 v7.0.13 (Primer-e, Quest Research Limited).

Histological methods and analysis

Corals preserved in 10% formalin and seawater were decalcified using 8% HCl and stored in 2% formalin solution for dissection and staining. Six pairs of infected and adjacent healthy tissue, and 4 control healthy samples ($n = 16$) were trimmed and processed using a Leica TP1020 automated tissue processor (Leica Microsystems, Mt Waverly, Australia) and embedded in paraffin on a Leica EG1150 H Heated Paraffin Embedding Module (Leica Microsystems). Histological sections were prepared following a modified protocol outlined in Peters et al. (2005). Serial histological Sects. (6–8 µm) were cut on a cryostat and stained with Gill's hematoxylin and eosin (H&E). Slides were passed through 100% ethanol for 30 s three times for rehydration, as per core facility protocols. Slides were viewed on an Olympus BX51 upright microscope with DP70 digital microscope system (Olympus Life Science Solutions, Victoria, Australia). To measure changes in reproductive and biological functioning, presence/absence of ova, number of mesenteries and polyps were recorded per 5 mm^2 and density of zooxanthellae per 1 mm^2 were counted on 3 sections of each tissue sample, using the Olympus cellSens Standard v1.8 software (Olympus), and the mean taken. To investigate changes in biological and reproductive components, between control, adjacent healthy and infected tissues, variables were standardised, and data square-root transformed to meet assumptions of normality and variance. A 1-way PERMANOVA main test was applied to a Bray–Curtis Similarity matrix, to test for significant differences between individuals (ID) and health status (Infected, Healthy, Control; fixed, nested in ID). The PERMANOVA used the following parameters: Type 1 sums of squares, 9999 permutations and unrestricted permutations of raw data. A pairwise PERMANOVA was used to test for significant differences between health status, using the same parameters. Descriptive statistics and data analysis were carried out in Primer 7.

Results

Local distribution and abundance of *Isopora palifera* and growth anomalies

A total 1322 *Isopora palifera* colonies were recorded on belt transects across 9 sites covering a total area survey of

6750 m^2. Coral cover surveys revealed 17 scleractinian coral genera present across 9 sites, with hard coral comprising of 44 ± 2.49% of substrate (Table 3). Per site, 7 genera accounted for approximately 95% of hard coral cover, proportional to site level coral cover (Fig. 3). *Isopora* was the sixth most prevalent genera of coral, accounting for 5.78 ± 0.93% to the total hard coral cover (Fig. 4). Comparatively, susceptible *Acropora* and *Porites* colonies were the two dominant genera, comprising of 24.89 ± 3.38% and 18.81 ± 2.04% of hard coral. *I. palifera* was present at all sites; however there was a significant difference between number of colonies per site (p = 6.963e−12, *df*: 8,36, F = 4.82; Fig. 5) whereby the highest mean number of colonies was found on the outer reef slope at Direction Island (DIO = 67.17 ± 8.23) and the lowest on the outer reef slope at Quarantine Station (QS = 6.83 ± 3.43).

At reef-wide scale, there was a significant difference in the number of infected and healthy colonies ($p = 2.632^{e-06}$, *df*: 1,36, F = 30.99), where 67% (n = 883) of colonies were healthy and 33% (n = 439) of colonies were infected with GAs. The average number of cases was reported at 16.26 per 750 m^2 (± 8.57 SE). Infected colonies were reported all survey sites except for Quarantine Station and Aquarium 1; however these sites exhibited lower numbers of *I. palifera* colonies (41 and 79 colonies, respectively).

At the site scale, the mean number of healthy colonies per site was greater than the number of infected (32.70 ± 4.12 and 16.26 ± 5.09 colonies, respectively). Direction Island Outside was the only site where the mean number of infected colonies was greater than the number of healthy colonies (78.33 ± 14.19 and 56.00 ± 3.51, respectively; Fig. 5a). Six of nine sites recorded over 5% of the population infected; however sites that recorded greater than 30 cases per 750 m^2 were only Direction Island Outside (78.33 ± 14.19) and Anchor Hole (30.33 ± 9.87; Fig. 5b). At a colony scale, growth anomalies were found on 41.25 ± 0.04% of the surface area of colonies infected.

Table 3 Substrate type (mean ± SE) calculated from point intersect transects across 9 sites

Substrate type	Contribution (%)
Hard coral	44.00 ± 2.4
Turf	19.70 ± 2.65
Soft coral	11.19 ± 2.49
Sand	10.00 ± 1.87
Coralline	9.11 ± 1.89
Rubble	2.44 ± 1.04
Macroalgae	1.85 ± 1.08
Red foliose algae	0.52 ± 0.52
Sponge	0.52 ± 0.25
Fresh dead coral	0.37 ± 0.24
Halimeda	0.22 ± 0.12
Clam	0.15 ± 0.10
Silt	0.07 ± 0.07
Encrusting algae	0.00
Holothurian	0.00

Geochemical differences between healthy and infected skeletons

There was a significant interaction effect in chemical composition between site and healthy and infected colonies ($p < 0.05$, Table 4). On further analysis, it was found that the trace element composition of infected skeletons differed significantly between all sites, which was not represented in healthy skeletons (Table 5). At site level only, there was a significant difference between Kats Kable and both Direction Island Outside, and Anchor Hole with regards to chemical composition (Table 6).

Mean barium, calcium, phosphorous and aluminium concentrations were greater in growth anomaly infected skeletons, whereas magnesium, uranium and potassium were higher in healthy skeletons (Table 7). Potassium, followed by barium, provided the greatest contribution to overall dissimilarity in the chemical composition of healthy and infected colonies (Table 8). Within healthy colonies, potassium and uranium had the highest contribution to similarity between samples (22.34%, 17.89%), whereas strontium and calcium had the greatest contribution to the similarity within infected colonies.

Biological differences between healthy and infected tissue

The density of mesenteries, polyps and zooxanthellae, and presence of oocytes in tissues was significantly affected by health status (p = 0.002, *df*: 2,47, Pseudo-F = 3.966). Infected tissues recorded the least quantities of the biological and reproductive components (Fig. 6); specifically oocytes were only present in one sample, in contrast to both sets of healthy tissues (Fig. 6).

Discussion

Disease outbreaks threaten the integrity, health, and productivity of coral communities across the globe, including at the remote oceanic atoll of CKI. Here, one third of the locally dominant *I. palifera* population is reported to be affected by GAs and is present at of 75% of sites surveyed. The finding of one third of the community affected,

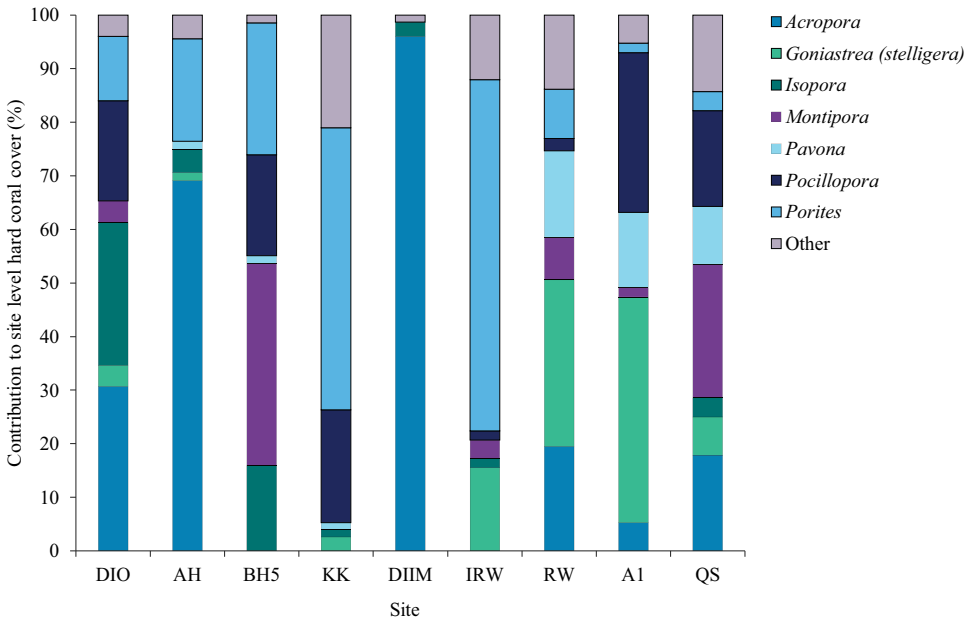

Fig. 3 Contribution of major scleractinian genera (> 5%) to the composition of hard coral cover at each site. Percentage calculated as proportion of total coral cover per site. Coral cover was surveyed by 3 × 25 m point intersect transect method (50 cm). Other includes *Astreopora, Cyphastrea, Diploastrea, Favites, Gardinoseris, Leptastrea, Lobactis, Lobophyllia, Pachyseris* and *Psammocora*. Additional count data are given in Online Resource 1

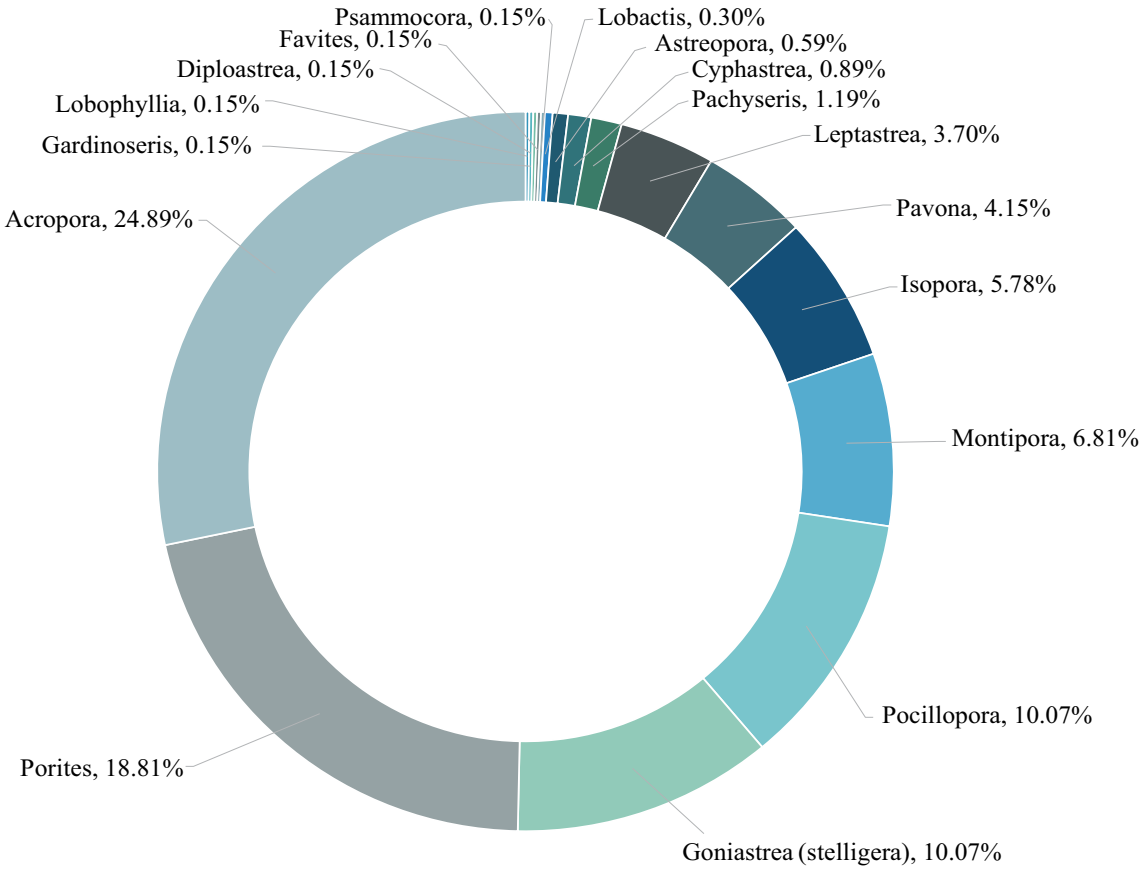

Fig. 4 Coral cover of sites surveyed at Cocos Keeling Islands by genera. Percentage calculated as proportion of hard coral cover, accounting for 44% of total substrate. Coral cover surveyed by 3 × point intersect transect method (50 cm)

exceeds two published outbreak threshold levels; 5% of the population (Willis et al. 2004) and 30 cases per 750 m^2 (Maynard et al. 2011). Furthermore, six sites reported greater than 5% of colonies were affected (Fig. 5). Conversely only two sites recorded greater than 30 cases per 750 m^2. Having established that this location is

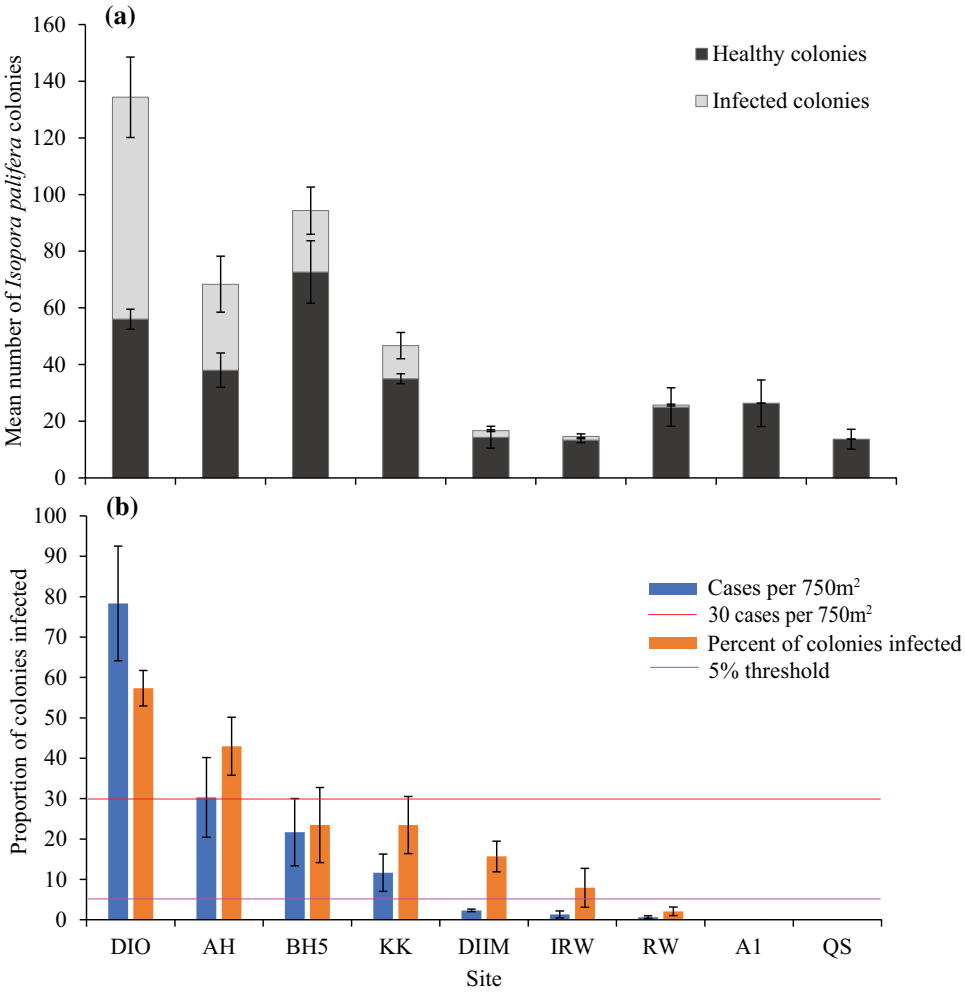

Fig. 5 **a** Number of healthy and infected *I. palifera* colonies per site (mean ± SE), **b** proportion of infected *I. palifera* colonies only, in comparison to published outbreak threshold levels (Dashed line) of either 5% (Willis et al. 2004) or 30 cases per 750 m^2 (Maynard et al. 2011)

Table 4 Two-way PERMANOVA of the difference in trace element composition between healthy and GA infected skeletons *$p < 0.05$ in response to both Health Status and Site

Source	*df*	SS	MS	Pseudo-F	P(perm)	Unique perms
Health Status	1	54.765	54.765	7.5805	0.0001*	9920
Site	2	12.962	12.962	1.7941	0.0297*	9939
Health Status x Site	2	17.267	17.267	2.3901	0.0025*	9913
Res	114	823.59	7.2245			
Total	119	952				

Data were normalised before analysis

experiencing an outbreak, it is important to establish how GAs may influence the growth and future viability of the *I. palifera* population.

Growth anomalies are diagnosed by changes to skeletal growth processes, specifically rapid skeletal extension, low skeletal density and changes to skeletal structures (Gateño et al. 2003; Domart-Coulon et al. 2006). Observation of coral skeletons, whilst not quantified in this study, indicated a loss of structure, and an increase in porosity (Fig. 7), however, geochemical analysis of healthy and infected skeletal subsamples provided further insight into the way the disease affects the skeleton. The investigation of trace element composition between healthy and infected corals revealed changes indicative of a shift in crystal structure from aragonite (healthy samples) to the less dense calcite (infected samples). More specifically, there was elevated barium and lower magnesium concentrations in infected samples suggesting the reprecipitation of calcite. (Webb et al. 2009; Spies and Takabayashi 2013). Furthermore, the reduction in uranium in infected corals may be the result of the recrystallisation process to neogenic (newly-formed) calcite (Littlewood et al. 2017). The

Table 5 Pairwise PERMANOVA investigating interaction effects between Health Status and Site for trace element analysis between healthy and infected skeletons $*p < 0.05$

Groups	*t*	P(perm)	Unique perms
Within level 'Healthy' of factor 'Health Status'			
DIO, AH	0.80886	0.7626	9934
DIO, KK	1.3175	0.078	9934
AH, KK	1.1927	0.1578	9930
Within level 'Infected' of factor 'Health Status'			
DIO, AH	1.7958	0.0069*	9933
DIO, KK	1.548	0.0316*	9937
AH, KK	2.5304	0.0001	9940

Data were normalised prior to analysis

Table 6 Pairwise PERMANOVA investigating the difference in trace element composition between sites only $*p < 0.05$

Groups	*t*	P(perm)	Unique perms
DIO, AH	0.89523	0.5794	9936
DIO, KK	1.4328	0.0345*	9938
AH, KK	1.8121	0.0015*	9932

Data normalised prior to analysis

Table 7 Percentage concentration of key trace elements in either healthy or GA-infected skeleton samples (mean ± SE)

Element	Infected	Healthy
Ca (%)	33.45 ± 0.50	31.81 ± 0.40
Sr (%)	0.66 ± 0.01	0.66 ± 0.01
Mg (ppm)	5535.23 ± 220.22	6298.24 ± 288.63
U (ppm)	169,567.33 ± 21,711.45	176,809.28 ± 48,181.73
K (ppm)	379.75 ± 16.58	543.00 ± 17.36
P (%)	0.15 ± 0.01	10.28 ± 10.16
Ba (ppm)	698.69 ± 19.32	562.56 ± 20.91
Al (ppm)	880.50 ± 26.55	806.01 ± 29.80

$n = 120$ (60 points each)

presence of calcite may be the result of the process of bacterial bioerosion where, within the GAs, the aragonite is being bioeroded by infecting bacteria and mobilised cations reprecipitating as an abiotic marine cement.

The process of bioerosion of carbonate structures and subsequent reprecipitation as alternate crystal forms has been reported in crustose coralline algae (Cox et al. 2017) and in fossil bones as the result of an aquatic biofilm (Pesquero and Fernández-Jalvo 2014), and is an essential part of the marine diagenetic environment(Moore and Wade 2013). Bioerosion in corals by intra-skeletal microborers, including algae, bacteria and fungi has been well documented (Tribollet and Payri 2001; Tribollet and Golubic 2011; Glynn and Manzello 2015) and has been linked to disease epizootics (Gleason et al. 2017). Whilst further analysis by scanning electron microscopy (SEM) is required to investigate the story proposed here, the changes to the skeletal growth processes may be more complex than previously thought and include both abiotic and biotic factors.

Spatial differences in the geochemistry of samples are to be expected as trace elements are accumulated from surrounding seawater, however, unexpectedly, such differences were only apparent in infected skeletons (Table 5). When investigating solely the difference between site, the composition of trace elements in samples collected from Kats Kable were dissimilar to the composition of those collected at other sites. This may be the reflection of the history of the site, which is a historic dump (Mccarthy 2005), and scrap materials may be leaching into the sediment and water; however this requires further information. The finding of a significant difference in trace elements is evident in infected skeletons, coupled with other GA characterisations (Squires 1965; Gateño et al. 2003; Domart-Coulon et al. 2006), suggest that the effect of the disease on skeletal growth processes extends to a molecular level.

The division of energy away from reproductive and autotrophic activities, towards rapid skeletal growth, appears to have occurred at the detriment of the biological and reproductive functioning of the coral. GA-infected tissues presented with a reduction in the density of polyps, mesenterial filaments and zooxanthellae, and an almost complete absence of oocytes. These findings are consistent with the results of GA studies in other scleractinian coral species (Domart-Coulon et al. 2006; Burns and Takabayashi 2011). More importantly, these findings suggest future declines in gamete availability are likely, however should be considered in the context of both the total proportion of colonies contributing to population fecundity (Palmer and Baird 2018), and the proportion of individual colonies impacted, as healthy tissue adjacent to infected tissue appeared to function normally. In this regard, reproductive potential of *I. palifera* at the Cocos (Keeling) Islands could be conservatively diminished by approximately 11%, based on GAs affecting a minimum of one-third of the surface area across one-third of the population. *Isopora* species encompassed, on average 6% of the total hard coral cover hence this outbreak threatens the future accretional potential of the CKI reefs. Furthermore, *I. palifera* is an important habitat forming species providing

Table 8 Results of SIMPER analysis demonstrating contributions of each element analysed to dissimilarity in trace element composition between healthy and infected skeletons

Variable	Group healthy Av. value	Group infected Av. value	Av. Sq. distance	Sq. dist/SD	Contrib. (%)	Cum. (%)
K	0.528	-0.528	2.54	0.82	14.95	14.95
Ba	− 0.401	0.401	2.31	0.76	13.56	28.51
Ca	− 0.228	0.228	2.09	0.70	12.28	40.79
Mg	0.189	− 0.189	2.05	0.49	12.09	52.88
Al	− 0.169	0.169	2.04	0.36	12.00	64.88
P	0.091	− 0.09100	2.00	0.13	11.76	76.65
Sr	0.0381	− 0.0381	1.99	0.77	11.68	88.33
U	0.0126	− 0.0126	1.98	0.16	11.67	100.00

Average squared distance = 17.76

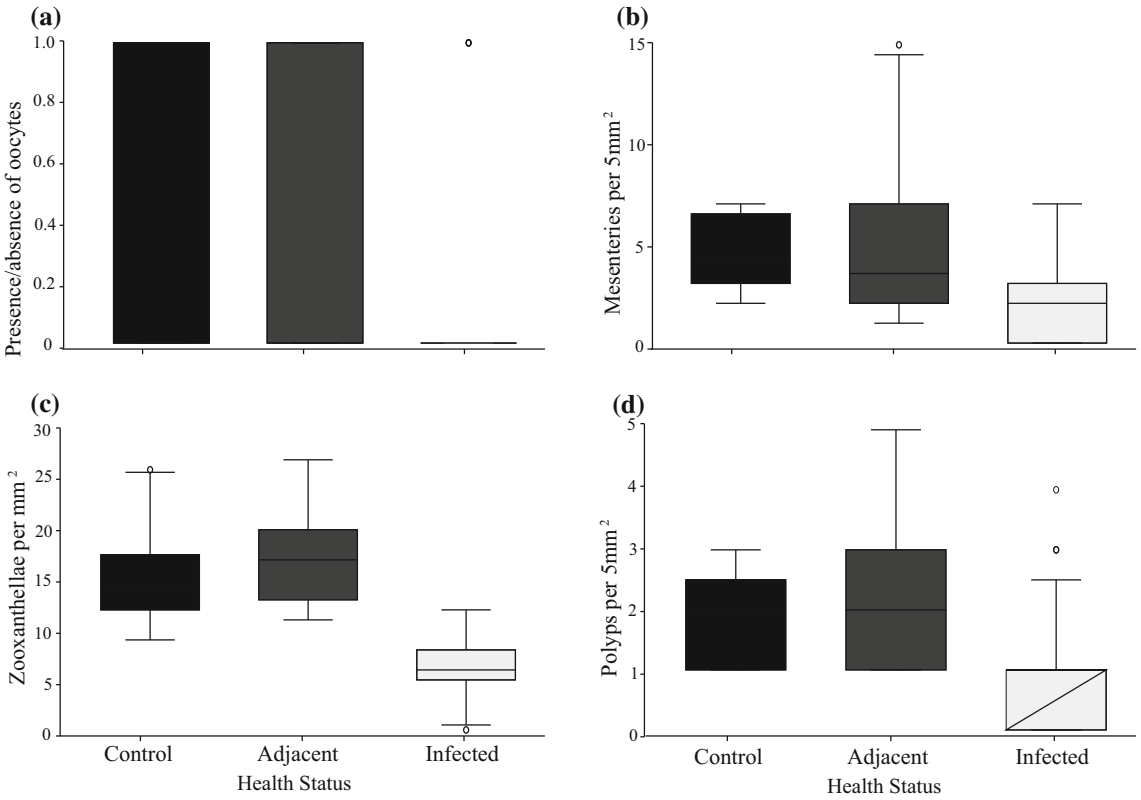

Fig. 6 Comparison of reproductive and biological components of infected and adjacent healthy tissue (n = 36) and control tissues (n = 12). **a** Presence/absence of ova detected, **b** mean number of mesenteries, **c** density of zooxanthellae per 1 mm^2, **d** mean number of polyps

vertical complexity for associated taxa; however fish may also play a significant role in the mobility of GAs.

The outbreak of GAs appeared to be confined to *I. palifera* colonies only, and the mode of transmission of this disease has yet to be established. As seen in other disease studies, fish have been highlighted as possible vectors by carrying pathogens to susceptible hosts (Aeby and Santavy 2006) or clean-up mechanisms through preferential feeding upon diseased tissue (Chong-Seng et al. 2011). In this case of GAs, it may be the latter, as fish scrapes were observed solely upon the seemingly softer lesions, and not the typically dense *I. palifera* skeletons. Whilst not quantified here, investigating the role of fish, in correlation with other transmission vectors may provide insight to the lifecycle of this disease. Previous studies have highlighted host density (Aeby et al. 2011), direct contact (Kaczmarsky and Richardson 2007) and coral cover (Williams et al. 2010) as key factors in this disease prevalence. Whilst this may be

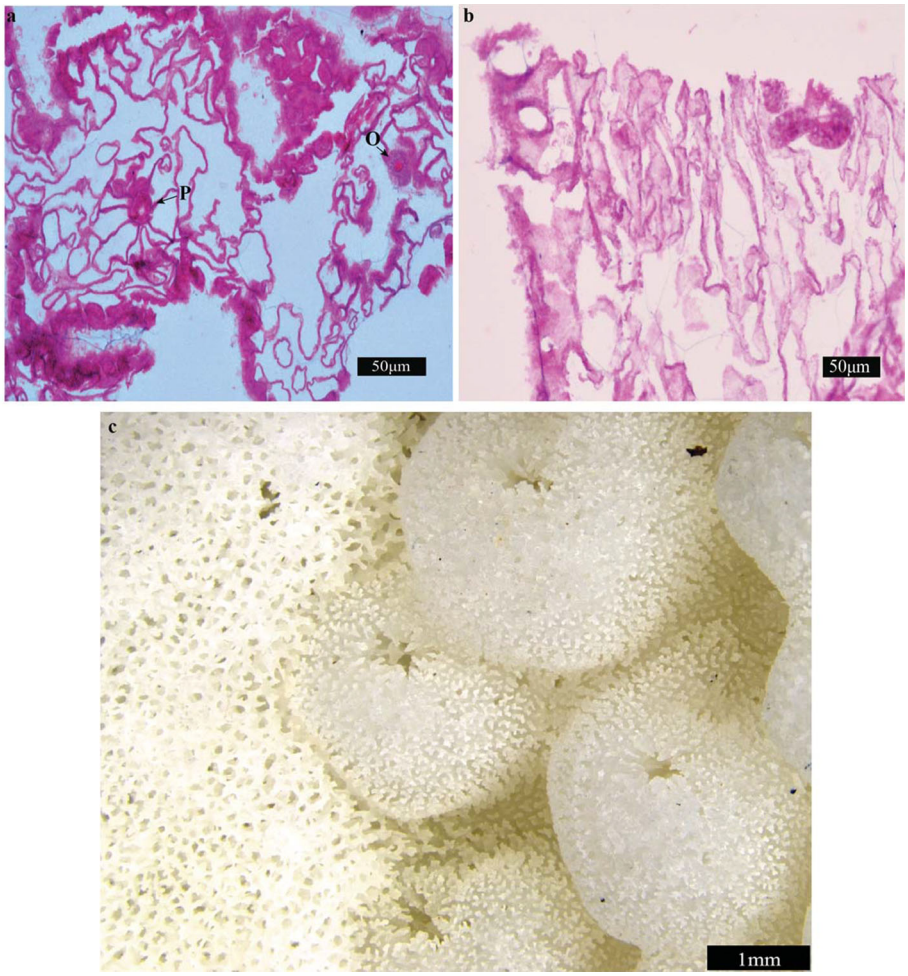

Fig. 7 Microscope images of histological tissue sections and skeletal lesion boundary. **a** healthy tissue samples with polyp (P) and primordial oocyte (O), note structure of gastrodermis and walls, **b** infected tissue sample with mesentery (arrow), and haphazard arrangement of tissues, **c** intersection of growth anomaly (left) and normal skeleton (right), note absence of polyp skeletal development in the infected portion of the skeleton

true for *I. palifera*, the abundance of *Acropora* and *Porites* species, normally disproportionally affected by GAs, was much greater than that of *I. palifera* (> 18% each) and was observed to be in direct contact with infected *I. palifera* colonies. Conflicting studies have discussed the role of direct contact in GA transmission and, by extension, the whether the disease is contagious (Kaczmarsky and Richardson 2007; Irikawa et al. 2011). The results of this study call in to question whether GAs are indiscriminatory or display species specific modes of transmission. Previously, research has focussed on describing the effects of GAs in species specific cases (Domart-Coulon et al. 2006; Work et al. 2008; Burns and Takabayashi 2011), and transmission only briefly discussed in the few long-term monitoring case studies (Irikawa et al. 2011).

The GA diagnosis provides sufficient detail to accurate identify the disease in situ, and understand the effects on the host; however the causal mechanism of this disease are yet to be established. GAs have been linked to a variety of anthropogenic and environmental factors (Aeby et al. 2011; Work et al. 2015), and increasingly the bacterial communities within the tissues have been highlighted as possible refuges for pathogenic bacteria that may be implicated in the etiology of GAs (Chiu et al. 2012; Ng et al. 2015; Rajasabapathy et al. 2020). Similar coral disease studies are reporting a variety of microbial causal agents, such as bacteria in *Montipora* white syndrome (Ushijima et al. 2014; Beurmann et al. 2017), terrestrial fungi linked with aspergillosis of sea fans (Kim and Harvell 2002) and cyanobacterial mats of black band disease (Richardson 2004). It is likely that an outbreak of GAs is the result of a combination of environmental, anthropogenic, and microbial factors, and the complexity of such interactions hinders the management of such a critical disease. Many questions still surround GAs, not just regarding etiology, but long-term progression of the disease, inter-species transmission, and the role of corallivorous fish.

Acknowledgements This project was funded by ARC Linkage Project (LP160101508). Coral samples were collected under Fisheries Exemption No. 3080". For fieldwork we thank Meg Weir and Deiter Gerhard from CocosDive and Taj Powell. We thank Dr. Merinda Nash and Dr. Jens Zinke for their guidance on geochemical analysis and interpretation. For advice and edits we give thanks to Dr. Kar-Chun Tan, Dr. Nicola Browne, Dr. Claus Christopherson, Dr.

Stephen Newman, and the Australian Coral Reef Society Student Writing Retreat, in particular Natalia Andrade Rodriguez. Finally, we acknowledge the valuable assistance from the TrEnD Laboratory, especially Dr. Katrina West and the Coral CORE group.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Aeby GS (2006) Baseline levels of coral disease in the Northwestern Hawaiian Islands. Atoll Res Bull

Aeby GS, Santavy DL (2006) Factors affecting susceptibility of the coral Montastraea faveolata to black-band disease. Mar Ecol Prog Ser 318:103–110

Aeby GS, Williams GJ, Franklin EC, Haapkyla J, Harvell C, Neale S, Page CA, Raymundo L, Vargas-Ángel B, Willis BL, Work TM, Davy SK (2011) Growth anomalies on the coral genera Acropora and Porites are strongly associated with host density and human population size across the Indo-Pacific. PLoS ONE 6:e16887

Andersson ER, Stewart JA, Work TM, Woodley CM, Schock TB, Day RD (2020) Morphological, elemental, and boron isotopic insights into pathophysiology of diseased coral growth anomalies. Sci Rep 10:8252

Arenas-Islas D, Huerta-Diaz MA, Norzagaray-López CO, Mejia-Piña KG, Valdivieso-Ojeda JA, Otero XL, Arcega-Cabrera F (2019) Calibration of portable X-ray fluorescence equipment for the geochemical analysis of carbonate matrices. Sediment Geol 391:105517

Beurmann S, Ushijima B, Videau P, Svoboda CM, Smith AM, Rivers OS, Aeby GS, Callahan SM (2017) Pseudoalteromonas piratica strain OCN003 is a coral pathogen that causes a switch from chronic to acute Montipora white syndrome in Montipora capitata. PLoS ONE 12:e0188319

Bruckner AW (2015) White syndromes of western Atlantic reef-building corals Diseases of Coral. Wiley, Hoboken, pp 316–332

Burns JHR, Takabayashi M (2011) Histopathology of growth anomaly affecting the coral, Montipora capitata: implications on biological functions and population viability. PLoS ONE 6:e28854

Bythell J, Pantos O, Richardson LL (2004) White plague, white band, and other "white" diseases Coral health and disease. Springer, Berlin, pp 351–365

Chiu JMY, Li S, Li A, Po B, Zhang R, Shin PKS, Qiu J-W (2012) Bacteria associated with skeletal tissue growth anomalies in the coral Platygyra carnosus. FEMS Microbiol Ecol 79:380–391

Chong-Seng KM, Cole AJ, Pratchett MS, Willis BL (2011) Selective feeding by coral reef fishes on coral lesions associated with brown band and black band disease. Coral Reefs 30:473–481

Cox TE, Nash M, Gazeau F, Déniel M, Legrand E, Alliouane S, Mahacek P, Le Fur A, Gattuso JP, Martin S (2017) Effects of in situ CO2 enrichment on Posidonia oceanica epiphytic community composition and mineralogy. Mar Biol 164:103

Domart-Coulon IJ, Traylor-Knowles N, Peters E, Elbert D, Downs CA, Price K, Stubbs J, McLaughlin S, Cox E, Aeby GS, Brown PR, Ostrander GK (2006) Comprehensive characterization of skeletal tissue growth anomalies of the finger coral Porites compressa. Coral Reefs 25:531–543

Gateño D, León A, Barki Y, Cortés J, Rinkevich B (2003) Skeletal tumor formations in the massive coral Pavona clavus. Mar Ecol Prog Ser 258:97–108

Gilmour JP, Cook KL, Ryan NM, Puotinen ML, Green RH, Shedrawi G, Hobbs J-PA, Thomson DP, Babcock RC, Buckee J, Foster T, Richards ZT, Wilson SK, Barnes PB, Coutts TB, Radford BT, Piggott CH, Depczynski M, Evans SN, Schoepf V, Evans RD, Halford AR, Nutt CD, Bancroft KP, Heyward AJ, Oades D (2019) The state of Western Australia's coral reefs. Coral Reefs. https://doi.org/10.1007/s00338-019-01795-8

Gleason FH, Gadd GM, Pitt JI, Larkum AWD (2017) The roles of endolithic fungi in bioerosion and disease in marine ecosystems I General concepts. Mycology 8:205–215

Glynn PW, Manzello DP (2015) Bioerosion and coral reef growth: a dynamic balance Coral Reefs in the Anthropocene. Springer, Netherlands, pp 67–97

Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DJ, Hofmann EE, Lipp EK, Osterhaus ADME, Overstreet RM, Porter JW, Smith GW, Vasta GR (1999) Emerging marine diseases - climate links and anthropogenic factors. Science 285:1505–1510

Hobbs J-PA, Allen GR (2014) Hybridisation among coral reef fishes at Christmas Island and the Cocos (Keeling) Islands. Raffles Bull Zool 30:220–226

Hobbs J-PA, Frisch AJ, Allen GR, Van Herwerden L (2009) Marine hybrid hotspot at Indo-Pacific biogeographic border. Biol Lett 5:258–261

Hoeksema BW, Cairns S (2020) Madrepora kauaiensis Vaughan, 1907. World List Scleractinia

Irikawa A, Casareto BE, Suzuki Y, Agostini S, Hidaka M, van Woesik R (2011) Growth anomalies on Acropora cytherea corals. Mar Pollut Bull 62:1702–1707

Kaczmarsky L, Richardson LL (2007) Transmission of growth anomalies between Indo-Pacific Porites corals. J Invertebr Pathol 94:218–221

Kim K, Harvell C (2002) Aspergillosis of sea fan corals: disease dynamics in the Florida Keys. In: Porter J, Porter K (eds) The Everglades, Florida Bay, and coral reefs of the Florida Keys: an ecosystem sourcebook. CRC Press, Boca Raton, pp 813–824

Lavers JL, Dicks L, Dicks MR, Finger A (2019) Significant plastic accumulation on the Cocos (Keeling) Islands Australia. Sci Rep 9:7102

Liao S, Tao C, Li H, Zhang G, Liang J, Yang W (2017) Use of portable X-ray fluorescence in the analysis of surficial sediments in the exploration of hydrothermal vents on the southwest Indian Ridge. Acta Oceanol Sin 36:66–76

Littlewood JL, Shaw S, Peacock CL, Bots P, Trivedi D, Burke IT (2017) Mechanism of enhanced strontium uptake into calcite via an amorphous calcium carbonate crystallization pathway. Cryst Growth Des 17:1214–1223

Maynard JA, Anthony KRN, Harvell C, Burgman MA, Beeden R, Sweatman H, Heron SF, Lamb JB, Willis BL (2011) Predicting outbreaks of a climate-driven coral disease in the Great Barrier Reef. Coral Reefs 30:485–495

Mccarthy M (2005) Gerhard cannon site (Direction Island, Cocos Keeling Islands) inspection report.

Moore CH, Wade WJ (2013) Marine diagenetic environment. Developments in sedimentology. Elsevier, Amsterdam, pp 93–131

Ng JCY, Chan Y, Tun HM, Leung FCC, Shin PKS, Chiu JMY (2015) Pyrosequencing of the bacteria associated with Platygyra carnosus corals with skeletal growth anomalies reveals differences in bacterial community composition in apparently healthy and diseased tissues. Front Microbiol 6:1142

Palmer C, Baird A (2018) Coral tumor-like growth anomalies induce an immune response and reduce fecundity. Dis Aquat Organ 130:77–81

Pesquero MD, Fernández-Jalvo Y (2014) Bioapatite to calcite, an unusual transformation seen in fossil bones affected by aquatic bioerosion. Lethaia 47:533–546

Peters E, Halas JC, McCarty HB (1986) Calicoblastic neoplasms in Acropora palmata, with a review of reports on anomalies of growth and form in corals. J Natl Cancer Inst 76:895–912

Peters E, Price KL, Horowitz DJB, Price KL, Horowitz DJB (2005) Histological preparation of invertebrates for evaluating contaminant effects. CRC Press

Pollock FJ, Morris PJ, Willis BL, Bourne D (2011) The urgent need for robust coral disease diagnostics. PLoS Pathog 7:e1002183

Rajasabapathy R, Ramasamy KP, Manikandan B, Mohandass C, Arthur James R (2020) Bacterial communities associated with healthy and diseased (skeletal growth anomaly) reef coral Acropora cytherea from Palk Bay. India Front Mar Sci 7:92

Raymundo L, Couch C, Harvell C (2008a) Coral disease handbook guidelines for assessment, monitoring & management.

Raymundo L, Work TM, Bruckner AW, Willis BL (2008b) A decision tree for describing coral lesions in the field. In: Raymundo L., Couch C.S., Harvell C.D. (eds) Coral diseases handbook: guidelines for assessment, monitoring and management. Coral reef targeted research and capacity building for management program, St Lucia, QLD, Australia, pp 17–32

Richards ZT, Newman SJ (2019) Outbreak of growth anomalies in Isopora palifera at Cocos (Keeling) Islands. Mar Biodivers 49:1071

Richardson L (2004) Black band disease. Coral health and disease. Springer, Berlin, pp 325–336

Sato Y, Bourne D, Willis BL (2009) Dynamics of seasonal outbreaks of black band disease in an assemblage of Montipora species at Pelorus Island (Great Barrier Reef, Australia). Proc R Soc B Biol Sci 276:2795–2803

Schatzlein D (2015) XRF technology for non-scientists.

Spies N, Takabayashi M (2013) Expression of galaxin and oncogene homologs in growth anomaly in the coral Montipora capitata. Dis Aquat Organ 104:249–256

Squires DF (1965) Neoplasia in a coral? Science 148:503–505

Tribollet A, Golubic S (2011) Reef bioerosion: agents and processes. Coral reefs: an ecosystem in transition. Springer, Netherlands, pp 435–449

Tribollet A, Payri C (2001) Bioerosion of the coralline alga Hydrolithon onkodes by microborers in the coral reefs of Moorea, French Polynesia. Oceanol Acta 24:329–342

Ushijima B, Videau P, Burger AH, Shore-Maggio A, Runyon CM, Sudek M, Aeby GS, Callahan SM (2014) Vibrio coralliilyticus strain OCN008 is an etiological agent of acute Montipora white syndrome. Appl Environ Microbiol 80:2102–2109

Webb GE, Nothdurft LD, Kamber BS, Kloprogge JT, Zhao JX (2009) Rare earth element geochemistry of scleractinian coral skeleton during meteoric diagenesis: a sequence through neomorphism of aragonite to calcite. Sedimentology 56:1433–1463

Weil E (2004) Coral reef diseases in the wider Caribbean Coral health and disease. Springer, Berlin, pp 35–68

Williams GJ, Aeby GS, Cowie ROM, Davy SK (2010) Predictive modeling of coral disease distribution within a reef system. PLoS ONE 5:e9264

Williams GJ, Work TM, Aeby GS, Knapp IS, Davy SK (2011) Gross and microscopic morphology of lesions in Cnidaria from Palmyra Atoll, Central Pacific. J Invertebr Pathol 106:165–173

Willis BL, Page CA, Dinsdale EA (2004) Coral disease on the Great Barrier Reef Coral health and disease. Springer, Berlin, pp 69–104

Work TM, Aeby GS, Coles S (2008) Distribution and morphology of growth anomalies in Acropora from the Indo-Pacific. Dis Aquat Organ 78:255–264

Work TM, Kaczmarsky L, Peters E (2015) Skeletal growth anomalies in corals. In: Woodley CM, Downs C, Bruckner AW, Porter JW, Galloway SB (eds) Diseases of coral. Wiley, Hobokin, pp 291–299

Zhang SJ, Mu H, Lun JCY, Qiu J-W (2017) Molecular pathology of skeletal growth anomalies in the brain coral Platygyra carnosa: A meta-transcriptomic analysis. Mar Pollut Bull 124:660–667