Coral Symbioses Under Stress:
Spatial and Temporal Dynamics of Coral-*Symbiodinium* Interactions

by

Danielle C. Claar
B.Sc., University of Hawaii at Hilo, 2012

A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

University of Victoria

Supervisory Committee

Coral Symbioses Under Stress:
The Spatial and Temporal Dynamics of Coral-*Symbiodinium* Interactions

by

Danielle C. Claar
B.Sc., University of Hawaii at Hilo, 2012

Supervisory Committee

Dr. Julia K. Baum (Department of Biology)
Supervisor

Dr. Steve J. Perlman (Department of Biology)
Departmental Member

Dr. Ryan Gawryluk (Department of Biology)
Departmental Member

Dr. Brian Starzomski (School of Environmental Studies)
Outside Member

Abstract

Supervisory Committee
Dr. Julia K. Baum (Department of Biology)
Supervisor
Dr. Steve J. Perlman (Department of Biology)
Departmental Member
Dr. Ryan Gawryluk (Department of Biology)
Departmental Member
Dr. Brian Starzomski (School of Environmental Studies)
Outside Member

Coral reefs, the planet's most diverse marine ecosystems, are threatened globally by climate change and locally by overfishing and pollution. The dynamic partnership between coral and their endosymbiotic algae (*Symbiodinium*) is the foundation of all tropical reef ecosystems. *Symbiodinium* provide coral with nutrients for growth, but stress can break down this symbiosis, causing coral bleaching. There are also life-history trade-offs amongst *Symbiodinium* types - some provide coral with more nutrition, while others are better able to cope with environmental stressors. Although these symbioses are believed to be a critical element of reef resilience, little is known about how local and global stressors alter these partnerships. In this thesis, I combine synthetic literature reviews and a meta-analysis, with field research, molecular analyses, bioinformatics, and statistical analyses to investigate environmentally-driven mechanisms of change in coral-symbiont interactions with the aim of advancing understanding of how corals will adapt to the stressors they now face.

First, I conducted a review of coral-*Symbiodinium* interactions, from molecules to ecosystems and summarized the current state of the field and knowledge gaps. Next, I conducted a meta-analysis of coral bleaching and mortality during El Niño events and created an open-source coral heat stress data product. I found that the 2015-2016 El Niño instigated unprecedented thermal stress on reefs globally, and that, across all El Niño events, coral bleaching and mortality were greater at locations with higher long-term mean temperatures. I provided recommendations for future bleaching surveys, and in a related perspectives piece, highlighted the importance of survey timing during prolonged coral bleaching events.

The latter three empirical chapters are based on my six field expeditions to Kiritimati (Christmas Island). Taking advantage of the atoll's natural ecosystem-scale experiment, I tagged, sampled and tracked over 1,000 corals across its chronic human disturbance gradient. Since corals can uptake *Symbiodinium* from the surrounding environment, I first investigated the effect of local disturbance and winter storm waves on *Symbiodinium* communities in coral, sediment, and seawater. Greater variability in *Symbiodinium* communities at highly disturbed sites suggests that local disturbance destabilizes symbiont community structure. Since local disturbance influences *Symbiodinium* community structure and coral-associated microbial communities, I next examined the covariance of coral-associated *Symbiodinium* and microbial communities for six coral species across Kiritimati's disturbance gradient.

Most strikingly, I found corals on Kiritimati that recovered from globally unprecedented thermal stress, experienced during the 2015-2016 El Niño, while they were still at elevated temperatures. This is notable, because no coral has previously been documented to recover from bleaching while still under heat stress. Only corals protected from local stressors exhibited this capacity. Protected corals had distinct pre-bleaching algal symbiont communities and recovered with different algal symbionts, suggesting that *Symbiodinium* are the mechanism of resilience and that protection governs their communities.

Together, this research provides novel evidence that local protection may be more important for coral resilience than previously thought, and that variability in symbiotic and microbial communities provides a potentially flexible mechanism for corals to respond to both local and global stressors.

Table of Contents

List of Tables

List of Figures

Acknowledgments

Although the Introduction and Conclusion are written in first person, all research within this dissertation has been conducted with and reviewed by an excellent group of collaborators, from whom I have learned much, and to whom I am very grateful. First, I would like to express my sincere gratitude to my advisor Julia Baum for accepting me as her Ph.D. student, and for helping me grow both scientifically and personally over the past five years. Julia has provided me with countless opportunities and always encouraged me to reach my full scientific potential. She taught me how to prepare for and conduct remote fieldwork, and I've learned much from her concise, compelling scientific writing style. I count myself lucky to have been her Ph.D. student.

I have been privileged to collaborate with many exceptional scientists during my Ph.D. research. Ruth Gates provided a welcoming lab space for me to learn molecular ecology techniques and hone my coral knowledge, as well as stimulating conversations and encouragement to think about the bigger picture. Ruth was a member of my committee for nearly all of my PhD, until her passing in late 2018. She was a beacon of hope for coral reefs, and she is dearly missed. Brian Starzomski's ecology course helped me develop a solid scientific foundation for my research; discussions with Brian have encouraged me to think deeply about ecological processes and mechanisms. Steve Perlman invited me to symbiosis discussions in his lab and helped me think more broadly about symbiosis in other systems. Ryan Gawryluk graciously joined my committee shortly before my defense and provided valuable input for the final draft of this dissertation. Andrew Baker provided valuable insight into coral bleaching and resilience and welcomed me into his lab to collaborate and learn qPCR. Hollie Putnam was an early influence on how I thought about symbiotic flexibility and bioinformatics, and she has advised me on many aspects of science and collaboration. I am especially grateful to her for the time we spent together at the Hawai`i Institute of Biology this past year. Ross Cunning has developed lab protocols and bioinformatic tools that I used throughout my thesis, and he has consistently provided valuable discussions about ecology and coral symbiosis. I particularly appreciate his hospitality when I visited Miami last year. Becky

Vega Thurber and Melissa Garren provided helpful in-depth comments on chapter 6 of this thesis. I have been fortunate to work with these amazing individuals, and I look forward to many more collaborations in years to come.

I would like to acknowledge the help of all field biologists, local collaborators, and lab scientists who made this work possible. Firstly, the Kiritimati Island Field Team from 2013-2016: Maryann Watson, James Robinson, Scott Clark, Torbyn Bridges, Jonatha Giddens, James Mortimer, John Burns, Jamie McDevitt-Irwin, Sean McNally, Sarah Friesen, Kristina Tietjen, Julia Baum, Lisa Szostek, and Kieran Cox. Your creativity under pressure solved many problems, and your careful science, hard work, and good humor allowed us to gather more data than I could have imagined before the start of my graduate work. I would like to express thanks to Kim Cobb and Pamela Grothe, who we worked with multiple times during our Kiritimati field work. I am grateful to our local Kiribati collaborators, who provided information, translation, supplies, and assistance, especially Aana T. Berenti and Ratita Bebe from MELAD (Ministry of Environment, Lands and Agricultural Development), Kiaueta Teboko Tarau and Taratau Kirata from the Ministry of Fisheries & Marine Resource Development, Puta Tofinga, Anami Tiouniti, Jacob and Lavinia Teem, Neera, Oldman, and Alfred Smith. I would also like to thank the hard-working and meticulous lab scientists who processed and sequenced my samples. Amy Eggers was a joy to work with and was immensely helpful in accurate processing and making protocol decisions throughout. Thanks to Clay Clark at UC Riverside, who helped ensure that my last sample set was quickly and accurately sequenced. Thanks also to the hard-working Baum Lab coral ID team, with a special thanks to Jessie Lund and Lisa Szostek for spending many hours learning and identifying corals from Kiritimati. I am particularly grateful to Jen Davidson for making my research in Hawaii possible, always coming through in a pinch, and for helping me take a day at the beach when I needed it most. I also appreciate the helpful comments of anonymous reviewers on our manuscripts that are currently published or in review.

I appreciate the colleagues and fellow students who have worked with and alongside me during my graduate studies. Kristina Tietjen has been a wonderful dive buddy, trusted confidante, and scientific collaborator. Without her, I would have never found my tagged corals, and I would have spent a lot more time wandering around lost.

Jamie McDevitt-Irwin provided meta-analysis and microbe expertise and has always been there for me with a hug and a listening ear. Thanks to all the past and present Baum Lab members who have shared ideas, skills, knowledge, and fun lab retreats. Thanks also to the Gates Lab for making my stay at HIMB enjoyable and productive. Thanks to my open science inspirations, James Robinson, Ross Cunning, and Easton White.

I am appreciative of all the individuals and funding agencies that have supported my Ph.D. research financially. The support of multiple UVic Graduate Scholarships/Fellowships and the Vanier Canada Scholarship allowed me to focus on my research throughout my Ph.D. I would also like to acknowledge funding from the Women Divers Hall of Fame, the American Academy of Underwater Sciences, UVic Centre for Asia-Pacific Initiatives, National Geographic, the National Science Foundation, the International Society for Reef Studies, the Hawai`i Community Foundation, and equipment grants from Sea-Bird Scientific and Divers Alert Network.

I have had an incredible support system which has helped me through the ups and downs of my Ph.D. research. I appreciate my colleagues and collaborators who have become close friends. I would like to thank my husband, Julian, who has been an incredible support for both my science and my wellbeing throughout my Ph.D. Being with you makes me smarter and happier, and I appreciate your unwavering support and everything you’ve done to help me succeed. Thanks to our labradoodle, Clünas, who has helped me press the “submit” button more than once. I am also extremely grateful for my friends Alanna Sutton, Kristin and Joe Day, and Ariel Webster for adventures and support, and for helping me keep things in perspective. Finally, I would like to thank my parents, David and Marie Claar, for introducing me to the ocean and to science at a young age, for giving me endless opportunities to learn and grow, and for always being there for me when things got tough. I couldn’t have done this without you.

Dedication

To my parents: who raised me in the country, and shared with me the wonder of the sea.

Chapter 1
Introduction

Environmental change shapes ecological interactions and evolution at all scales (Levin 1992, Walther et al. 2002, Tylianakis et al. 2008, Gilman et al. 2010). Changes in the environment can act as macroevolutionary drivers of diversity dynamics, shaping ecosystem structure and altering the assemblage and distribution of species globally (Ezard et al. 2011, Condamine et al. 2013). The interaction between environmental stress and ecological communities is a persistent feature in biology (Menge & Olson 1990), but recently, human influences have introduced novel environmental changes (Williams & Jackson 2007), and accelerated processes such as climatic warming (Halpern et al. 2015). Since humans have instigated cascading effects on ecosystems at a global scale (e.g. Harley et al. 2006, Baum & Worm 2009, Post et al. 2009), it is important to understand the mechanisms of how these changes impact vital ecosystems.

Environmental stressors often act in concert to shape ecosystem processes (Vinebrooke et al. 2004, Crain et al. 2008). A stressor is defined as: “any natural or anthropogenic pressure that causes a quantifiable change, whether positive or negative, in biological response” (Côté et al. 2016). Ecosystems can be simultaneously or sequentially impacted by different types of stressors, including physical stress (e.g. storms, natural warming cycles), biological stress (e.g. disease, competition), and human disturbance (e.g. overharvesting, pollution, climate-change amplified warming) (Menge & Sutherland 1987, Hughes & Connell 1999). Environmental stressors can alter ecosystem structure at a large scale, by filtering species assemblages and reorganizing communities (Belyea & Lancaster 1999, Lebrija-Trejos et al. 2010). Multiple stressors can act to not only shape spatial distributions of species, but can act antagonistically, additively, or synergistically to change ecological communities (Brook et al. 2008, Ban et al. 2014, Côté et al. 2016). Furthermore, when a community is exposed to multiple, compounded stressors, 'ecological surprises' can occur, whereby ecosystem structure fundamentally changes in an unexpected way (Paine et al. 1998).

Environmental stressors can affect the inherent resilience capacity in a system. Here, I define resilience following Holling (1973) as the capacity of a system to undergo both external and internal stressors while still maintaining structure, function, and feedbacks (Holling 1973, Graham et al. 2013). Resilience can be divided into two parts: resistance and recovery. Resistance is the ability of an organism or system to withstand environmental stressors while retaining vital functions, whereas recovery is the ability of an organism or system to regain these functions after they have been altered by stress or temporarily lost (Hodgson et al. 2015). Multiple stressors typically degrade resilience capacity (Nyström et al. 2000, Hughes et al. 2003, 2005, 2010). For example, in moss patch landscapes, disturbance and habitat loss together can cause a significant loss of ecosystem resilience that exceeds both individual and additive effects (Starzomski & Srivastava 2007). However, in some cases, one stressor may bolster resilience to another by selecting for communities that are co-tolerant to more than one type of stressor (Hughes & Connell 1999, Darling et al. 2010). This is important because some human-caused environmental stressors are easier to address and manage than others. Consequently, understanding the relative importance of, and interactions between, different stressors can inform management priorities and targeted conservation strategies.

Stress can change fundamental ecological processes, such as competition and predation (Menge & Sutherland 1987), and recent research has also focused on the mechanisms underlying how stress influences mutualisms and symbioses, and how these interactions shape ecosystems (Bruno et al. 2003). For example, in forest ecosystems, soil heating during fire can simplify the community structure of ectomycorrhizal fungi on pine (*Pinus muricata*) seedlings by decreasing fungal diversity and increasing the abundance of a select number of fungal taxa, which may help to maintain diversity in fungal communities (Peay et al. 2009). In tidal ecosystems, drought caused by climate extremes can trigger the breakdown of a facultative mutualism between seagrass and burrowing clams, accelerating ecosystem degradation (de Fouw et al. 2016). In insects, heat stress can influence defensive symbioses (Corbin et al. 2017, Vorburger & Perlman 2018), for example, decreasing aphid resistance to parasitoid wasps by influencing the secondary bacterial symbiosis (Bensadia et al. 2006). These cases exemplify progress made to understand how stress influences these interactions, but many questions remain regarding the effect of stress on symbiotic interactions.

This dissertation focuses on the broad themes of environmental stress, ecological communities, and resilience in the context of coral symbioses. Symbioses are vital to reef-building corals and are foundational to coral reef ecosystems (van Oppen & Gates 2006), which are highly diverse and exceptionally threatened. A suite of stressors, including fishing, pollution, and disease threaten the resilience of the world’s coral reefs (Hughes et al. 2003, Wiedenmann et al. 2012, Vega Thurber et al. 2014). Coral reefs are also increasingly threatened by stressors associated with climate change, namely bleaching and ocean acidification, which can disintegrate the coral reef itself and disrupt critical symbiotic relationships (Hoegh-Guldberg et al. 2007, Pandolfi et al. 2011). Moreover, the tight coupling between ecosystem health and human welfare in developing countries (Costanza et al. 1997, Barnett & Adger 2003, Bellwood et al. 2004), where reefs form the basis of many economies, heightens the need to understand what ecological properties confer resilience to these ecosystems.

Corals live in symbiosis with single-celled photosynthetic dinoflagellates (*Symbiodinium*, previously zooxanthellae) that reside inside the coral tissue (Muscatine & Cernichiari 1969). There are several types of *Symbiodinium* that provide coral with nutrients necessary for growth; and the abundance of each type can change over time (Rowan 1995, Baker 2003). There are trade-offs for coral to hosting different types of *Symbiodinium* – some provide the coral with a greater proportion of their metabolic products but have lower physiological tolerances, while others are more ‘selfish’ with their metabolic products, but better able to cope with stressors (e.g. increased water temperatures) (Sachs & Wilcox 2006, Stat & Gates 2011). Thus, although these relationships have developed over evolutionary time (~160 million years, LaJeunesse et al. 2018), the resilience of the coral symbiome is constantly shaped by dynamic coral-symbiont interactions (Stat et al. 2006). Despite the foundational nature of coral-algal symbiosis to reefs worldwide, little is known about how combined local and global stressors alter the resilience of this partnership (but see Wooldridge & Done 2009, Wooldridge 2009, Wiedenmann et al. 2012a). Elucidating the mechanisms underlying changes in coral-symbiont interactions is essential to understanding the ability of the coral symbiome to adapt to the multiple stressors they now face.

The first three chapters of this dissertation focus on coral symbioses and bleaching at a global scale. In Chapter 2, my co-authors and I review the current state of knowledge for coral-algal symbioses, focusing on the history of the field as well as emerging methods to address symbiotic complexity (Claar et al. 2017). Despite the rapid advances made in this field during recent years, I outline some major remaining knowledge gaps regarding coral-*Symbiodinium* community dynamics. Since the publication of this chapter, a new manuscript has been published with a comprehensive taxonomy that divides *Symbiodinium* into several genera in the family Symbiodiniaceae (LaJeunesse et al. 2018), reflecting ongoing scientific development in this field. Next, in Chapter 3, I evaluate the global consequences of pulse warming during El Niño in the context of local thermal regimes (Claar et al. 2018). To do this, my co-authors and I conducted a re-analysis of satellite sea surface temperature (SST) to quantify coral heat stress and local SST climatology. We demonstrate that the 2015/2016 El Niño, paired with anthropogenic warming, instigated wide-spread heat stress across the Pacific Ocean. This warming was associated with the 3rd documented global coral bleaching event (Eakin et al. 2016), altering coral reef ecosystem structure across multiple regions. Additionally, by conducting a meta-analysis of published El Niño and coral research, I show that, as expected, El Niño warming increases coral bleaching and coral mortality, and that locations with warmer long-term mean temperatures may be more susceptible to El Niño warming. I also note that significant gaps remain regarding El Niño warming impacts on coral reefs due to inconsistent data collection and reporting. Therefore, I provide general guidelines for the publication of coral bleaching studies that I hope will be utilized in future studies, including those from the 2015/2016 El Niño event. Next, in

Chapter 4, I expand our consideration of one particular aspect of coral bleaching studies: the timing of field surveys (Claar & Baum 2018). I provide a theoretical framework and an example from the Central Pacific Ocean to argue that, as warming events increase in duration and severity, survey timing becomes more important due to variability in coral species' bleaching trajectories. For an example, during a long warming event, early surveys may miss bleaching that occurs later in the event, while late surveys may miss initial mortality rates for sensitive species. I suggest multiple field surveys where possible, but minimally recommend that survey timing, in the context of the complete warming event, be recorded and considered in the interpretation and analysis of survey results.

After evaluating the progress and challenges of current research on coral symbiosis, bleaching, and survival at a global scale, the latter three chapters focus in on the effects of local human disturbance on coral symbiosis dynamics, building on field research I conducted on Kiritimati atoll (Christmas Island). Situated in the remote equatorial Pacific Ocean (01°52'N 157°24'W), Kiritimati is one of the northern Line Islands. The atoll is part of the Republic of Kiribati, an island-nation identified as having high reef dependence, high exposure to impending anthropogenic threats, and low capacity to adapt to these threats (Burke et al. 2011). Kiritimati's distinctive features make it highly suitable as a focal system to disentangle the mechanisms that underlie reef resilience in the face of local and global stressors. Kiritimati is unique because there is significant spatial variability in the level of local anthropogenic stressors impacting the reef, creating one of the most extreme disturbance gradients in the world over a very small spatial scale (Walsh 2011, Watson et al. 2016). Coral reef ecosystems on Kiritimati are faced with spatially heterogeneous local stressors: reefs on the Northwestern face of the lagoon are subject to localized human disturbances including poor water quality, terrestrial runoff and overfishing (Sandin et al. 2008), while reefs in Bay of Wrecks, Vaskess Bay, and most of the North Coast were nearly pristine (Walsh 2011, Watson et al. 2016). In addition to a gradient of local stressors, Kiritimati was at the epicenter of the major 2015/2016 El Niño event (Eakin et al. 2016). During this event, Kiritimati experienced ten months of continuous heat stress, exceeding current records of cumulative heat stress on any modern coral reef. The distribution of study sites on Kiritimati across the gradient of human impact provided a natural experiment that allowed us to test the extent to which local human disturbance affects the stability of coral symbioses in the context of acute warming.

In Chapter 5, I quantified changes in *Symbiodinium* communities in three ecological compartments (i.e. coral, water, and sediment) before and after a significant storm swell event at two different levels of local human disturbance (Claar et al. *in review*). I show that increased levels of human disturbance change symbiont community composition during baseline conditions (i.e. no pulse stress influence), and that this effect is stronger than the mechanical mixing that occurred during the pulse stress of the storm event. Specifically, *Symbiodinium* communities at disturbed sites had higher beta diversity (i.e. multivariate community spread) than those at lower disturbance sites. This finding follows the ‘Anna Karenina’ principle (Diamond 1997, Zaneveld et al. 2017), which suggests that the structure of natural communities under stress follow Leo Tolstoy’s adage, “all happy families look alike; each unhappy family is unhappy in its own way”. I found this pattern of increased beta diversity with high levels of human disturbance to hold true for not only coral-associated *Symbiodinium* communities, but also for free-living *Symbiodinium* communities (i.e. those in the water and sediment). Although this study demonstrated differences in symbiont community structure based on human disturbance, open questions remain regarding the consequences of these differences.

These results motivated additional research to understand how the broader coral symbiome (including both *Symbiodinium* and microbial communities) responds to multiple levels of local human disturbance. Coral-associated microbial diversity on Kiritimati is different between very high and very low disturbance for two coral species (McDevitt-Irwin et al. *accepted*). In Chapter 6, I expand on this study to find that both microbial alpha and beta diversity are higher at a very high level of human disturbance than at three moderate and lower levels of local human disturbance across seven coral species (Claar et al. *in prep*). I also tested for concordance, which represents similarity in multivariate community shape, and can indicate co-occurrence or similar responses of both communities to environmental drivers. I found that when all coral species were considered together, there was concordance between microbe and *Symbiodinium* communities, but when each coral species was considered separately, there was only concordance for two massive coral species *H. microconos* and *F. pentagona*. I also found that the presence of certain *Symbiodinium* (clades A, D, and G) was associated with either an increase or decrease in the abundances of several microbial taxa which may be indicative of changes in the symbiotic state. The results of this chapter suggest that *Symbiodinium* and microbial communities are similarly affected at high levels of chronic disturbance, and that there are links between these communities at an island scale.

To better understand the consequences of local disturbance-influenced *Symbiodinium* community structure, I next leveraged a natural experiment (the 2015/2016 mega El Niño event) to assess how these changes influence coral resilience to a major pulse stressor. It is generally accepted that local human disturbance can influence coral resilience by inhibiting recovery mechanisms: for example, increased fishing can lead to decreased herbivorous fish biomass, leading to an increase in competing algae and a decreased probability of coral settlement and regrowth (Mumby & Harborne 2010). However, evidence for potential linkages between coral disturbance and coral resistance to stress have been elusive. In Chapter 7, I show that human disturbance negatively alters symbiont communities (Claar et al. *in prep*). Corals (*Platygyra daedalea*) from protected locations had "more beneficial" symbioses, which were associated with higher survival rates compared to highly impacted locations. These corals were so well protected that they recovered from unprecedented heat stress (reaching an unprecedented 25 Degree Heating Weeks) while they were still being exposed to temperatures above their bleaching threshold. As far as we know, this is the first documentation of coral recovery from bleaching while the colonies are still under heat stress. I view this result as hopeful in the context of understanding how corals will respond to and recover from future heat stress events.

In sum, this thesis aims to evaluate the independent and interactive effects of local human disturbance and pulse warming on coral symbioses. With accelerating stressors threatening foundational ecosystems worldwide, it is critically important to understand how human-induced change influences ecological community structure. To that end, the research contained in this dissertation aims to elucidate mechanisms of coral symbiosis, stress, and survival in a changing world.

Chapter 2

Embracing complexity in coral-algal symbioses

Danielle C. Claar[1], Nicholas S. Fabina[2], Hollie M. Putnam[3], Ross Cunning[3], Emilia Sogin[3], Julia K. Baum[1], and Ruth D. Gates[3]

[1] Department of Biology, University of Victoria, Victoria, BC, Canada
[2] Department of Evolution and Ecology, University of California, Davis, CA, USA
[3] Hawaii Institute of Marine Biology, School of Ocean and Earth Science and Technology, University of Hawaii, Kaneohe, HI, USA

Published as: **Claar DC**, Fabina NS, Putnam HM, Cunning R, Sogin EM, Baum JK, Gates RD (2017) Embracing complexity in coral-algal symbioses. In: Grube M, et al. (eds) *Algal and Cyanobacteria Symbioses*.

Abstract

A major determinant of coral reef resistance and resilience is the intracellular symbiosis between scleractinian (reef-building) corals and dinoflagellates in the genus *Symbiodinium*. The inherent complexity of coral-*Symbiodinium* interactions, however, presents a significant challenge to understanding and predicting reef dynamics. Research focuses on the dynamics of coral-algal symbioses from the molecular to the ecosystem levels and new methods, including next generation sequencing, real-time PCR, mathematical and computational analyses, and metabolomics, are all providing novel insight into the mechanisms that initiate, sustain and disrupt coral-*Symbiodinium* symbioses. As these approaches continue to be developed and synthesized, our understanding of complex coral-*Symbiodinium* interactions is becoming progressively more comprehensive. This chapter focuses on recent progress in the field and highlights novel approaches to embracing complexity in coral-algal symbioses.

Main Text

Coral-*Symbiodinium* Interactions and Reef Resilience

Coral reef ecosystems must be resilient if they are to persist in a changing world, in which they are subject to increasing local and global anthropogenic stressors. The symbiosis between corals and *Symbiodinium* is fundamentally important to coral reef resistance and resilience, but the intrinsic complexity of coral-algal symbioses presents a significant challenge to predicting the future of reef resilience. The mechanisms governing in the initiation, maintenance, and dissolution of these symbioses are intricate, and their consequences for reef communities may be mediated by both community composition and environmental context. In response to this complexity, researchers have used a variety of physiological, ecological, and genomic approaches to explore coral-*Symbiodinium* associations (Hughes et al. 2003, West & Salm 2003, McClanahan et al. 2012). Scientific progress is accelerating due to new methodological tools and analytical approaches, and interdisciplinary syntheses are poised to play a central role in understanding how this symbiosis determines the structure

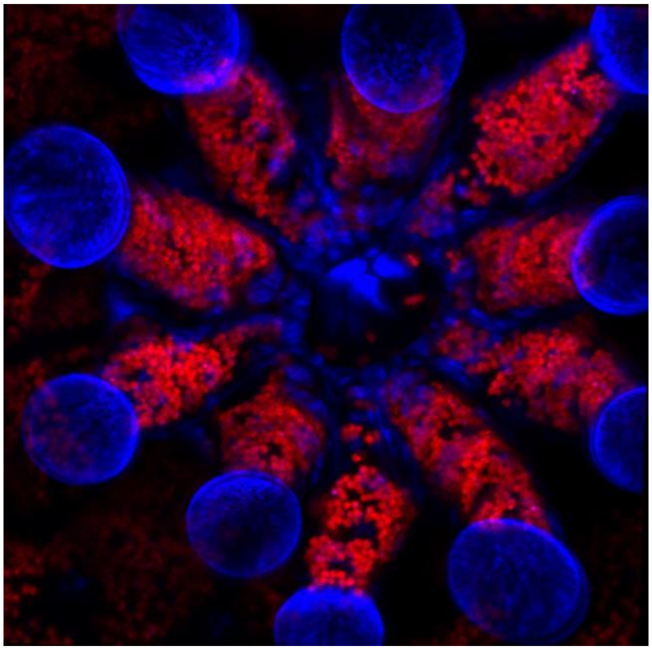


Figure 2.1. *Pocillopora damicornis* polyp visualized using a scanning laser confocal microscope. Image shows natural fluorescence of the coral holobiont, *Symbiodinium* in red, host pigments in blue.

and diversity of future reef communities. In this chapter, we present an overview of the evolution of coral-*Symbiodinium* research, current knowledge, and emerging techniques to elucidate the complex relationships between coral host and algal symbiont.

Coral-*Symbiodinium* Symbiosis

Symbiodinium (prev. zooxanthellae) are algal endosymbionts and are integral to coral persistence (Figure 2.1; Stat, Carter, & Hoegh-Guldberg, 2006). These photosynthetic dinoflagellates are found globally in association with a plethora of marine invertebrates, including giant clams, soft corals, hard corals, and anemones (LaJeunesse 2002). *Symbiodinium* are also found free-living within the water column, sediment, or other reservoirs (Manning & Gates 2008). These free-living populations can persist over long periods of time, while individual cells may be acquired by nearby hosts.

The relationship between *Symbiodinium* and scleractinian (reef-building) corals is one of the best-studied host-*Symbiodinium* interactions. In the coral-*Symbiodinium* interaction, these prolific symbionts provide organic carbohydrates to their host in exchange for essential nutrients (e.g. phosphates, nitrates, and inorganic carbon) and a relatively stable environment (Muscatine & Cernichiari 1969, Lewis & Smith 1971, Trench 1993). This symbiotic relationship occurs within the context of other ecological interactions. Each coral colony acts as a landscape for microorganisms, with skeletal characteristics and gross morphology creating light and temperature microhabitats across the colony’s surface (Kühl et al. 1995, Helmuth et al. 1997, Yost et al. 2013). These microhabitats are also exploited by a variety of bacteria, endolithic algae, and other microorganisms that are just beginning to be studied (Knowlton & Rohwer 2003, Stat et al. 2012). The sum of the micro- and macroorganisms associated with the coral host is called the holobiont. Together, the holobiont responds to environmental conditions and interacts with the broader reef community (Gates & Ainsworth 2011).

Corals provide the foundation for tropical reef ecosystems and the maintenance of their symbioses are vitally important to supporting tropical marine ecosystem biodiversity, function, and resilience (van Oppen & Gates 2006). Thus, the study of coral-*Symbiodinium* interactions is not only fundamentally important from an ecological perspective, but also provides information that is directly applicable to conservation practitioners.

Early Investigations of Coral-*Symbiodinium* Interactions

Research on *Symbiodinium* began almost a century ago, with Boschma's first studies of *Symbiodinium* (Boschma 1925a). At that time, it was believed that corals hosted zooxanthellae (*Symbiodinium*) in their tissues as a sort of predation interaction akin to farming – evidence of degraded symbionts in the gut cavity led Boschma to posit that corals derived nutrition by selectively consuming zooxanthellae (Boschma 1925b). *Symbiodinium* were little studied over the next 45 years, such that the true symbiotic nature of coral-zooxanthellae interactions was not discovered until 1969 (Muscatine & Cernichiari 1969). This discovery was quickly followed by the description of uptake of photosynthetic product by corals using labeled ^{14}C (Lewis & Smith 1971) and carbon stable isotope ratios (Land et al. 1975). Subsequently, the symbiont's role in facilitating coral calcification was described (Goreau 1963, Pearse & Muscatine 1971), and early photobiology studies, which quantified symbiont photoadaptation by depth and ambient light intensity (Jokiel et al. 1982, Dustan 1982), suggested that the coral host had the capability to control symbiont density in bright environments (Drew 1972). Most early *Symbiodinium* research focused on physiology, and as recently as 1979 it was believed that there was only one type of *Symbiodinium microadriaticum* that had a global distribution (Kinzie & Chee 1979).

Improved Molecular Techniques

As the field progressed, improved molecular techniques began to illuminate the diversity of *Symbiodinium* taxa (Figure 2.2). First, the catch-all name zooxanthellae (used to describe a group of yellow-brown symbiotic dinoflagellates; Brandt, 1881) was exchanged for *Symbiodinium* (a more specific, genetically delineated taxon; Freudenthal, 1962). Beginning in the 1990s, scientists recognized that genetic diversity within *Symbiodinium* was likely far greater than originally thought, and equivalent to diversity among orders in other dinoflagellate groups (Rowan & Powers 1991). Until very recently, however, coarse molecular techniques and extremely low sample size have limited analyses of *Symbiodinium* community structure. In fact, for nearly 80 years, all

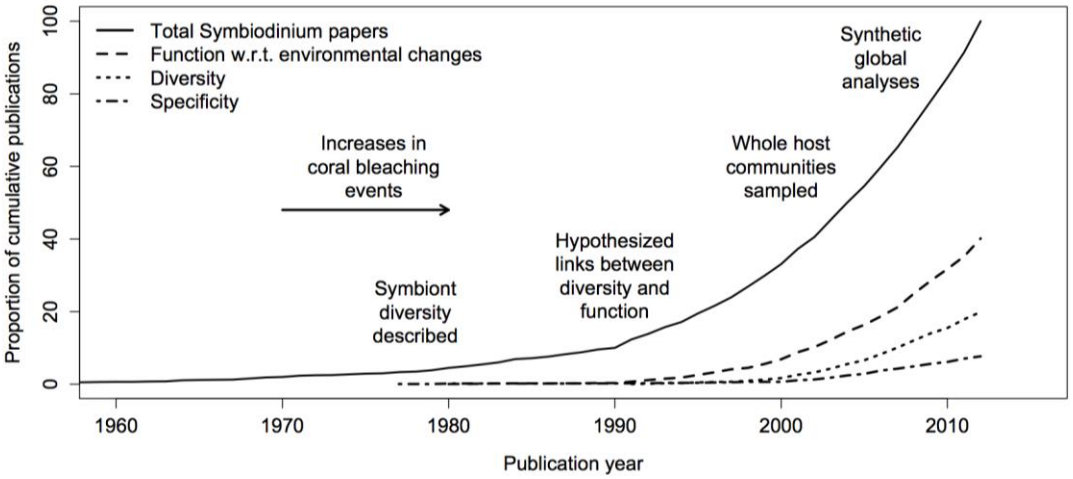


Figure 2.2. Growth of coral-*Symbiodinium* research from 1960 through 2013, including major discoveries and developments in the field. Figure created from Web of Science search of coral-*Symbiodinium* literature from pre-1960 until 2013.

coral-*Symbiodinium* studies found absolute specificity between a single *Symbiodinium* clade and a particular coral species (Rowan & Powers 1991, Trench 1993). As molecular techniques improved, multiple *Symbiodinium* clades were found within coral species, and subsequently within individual coral colonies (Rowan 1995). Recent research has confirmed the presence of this rich diversity (Stat et al. 2008, Quigley et al. 2014, Thomas et al. 2014).

There are currently nine described clades (clades A through I, Figure 2.3); each of which is further divided into multiple genetic strains or types (Rodrigues-Lanetty et al. 2001, van Oppen et al. 2005); (Lesser et al. 2013)). The lack of formally described species, and inconsistency in name usage arising from variable subclade naming systems, has resulted in much debate about species classifications within *Symbiodinium* (Stat et al. 2012), except see (LaJeunesse 2001). Despite the absence of a consensus naming system, clades are generally divided into over 100 genetically delineated types with many having distinct geographic distributions, host preferences, and abiotic optima (Fabina et al. 2012). As many distinct types of *Symbiodinium* are now recognized, the diversity and abundance of types within corals is thought to be integral to coral holobiont

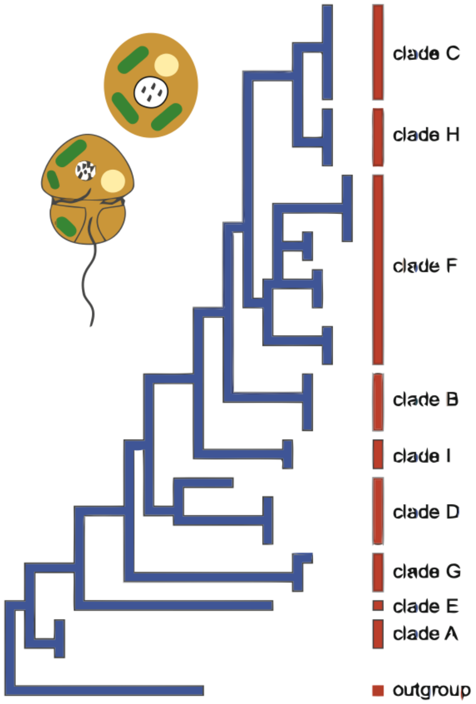


Figure 2.3. *Symbiodinium* clade phylogeny with illustrations of the cyst and zoospore stages. Phylogeny of *Symbiodinium* inferred using the 28s rDNA marker. From Lesser et al. 2013.

fitness. Consequently, taxonomic research is using novel genetic techniques to evaluate the relationship between *Symbiodinium* type and phenotypic traits or symbiotic outcomes.

Coral Bleaching and Ecological Stress Response

Paralleling improvements to molecular techniques, the extreme coral bleaching that occurred around the world during the 1982-83 and 1997-98 El Niño events renewed interest in understanding how coral-symbiont interactions mediate coral responses to environmental stressors. These widespread bleaching events were unprecedented at the time, and coral mortality reached 95% in some regions (Glynn 1993). Coral bleaching occurs when the symbiotic relationship breaks down, resulting in a loss of photosynthetic capacity and/or *Symbiodinium* loss from the coral colony (Gates et al. 1992, Douglas 2003). Mild or short-term bleaching can reduce coral growth rates and hinder other biological functions, while severe or prolonged bleaching can kill corals. While coral mortality was extensive during these early bleaching events, some coral species and individuals appeared to be more resistant to bleaching or more likely to recover after bleaching. These differences prompted researchers to search for the mechanisms underlying this variability, leading to a new wave of *Symbiodinium* studies in the late 1990s.

One of the earliest and most controversial hypotheses relating *Symbiodinium* composition to bleaching responses was the Adaptive Bleaching Hypothesis (ABH). Briefly, the ABH predicts that corals bleach in order to rid their tissues of suboptimal *Symbiodinium* types, allowing them to uptake new symbionts (“switching”) or adjust the relative proportions of symbionts within their tissues (“shuffling”) (Baker 2003). Both switching and shuffling were proposed as potential mechanisms by which corals could maximize holobiont fitness and quickly adapt to changing environments (Buddemeier & Fautin 1993, Baker 2001, Buddemeier et al. 2004). A strength of the ABH is its explicit hypotheses and assumptions, but empirical and theoretical tests have provided only equivocal support. While many corals house background symbionts in low levels (Correa et al. 2009), symbioses with background or exogenous symbionts are often unstable (Coffroth et al. 2010). Moreover, hosts with flexible symbioses may be more sensitive to environmental changes (Putnam et al. 2012). Although the ABH provides a framework for exploring the functional importance of *Symbiodinium* diversity, it appears that the complexity of the coral-*Symbiodinium* system necessitates more nuanced answers.

Beyond the acclimation capacity of symbiont switching and shuffling, there are several other mechanisms that can facilitate the adaptation of the coral holobiont. After a period of studying how symbionts affect bleaching, focus turned to the role of the host in coral bleaching and holobiont community dynamics (reviewed in Baird et al. 2009). Host adaptations can include protective fluorescent pigments (Salih et al. 2000), mycosporine-like amino acids (MAAs, (Banaszak et al. 2000)), antioxidant systems (reviewed in Lesser, 2006), and synthesis of heat shock proteins and other physiological mechanisms (Gates & Edmunds 1999). Coral host populations can show significant genetic divergence on the scale of <10 km, suggesting that, for some species, host variability may play a prominent role in spatial patterns of holobiont thermotolerance (Kenkel et al. 2013). Utilization of host-adaptive strategies tends to be species specific, and the combination of host acclimatization and adaptation with *Symbiodinium* community structure shapes the resilience of the coral holobiont.

Symbiont Specificity and Stability

The extent to which symbiont dynamics drive coral stress responses is determined by the specificity and stability of interactions. Despite the fact that many adaptive mechanisms are species-specific, coral-symbiont interactions can be divided into the broad categories of generalists and specialists (Putnam et al. 2012). Generalist coral species, including many *Acropora* species, can harbor a diverse array of *Symbiodinium* types either simultaneously, in succession within individual coral colonies, or across subpopulations. In contrast, specialist coral species, including massive corals such as *Porites*, harbor fewer symbiont types. One potential advantage of generalism would be the potential to rapidly switch or shuffle symbiont types to optimize environmental responses, as described by the adaptive bleaching hypothesis (Baker 2003, Berkelmans & van Oppen 2006). However, recent research has suggested that generalists may also be more susceptible to changing environmental conditions due to opportunistic symbionts (Putnam et al. 2012). Specialist corals may benefit from more efficient symbiotic associations, but the mechanisms involved, and their exact consequences, are still poorly understood.

Destabilization of symbiotic interactions during coral bleaching is often associated with and followed by changes in *Symbiodinium* community structure. Symbiont total diversity, as well as the symbiont dominance hierarchy and community evenness all may change, as a result of active coral regulation, differential *Symbiodinium* survival, symbiont competition, or other mechanisms. Bleached coral tissues may provide niche space for atypical symbionts, such as a clade B type observed in bleached *Pocillopora*, although these may be quickly replaced by more stable clade C associations during recovery (LaJeunesse et al. 2010). This process of community destabilization and reassembly may lead to "switching" or "shuffling" to more heat-tolerant symbionts in some cases, but not others. A recent study found a positive relationship between the history of thermal stress anomalies (TSA) and prevalence of clade D (heat tolerant) *Symbiodinium* in the more generalist *Montipora capitata*, but not in the specialists *Porites lobata* and *P. compressa*, suggesting these dynamics may be species-specific (Stat et al. 2013). Shifts toward heat-tolerant symbionts also depend on bleaching severity and recovery temperature (Cunning et al. 2015), indicating that ecological context also influences stability and dynamism in symbioses. The persistence of changes in *Symbiodinium* communities (such as increases in clade D) may also depend on frequent or sustained environmental pressure (Baird et al. 2007); otherwise, they may revert to the originally dominant symbiont (Thornhill et al. 2006). This shift back to the original *Symbiodinium* community is usually attributed to a more evolutionarily derived symbiosis that provides increased nutrition to the host and supports a higher coral growth rate (Little et al. 2004). Research is actively investigating the drivers and dynamics of *Symbiodinium* community structure, but many questions remain regarding the processes that initiate and sustain changes in host-symbiont interactions.

Studies attempting to elucidate the mechanisms underlying coral diversity patterns suggest that environmental drivers are a strong determinant of geographic distribution of coral-*Symbiodinium* associations. *Symbiodinium* communities often demonstrate zonation by ambient light levels that are modulated by reef depth (Iglesias-Prieto et al. 2004, Frade et al. 2008, Finney et al. 2010). While light is a strongly limiting factor in symbiont community structure, *Symbiodinium* are not limited to bright light environments. Coral-*Symbiodinium* communities inhabit a wide range of abiotic conditions, from bright shallow waters to nearly-dark mesophotic waters (Chan et al. 2009, Cooper et al. 2011, Wagner et al. 2011, Bongaerts et al. 2013), with considerable temperature variability and a range of other environmental factors.

As we have learned more about the distribution and complexity of coral symbioses, recent research has focused increasingly on the response of symbiont communities to multiple stressors. The deleterious effects of thermal stress have been repeatedly measured (e.g. Abrego et al. 2012, Stat et al. 2013, Baker et al. 2013), as have the additive effects of thermal stress with turbidity (Cooper et al. 2011), nitrogen (Béraud et al. 2013), ocean acidification (Ateweberhan et al. 2013), precipitation (Edge et al. 2013) and a variety of other environmental stressors (Maina et al. 2008). Integrating multiple approaches will be necessary to develop a synthetic understanding of the complex interactive effects of multiple stressors on coral symbioses.

Next Generation Sequencing Approaches

Our knowledge of symbiont specificity and stability has grown rapidly in the past decade, and we are now poised to exponentially expand our ecological understanding of the coral-*Symbiodinium* symbiosis with the application of Next Generation Sequencing (NGS) approaches. NGS approaches provide in-depth insight into novel symbiont genetic diversity, and current fingerprinting and sequencing efforts have identified hundreds of sequence types (Franklin et al. 2012, Tonk et al. 2013). There are, however, data limitations for geographic extent, sample coverage, and sequencing depth for the majority of coral species on reefs today, reducing our ability to test hypothesis of specificity and stability more globally. The power of NGS arises from its massively parallel, high throughput sequencing capacity. There are now multiple sequencing platforms from manufacturers such as Illumina, Roche, and Life Technologies that can generate millions of sequences in a single run, at the cost of a fraction of a cent per base (Glenn 2011). Importantly, these platforms can sequence marker amplicons from multiple samples simultaneously, allowing for sequenced genetic identification and deep coverage of diverse environmental samples (e.g. Nelson et al. 2014; Quigley et al. 2014). The early adoption of NGS amplicon sequencing in the broader microbial field has resulted in an explosion of data and has highlighted the importance of these techniques to identify the "rare biosphere", the multitude of low-abundance populations that account for a majority of microbial phylogenetic diversity (Sogin et al. 2006).

With the recent rapid expansion in *Symbiodinium* knowledge and the critical need to understand resilience and resistance mechanisms in corals related to the symbiosis, NGS is the logical next step in *Symbiodinium* genetic identification. The first application of NGS to the coral-*Symbiodinium* has highlighted the application of NGS to analyze archived DNAs in order to facilitate long term genetic and ecological comparisons (Edmunds et al. 2014). Studies have also identified the power of NGS to detect cryptic genetic diversity (Kenkel et al. 2013, Quigley et al. 2014). With the appropriate choice of gene region, the application of NGS can provide rapid and thorough investigation of *Symbiodinium* alpha and beta diversity to inform ecological analysis, including further tests of the ABH and other symbiotic specificity and stability questions.

High-Resolution Quantification

While symbiont diversity is a critical mediator of symbiosis function, symbiont abundance may also play an important role. Total *Symbiodinium* abundance, as well as the relative abundance of different *Symbiodinium* clades, can vary amongst coral colonies and species, across environmental gradients, and over time (Fagoonee et al. 1999, Fitt et al. 2000, Moothien-Pillay et al. 2005). The drivers and consequences of these dynamics remain poorly understood. Quantifying the dynamics of specific *Symbiodinium* types in mixed communities has been especially difficult, because traditional approaches to enumerating symbionts by counting with a hemocytometer and normalizing to skeletal surface area (e.g. cells cm^2) cannot differentiate among *Symbiodinium* types due to their morphological similarity. New molecular techniques, such as real-time PCR, overcome this limitation by quantifying symbiont clades based on genetic sequence variation, and normalizing their abundance to numbers of coral cells (e.g. a symbiont to host cell ratio; Mieog et al. 2009). These new techniques raise important issues regarding the suitability of different metrics for normalizing symbiont abundance, which will become increasingly important as next-generation sequencing provides further opportunities to quantify *Symbiodinium*.

Quantitative analyses of *Symbiodinium* dynamics within a community ecology framework can address questions regarding symbiont competition, host regulation, environmental control, and the links between symbiont abundance and symbiosis function. Symbiont abundance is likely to influence physiological performance of both partners by mediating the physical and chemical environment within the coral tissue microhabitat. For example, symbionts may reduce internal light environments (Enríquez & Pantoja-Reyes 2005, Wangpraseurt et al. 2012) and concentrations of nutrients and dissolved inorganic carbon (Wooldridge 2009), and increase intracellular pH (Venn et al. 2009a). Greater symbiont abundance is also linked to higher rates of photosynthesis and respiration in coral colonies (Hoogenboom et al. 2010), and increased sensitivity to environmental stress and bleaching (Cunning & Baker 2013). Furthermore, these relationships suggest that any environmental factor that alters symbiont abundance will impact coral physiology and performance. Thus, quantitative analysis of symbiont community dynamics in variable environments may help resolve complexity and variation in performance, while greatly enhancing our understanding of the basic biology and ecology of coral-algal symbioses.

Modeling coral-*Symbiodinium* associations

Simulation and theoretical modelling approaches to understanding coral-*Symbiodinium* associations have been helpful in supplementing empirical approaches. Indeed, one of the strengths of quantitative approaches is the ability to simplify otherwise complex systems and allow for exploration and description of individual components. One of the first models of coral-*Symbiodinium* associations examined how symbiont populations recover from bleaching events (Jones & Yellowlees 1997). The authors used experimental data to parameterize symbiont growth, expulsion, and carrying capacity within a discrete time model. The model was only able to faithfully reproduce symbiont recovery dynamics when all three components (growth, expulsion, and density dependence) were considered, which provided a foundation for later empirical and theoretical studies.

Another early modeling paper explored how elevated temperatures could modify symbiont abundance and diversity (Ware et al. 1996), particularly in the context of the ABH (Buddemeier & Fautin 1993). Very little was known about the host, symbiont, and holobiont responses to thermal stress, so the model was structured to explore the hypotheses of ABH. By simulating a community of symbionts with varying temperature tolerances to circumannual and abnormal temperature variations, Ware and colleagues (1996) showed that minor changes in temperature could cause changes in relative symbiont abundances, seasonal variation could lead to cyclical symbiont dynamics, and that symbiont responses to thermal variation can depend on thermal history. Although the model was necessarily simplified, it provided a robust framework for testing the assumptions and predictions of a provocative hypothesis.

A more recent and sophisticated model building upon the approaches of (Ware et al. 1996, Jones & Yellowlees 1997), explicitly included solar insolation and sea surface temperature dynamics, and determined symbiont growth and loss (expulsion or mortality) rates by their environmental tolerances and ability to access light resources (Figure 2.4, van Woesik et al. 2010). The authors highlighted two reef locations, in Florida and the Bahamas, to show that different symbiont phenotypes were competitive dominants in each location. Moreover, elevated temperatures would shift symbiont dominance hierarchies in historically cooler locations, and merely increase the relative abundance of

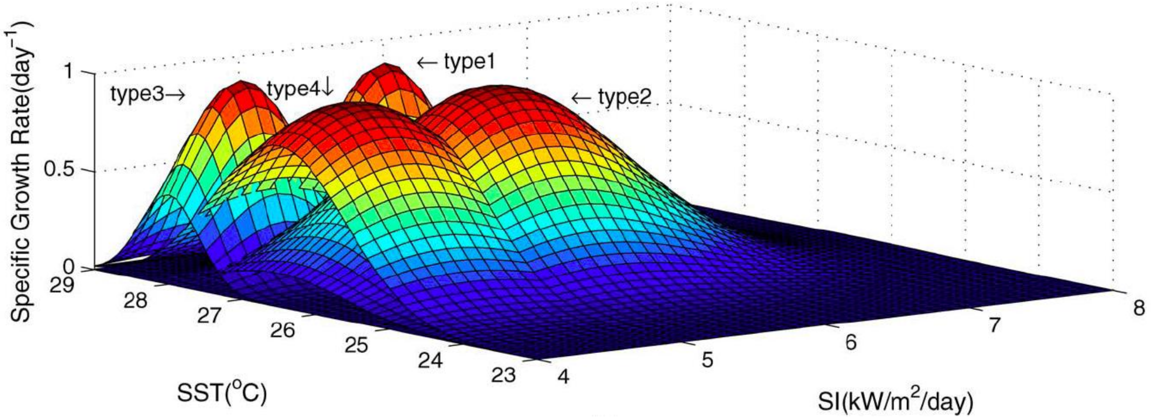


Figure 2.4. Growth rate of four *Symbiodinium* types in relation to Sea Surface Temperature (SST) and Solar Insolation (SI) from van Woesik et al 2010.

thermally tolerant symbionts in warmer locations. Others have modeled coral-*Symbiodinium* associations to understand their effects on benthic community dynamics. A pair of papers in 2009 and 2010 projected coral responses to climate change as a function of symbiotic and competitive dynamics (Baskett et al. 2009, 2010). Specifically, the authors modeled competition between thermally-tolerant massive corals and thermally-sensitive branching corals with a quantitative genetics framework for evolution of thermal tolerance in their *Symbiodinium* types. In the latter paper, competition between corals and macroalgae was incorporated to determine how alternative stable states could influence results. Baskett and colleagues found that shifts in symbiont community diversity could have a greater impact on coral persistence than evolution of symbiont thermal tolerance. Moreover, the nature of the climate scenario tested (e.g., business as usual or decreased emissions) could have a major impact on model outcome. The authors concluded that careful prioritization of management targets and mitigation of other environmental changes would be critical to reef persistence in future climates.

Recently, researchers have described communities of corals and *Symbiodinium* using network theory, using networks to simulate various scenarios (Fabina et al. 2012, 2013). Coral-*Symbiodinium* communities can be composed of tens or hundreds of host species and symbiont types, so quantitative frameworks for simplifying and describing these communities can lead to new insights. For instance, symbiont transmission mode is tightly linked to the specificity of symbiont assemblages, with horizontally transmitting corals having more generalist symbionts on average (Fabina et al. 2012). Moreover, total symbiont diversity appears to be highly correlated with these patterns - even more so than dominant symbionts (Rowan & Powers 1992). Simulations under various scenarios have shown that coral persistence depends on coral-*Symbiodinium* association patterns, *Symbiodinium* diversity, and the functional outcomes associated with thermally tolerant and nutritionally beneficial types (Fabina et al. 2012). In each of these cases, quantitative approaches have produced novel insights, elicited new ideas, and focused scientific and applied goals.

Nutrient Transfer between Symbionts and Hosts

Understanding functional differences in the taxonomically diverse assemblages of *Symbiodinium* communities found in reef building corals is important to describing how these unions will persist in time. At the base of the relationship between corals and *Symbiodinium* is the transfer of small compounds, called metabolites. Metabolites are small molecules resulting from cellular processes that are generally involved in growth, nutrition and reproduction. These molecules can be modified by the organism to serve a specific metabolic (primary metabolites) or ecological role (secondary metabolites). Early investigations of *Symbiodinium* revealed that the photosynthate, or bulk carbon compounds being passed from *Symbiodinium* to the host, was composed of a suite of metabolites, including large quantities of glycerol with smaller amounts of glucose, glycolic acid, and alanine (Muscatine 1967, Muscatine & Cernichiari 1969, Trench 1971a, b, Black & Burris 1983). However, this research assumed that all *Symbiodinium* partners were physiologically equal across coral hosts. Moreover, researchers typically used a suite of tools (e.g. thin layer chromatography) that have now been replaced by more sensitive and comprehensive techniques. Despite historical limitations in metabolite detection and analysis, targeted research was able to detect a variety of metabolites in coral and *Symbiodinium* extracts (for full review see Gordon & Leggat 2010). These compounds include various sugars, amino and organic acids, as well as lipids, all of which are thought to play important roles in maintaining the coral-*Symbiodinium* union (Muscatine 1967, Trench 1971a, Gates et al. 1995, Luo et al. 2009). Despite the importance of small compounds to coral reef survival and recent evidence suggesting that bulk photosynthate varies among *Symbiodinium* types (Stat et al. 2008), our understanding of quantitative differences in the photosynthate transfer remains limited.

Researchers are currently investigating the feasibility of using metabolomic techniques to detect metabolites in reef corals. These metabolite-profiling tools, which take advantage of mass spectrometry (MS) and nuclear magnetic resonance (NMR) analytical methods, have the capacity to detect and quantify many (100+) metabolites in a single run. Consequently, these tools can describe subtle, complex physiological responses of individuals to perturbations in environmental conditions. While metabolomics has yet to be applied towards describing metabolite production across *Symbiodinium* types, the increased sensitivity that these methods afford is changing the current understanding of the mechanistic relationship of metabolite exchange between the alga and the coral host. For instance, a recent gas chromatography-MS based analysis provided evidence that glucose, and not glycerol, is the major metabolite transferred between *Symbiodinium* and cnidarians. In combination with previous research, this study concluded that *Symbiodinium* derived glucose is used as a major osmolyte in coral tissues to combat stress (Burriesci et al. 2012). Researchers have also applied metabolomic profiling approaches to better understand fatty acid synthesis in reef corals. Dunn and colleagues (2012) used a combination of stable isotopic incubations and liquid-chromatography-MS analytical techniques to track and model lipid production dynamics.

In doing so, they found that cnidarian hosts may not be using symbiont-derived fatty acids directly following photosynthate transfer (Dunn et al. 2012). Finally, through a more targeted approach using ^{1}H-NMR techniques, researchers investigated the production of dimethylsulphoniopropionate (DMSP) on coral reefs. DMSP is an important cellular osmolyte and a central component of the marine sulfur cycle, and it was thought that DMSP production in reef corals was controlled solely by *Symbiodinium*. However, when Raina and colleagues (2013) applied this metabolite profiling approach towards studying the metabolite composition of *Symbiodinium*-free *Acropora* juveniles, they detected high concentrations of DMSP, suggesting that the coral host contributes a significant fraction of the coral holobiont's total DMSP production (Raina et al. 2013).

Metabolite profiling tools that are now becoming available for coral biology will enable researchers to more accurately describe cnidarian-dinoflagellate symbioses. By qualitatively and quantitatively describing metabolite transfer from the symbionts to host across a range of unions, researchers will be better poised to characterize the functional role each *Symbiodinium* type plays in the coral holobiont.

Conclusion

Future Outlook: Coral-*Symbiodinium* Interactions

In less than 100 years, our understanding of the symbiotic relationship between coral and *Symbiodinium* has grown from basic descriptions of symbiosis and nutrient transfer to empirical and modelling studies that define the response of complex communities across variable environmental dynamics. Due to recent and cumulative developments in the field of coral symbioses through experimental, molecular, and statistical techniques, coral-*Symbiodinium* interaction research is at the brink of explaining the mechanisms behind coral bleaching and resilience in the context of environmental and ecological complexity. Despite the great strides made in coral-*Symbiodinium* ecology, many unanswered questions remain. Active research within coral symbioses is progressing, but there is also considerable scope for this field to take advantage of new technologies and techniques. Complexity is the essence of coral symbioses, and only by embracing it can we move forward towards understanding their interactions in the dynamic marine environment.

Chapter 3
Global patterns and impacts of El Niño events on coral reefs: a meta-analysis

Danielle C. Claar[1*], Lisa Szostek[1], Jamie M. McDevitt-Irwin[1], Julian J. Schanze[2], Julia K. Baum[1]

[1] Department of Biology, University of Victoria, Victoria, British Columbia, Canada
[2] Earth and Space Research, Seattle, Washington, United States of America

Published as: **Claar DC**, Szostek L, McDevitt-Irwin J, Schanze JJ, Baum JK (2018) Global patterns and impacts of El Niño events on coral reefs: a meta-analysis. *PLoS ONE* 13(2) e0190957.

Abstract

Impacts of global climate change on coral reefs are being amplified by pulse heat stress events, including El Niño, the warm phase of the El Niño Southern Oscillation (ENSO). Despite reports of extensive coral bleaching and up to 97% coral mortality induced by El Niño events, a quantitative synthesis of the nature, intensity, and drivers of El Niño and La Niña impacts on corals is lacking. Herein, we first present a global meta-analysis of studies quantifying the effects of El Niño/La Niña-warming on corals, surveying studies from both the primary literature and International Coral Reef Symposium (ICRS) Proceedings. Overall, the strongest signal for El Niño/La Niña-associated coral bleaching was long-term mean temperature; bleaching decreased with decreasing long-term mean temperature (n = 20 studies). Additionally, coral cover losses during El Niño/La Niña were shaped by localized maximum heat stress and long-term

mean temperature (n = 28 studies). Second, we present a method for quantifying coral heat stress which, for any coral reef location in the world, allows extraction of remotely-sensed degree heating weeks (DHW) for any date (since 1982), quantification of the maximum DHW, and the time lag since the maximum DHW. Using this method, we show that the 2015/16 El Niño event instigated unprecedented global coral heat stress across the world's oceans. With El Niño events expected to increase in frequency and severity this century, it is imperative that we gain a clear understanding of how these thermal stress anomalies impact different coral species and coral reef regions. We therefore finish with recommendations for future coral bleaching studies that will foster improved syntheses, as well as predictive and adaptive capacity to extreme warming events.

Introduction

Climate change poses an imminent threat to the persistence of the world's coral reefs. Anthropogenic ocean warming is fundamentally altering marine ecosystems (Hoegh-Guldberg & Bruno 2010), exacerbating chronic local stressors such as overfishing, eutrophication, and coastal pollution, and threatening the resilience of marine ecosystems (Hughes et al. 2003, Hoegh-Guldberg et al. 2007). With increasing anthropogenic stressors, many coral reef ecosystems have transitioned from “safe operating spaces” – ecosystem states that are resilient to periodic stress events – towards “zone(s) of uncertainty” in which natural variability limits our prediction of ecosystem response, and “zone(s) of high risk” in which the ecosystem and its associated functions are already degraded (Norström et al. 2016). Global surface warming manifests not only as gradual increases in overall temperature, which are predicted to exceed 2°C by 2100 (Allen et al. 2014), but also as intense pulse heat stress events such as the warm phase of the El Niño Southern Oscillation (ENSO). ENSO is a quasi-periodic fluctuation in oceanographic and atmospheric conditions, which transitions among El Niño, neutral, and La Niña conditions. El Niño is associated with increases in sea surface temperature (SST) that are primarily centered in the Central and Eastern Tropical Pacific Ocean (Trenberth & Hoar 1997). Short-term positive warm temperature events caused by both El Niño and La Niña have instigated coral bleaching, so including both types of events allows us to investigate ENSO-related warming both in the Eastern/Central Pacific and in the Western Pacific, respectively. Major El Niño events have triggered three global coral bleaching events over the past four decades, the most intense of which unfolded over the course of 2015 and 2016 (Heron et al. 2016). Extreme El Niño events are predicted to double in the future due to greenhouse warming (Cai et al. 2014), and the frequency of severe coral bleaching events is expected to increase even under moderate warming scenarios (van Hooidonk et al. 2016). This projected increase in pulse warming events further threatens reefs which are already facing a multitude of stressors.

Short-term thermal stress events, such as El Niño, primarily impact corals by inducing coral bleaching. Under normal conditions, the coral animal lives in symbiosis with endosymbiotic *Symbiodinium* (previously, zooxanthellae), single-celled algae that reside in the coral's tissue and provide metabolic products necessary for coral survival (Muscatine & Porter 1977, Glynn 1993). Coral bleaching results from a loss of *Symbiodinium*, which can occur under stressful conditions as the symbiosis breaks down leading to the coral losing pigmentation as *Symbiodinium* lose photosynthetic functionality and are ejected from the coral tissue (Brown 1997). If the symbiosis is not reestablished before the coral is depleted of metabolic products and energy reserves, subsequent mortality can occur (McClanahan 2004). Coral bleaching can occur in response to a variety of environmental stressors, the most documented of which is increased water temperatures (Hoegh-Guldberg 2004). Substantial natural gradients of environmental stressors, including sea surface temperature regimes, exist at varying spatial and temporal scales (Maina et al. 2011, Gove et al. 2013), and corals begin to bleach when ocean temperatures exceed local thermal thresholds. Coral bleaching severity and the extent of subsequent coral mortality is highly variable both within and across regions. Moreover, coral bleaching can affect any reef, occurring not only in areas with coastal human populations but also on remote reefs (Aeby et al. 2003) and in protected areas (Hughes et al. 2017). While bleaching variability may be partially attributed to coral species differences, many other factors appear to influence the magnitude of changes in the coral reef community after pulse warming events.

Coral bleaching and mortality caused by extreme El Niño/La Niña events can induce catastrophic changes in foundational coral reef ecosystem structure (Glynn 1996, Graham et al. 2015). The impact of extreme El Niño events on coral communities worldwide has been observed and quantified since the early 1980s (Glynn 1984, Williams & Bunkley-Williams 1990, Stone et al. 1999). Notable regional examples include estimates of up to 95% coral mortality in some locations in the Eastern Pacific (Glynn 1990) and nearly 100% local coral mortality at some sites in Indonesia (Brown & Suharsono 1990) during the 1982-83 El Niño event, and up to 90% coral mortality on individual shallow Indian Ocean reefs during the 1997-98 El Niño event (Wilkinson et al. 1999). Many early studies and bleaching reports were based upon underwater visual observations of bleaching severity and coral mortality. When conducted without replication, visual estimates can preclude quantitative meta-analysis because they may be prone to intra-observer variability and may not include statistical measurements such as error (e.g. standard deviation, standard error, or confidence intervals) necessary for many quantitative analyses.

Depending on local conditions and the frequency of thermal stress, there is evidence that coral reefs can recover, even after an extreme El Niño/La Niña event (Stone et al. 1999, Arthur et al. 2006, Guzman & Cortes 2006, Golbuu et al. 2007). Despite the potential for recovery, the effects of a single El Niño/La Niña event can be permanent. For example, one reef-building hydrocoral became locally extinct, while another was driven to probable extinction as a direct result of the 1982/1983 El Niño event (Glynn &

de Weerdt 1991), and Panamanian reef structure damaged by the 1982/1983 El Niño had not returned to pre-El Niño levels nearly twenty years later (Eakin 2001). Even when the damage is not permanent, reefs can take more than a decade to recover from the impacts of an intense El Niño (Kelmo & Attrill 2013). Despite these widely recognized and extensively cited effects of ENSO-related pulse warming events, a quantitative global analysis of the effects of El Niño/La Niña on coral communities has not yet been conducted. Our research builds upon previous reviews which investigated the impact of single El Niño events on coral reefs (Glynn 1988, Glynn & D'Croz 1990, Davey & Anderson 1998), differential bleaching of corals between two El Niño events in one region (Lix et al. 2016), and the long-term recovery of coral reefs after El Niño (Baker et al. 2008).

Here, we address three questions of relevance to understanding climate change impacts on coral reefs: 1) How much coral bleaching and mortality have been observed and documented during previous El Niño/La Niña events?, 2) Does the coral stress metric Degree Heating Weeks (DHW) accurately predict El Niño/La Niña-driven changes in coral bleaching and cover on a global scale, and what other factors are influential?, and 3) How does the 2015-2016 El Niño event compare to prior events in terms of severity (maximum DHW) and geographic extent? We conducted a global meta-analysis to quantify the effects of El Niño/La Niña events on coral communities. Although it is widely accepted that the increase of SST that results from El Niño/La Niña events induces coral bleaching, the consistency of the effects of El Niño/La Niña fluctuations on coral bleaching and mortality has yet to be quantified through multiple ENSO oscillations at a global scale. To evaluate the relationships between DHW and coral bleaching and mortality, we build upon previous research (Box 1) using the Reynolds OI Level 4 AVHRR 0.25° sea surface temperature product to compute a comprehensive coral heat stress data set. This method allows for calculation of remotely-sensed coral heat stress for any date since 1982 using a consistent data set and climatology, allowing comparison of coral heat stress indices and response throughout the majority of published El Niño/La Niña-related coral bleaching events.

Box 1. Degree Heating Week (DHW) products and coral bleaching prediction.

Understanding how El Niño- and La Niña-related heat stress affects coral reefs at a global scale requires quantification of the magnitude of thermal stress for each reef location. Quantification of thermal stress on coral reefs using satellite observations began with the definition of ocean "hot spots" (Goreau & Hayes 1994) and in situ validation of satellite temperature observations at the reef scale (Montgomery & Strong 1994). In tandem with the development of "hot spot" analyses, researchers developed the concept of Degree Heating Weeks (DHW) as a cumulative metric of thermal stress on coral reefs (Gleeson & Strong 1995, Glynn 1996). The US National Oceanographic and Atmospheric Administration (NOAA) Coral Reef Watch Program (CRW) further developed these methods, using high-resolution (9 km satellite pixel) HotSpot anomaly mapping (Toscano et al. 2002) which improved correlation of satellite temperature observations with in situ measurements of coral reef temperature fluctuations (Toscano et al. 2002). NOAA CRW DHW products now include both an updated 50-km product (NOAA Coral Reef Watch 2000), and a newly-released 5-km product (NOAA CRW 2013). The NOAA CRW products have been successful in detecting many coral bleaching events around the globe (e.g. Liu et al. 2003; Liu et al. 2006), and can now be used to forecast upcoming thermal stress on coral reefs (Liu et al. 2008). Despite these major developments in understanding and predicting thermal stress and coral reef bleaching, a study that compared two DHW metrics to ReefBase bleaching reports found poor congruence between DHW magnitude and bleaching events both expected and observed, which was attributed to localized temperature variability and related coral adaptation (Boylan & Kleypas 2008). The extent of coral bleaching in a given location has been suggested to vary in relation to historical variability in maximum SST and local climatological maximums, although lack of adequate bleaching reports has hindered our understanding of these relationships and of global coral bleaching trends (Donner 2011). Most recently, nowcasting and predictive tools have allowed managers to prepare for and respond to coral bleaching events, and cutting-edge climatological heat stress models and analyses are continually being developed at the US NOAA CRW program (Heron et al. 2014) and the Australian Bureau of Meteorology (Garde et al. 2014). As local and regional coral bleaching reports are increasingly quantified, analyzed, and published, we have the ability to better understand how events such as El Niño and La Niña affect coral reefs at a global scale.

Methods

Literature search and data extraction

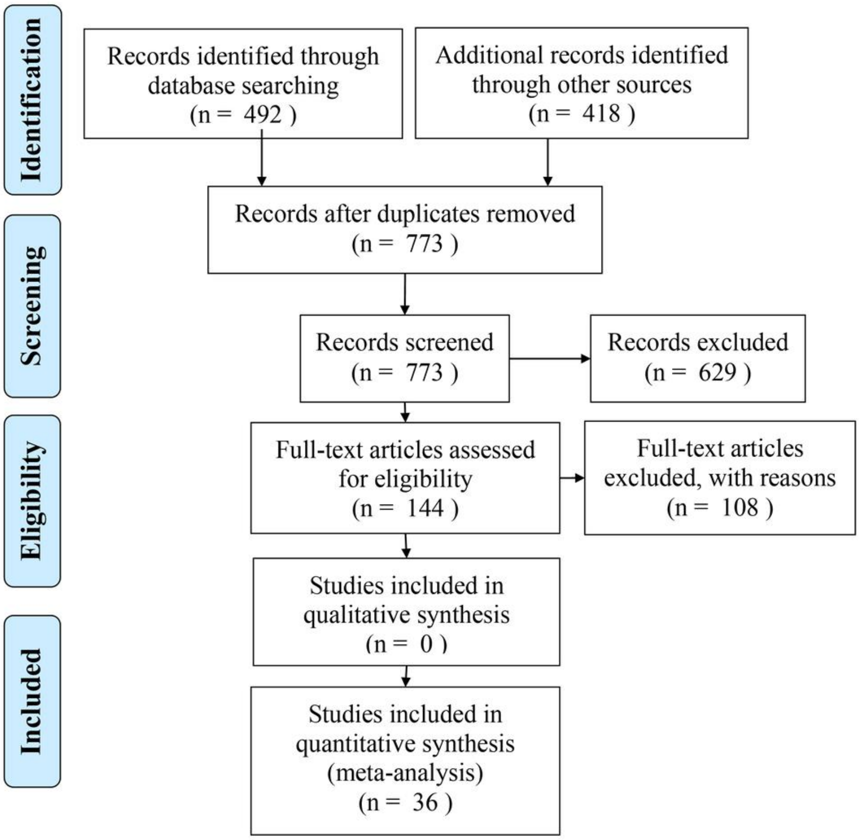
In order to identify primary literature regarding the effects of ENSO events (El Niño and La Niña) on coral health, we conducted a systematic literature search using all databases in ISI Web of Science using the following search terms: (coral*) AND (mortal* OR bleach* OR cover* OR health*) AND (El Niño OR El Nino OR ENSO). We performed the search first on December 3, 2014 and updated it on March 20, 2015 and again on August 27, 2016. We performed a second related search in ISI Web of

Science using the search terms: (coral*) AND (mortal* OR bleach* OR cover* OR health*) AND (La Niña OR La Nina) on September 16, 2016. Using a database search method (such as the ISI Web of Science keyword search employed in this study) is a common meta-analysis approach (Koricheva & Gurevitch 2014). In order to access relevant papers that were not published in the primary literature, we also queried the International Coral Reef Symposium (ICRS) Proceedings on ReefBase in three separate searches, first using the term 'El Niño', second using the term 'bleaching', and finally using the term 'La Niña'. Additional primary literature was included from bibliographies of relevant reviews or from within meta-analyses. To avoid data duplication and pseudo-replication, we did not include data from any summary reports. Although this may have limited our usable data to some extent, many reports either contained data that appeared elsewhere in the primary literature or that otherwise did not pass our selection criteria. An overview of all reviewed and included publications is given in Appendix A S1 Table, and all extracted data are available in Appendix A S2 and S3 Tables and at https://github.com/baumlab/Claar_ElNinoMetaAnalysis (DOI: 10.5281/zenodo.1134085).

We then systematically evaluated each paper returned from our searches (Figure 3.1). We first read the abstract to determine the relevance of the paper to our research questions. If deemed relevant (i.e. the focus of the study was coral responses to an El Niño/La Niña event), we then examined the full text to evaluate if the data presented could be extracted and utilized. Selected studies had to: 1) provide information on coral responses (i.e. changes in bleaching or coral cover) to El Niño or La Niña including the sample size of the study and some measure of the variance (i.e. standard deviation or error, 95% confidence intervals), and 2) compare the coral responses before and during an El Niño/La Niña event or before and after an El Niño/La Niña event. Note that for coral bleaching we also accepted studies that had not reported the extent of bleaching prior to the heat stress event. Due to our exclusion criteria, we included only field-based studies, and we did not include any laboratory manipulation studies. We obtained the dates of El Niño/La Niña events from the Australian Government Bureau of Meteorology (http://www.bom.gov.au/climate/enso/outlook/) and El Niño/La Niña data was accepted from the beginning of El Niño/La Niña heat stress up to two years after the end of the El Niño/La Niña event. It is beyond the scope of this manuscript to evaluate long-term recovery of coral bleaching and cover (see Baker et al. 2008 for an excellent review), so articles were also rejected if they quantified coral bleaching and recovery two years or more after the conclusion of the El Niño/La Niña event. Other reasons for rejecting an article included inaccessibility (e.g. not available through Web of Science, Google Scholar, ResearchGate, or author websites) or containing exclusively secondary data (e.g. reviews, meta-analyses).

a)

PRISMA 2009 Flow Diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.

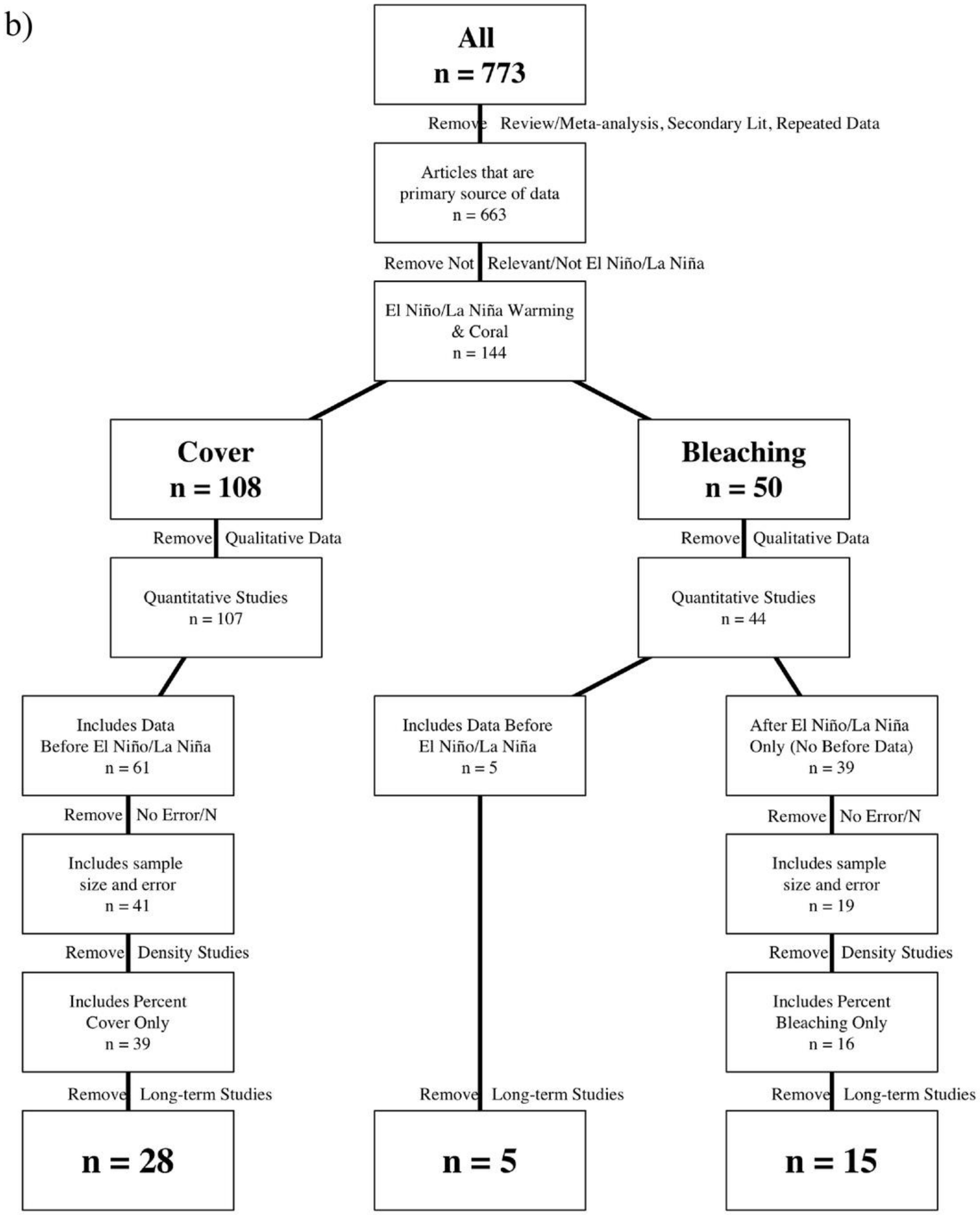


Figure 3.1. a) PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 flow diagram; and b) study-specific flowchart, both showing exclusion steps starting from studies returned from the full Web of Science and ICRS Proceedings literature search. Reviews and other meta-analyses, as well as secondary literature, or data which were repeated in more than one manuscript were excluded. Manuscripts which were not related to El Niño/La Niña warming, or otherwise not relevant to the current study were excluded. Relevant reviews were divided into manuscripts which address El Niño/La Niña-related changes in coral cover, and coral

bleaching (n = 7 studies included both). Qualitative studies were removed, as they could not be included in analyses. Coral cover studies were then excluded if they did not include before-El Niño/La Niña data. Finally, studies were excluded if they did not include either sample size or a measurement of error, did not quantify a standardized metric, or were conducted more than 2 years after the El Niño/La Niña warming event. A PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist is available in Appendix A Figure S1.

For each of the studies that met our selection criteria, we extracted the data required to calculate effect size (i.e. mean, variance, and sample size). For studies with time-series data, we defined the before value as the data point closest to the commencement of the El Niño/La Niña event, and the El Niño/La Niña value as data collected from the peak of the El Niño/La Niña event until two years after the El Niño/La Niña event. In cases where data was presented as a timeline, we extracted the earliest data point corresponding to El Niño/La Niña impact. If data were presented exclusively in a graph, we used the software GraphClick (Boyle et al. 2013) to obtain values from the figures. For studies that reported sampling error graphically, but the error bars were too small to be measured, we substituted in a small value (0.1) so that these measurements could be included in downstream analyses. If studies included both during (i.e. start of warming effects until six months after the peak) and post (i.e. six months to two years after peak warming) data points, we took each time frame as a separate data point. Where standard deviation was not reported, we calculated it from standard error or 95% confidence intervals. Since baseline coral bleaching outside of El Niño/La Niña events may not be zero, and some coral bleaching studies did not provide a before El Niño/La Niña estimate of bleaching (i.e. an estimate of local baseline bleaching), we simulated baseline bleaching levels using the conservative mean of 5% baseline bleaching (±15%

SD). Very few studies included before-El Niño/La Niña bleaching incidence or severity (n = 6), so we expanded our search to analyze published studies which did not provide before-impact data, but only provided values that were during or after the event ($n_{\text{after only}} = 23$) (Figure 3.1). We conducted two separate analyses on the full bleaching data set, with one using the extracted before-El Niño/La Niña values (with simulated before El Niño/La Niña values only for those instances where before-event bleaching was not reported), and the other using the simulated before-El Niño/La Niña values across the entire data set. We also extracted metadata from each selected paper including El Niño/La Niña year, coral taxonomic classification, sampling date, sampling depth, and latitude/longitude. In cases where geographical coordinates were not provided or were provided as the center of a region, we approximated the latitude and longitude from the text using Google Earth.

DHW calculations based on a consistent 33-year climatology

To evaluate the effects of sea surface temperature (SST) on coral reefs, we extracted a coral stress temperature data set spanning the time period of this analysis. While there are a number of pre-compiled products that provide degree-heating-week (DHW) and instantaneous bleaching thermal stress (Liu et al. 2013), they are not readily available for the full period of 1982-2016. To facilitate this analysis, we derived a new data product from the Reynolds OI 0.25° Level-4 SST analysis. This product is based on blended data from the Advanced Very High Resolution Radiometer (AVHRR) missions as well as *in situ* data and is published by the Group for High Resolution Sea Surface Temperature (GHRSST) (National Climatic Data Center 2007, Reynolds et al. 2007). We chose this optimally interpolated product because it provides gapless, quality-controlled and in situ

Table 3.1 Definition of derived variables included in our new data product. All variables are computed for a user-provided latitude/longitude and date, which can include any time point since 1984. With the exception of the first three months of 1982, we also calculate these same parameters for 1982-1983, although the beginning of usable data is in January 1982, so calculation of maximum DHW and time lag are restricted to within this window. Data are accessible at www.CoralStress.org.

Data Type	Abbreviation	Description
Current Degree Heating Week (DHW)	DHWnow	Current DHW calculated at the queried date, where any daily temperature exceeding the MMM by more than 1°C contributes to the DHW total, which is totaled over the previous 84 days (12 weeks).
Maximum DHW during El Niño	MaxDHW	Maximum DHW during the current year and the past three years.
Time Lag since Maximum DHW	TimeLag	Paired with MaxDHW. Time (in days) between queried date and maximum DHW. Only the time since the MaxDHW the corals have experienced are considered.
Current Sea Surface Temperature	SSTnow	Current sea surface temperature (SST) at the queried time.
Long-term Mean Temperature	SSTmean	Mean SST calculated over the period 1982-2014.
Long-term Temperature Variance	SSTvar	Variance of SST calculated over the period 1982-2014.
Long-term Temperature Standard Deviation	SSTstd	Standard deviation of SST calculated over the period 1982-2014.
Maximum Monthly Mean	MMM	Maximum monthly mean SST, computed by calculating the mean temperature of each month during the period 1982-2014 and selecting the month with the highest mean SST.
Month in which MMM occurs	MMMmon	Month in which the MMM occurs, ranges from January=1 to December=12.

verified (and enhanced) estimates, which minimizes known issues with satellite-only datasets, such as erroneous cold pixels, gaps due to clouds, and land contamination. We believe that the GHRSST Level-4 AVHRR product provides the best compromise between robustness and resolution for the present analysis. We performed all computations in MATLAB using daily maps at a spatial resolution of 0.25° (approximately 25-30 kilometers).

The first parameter we derived from SST was the instantaneous bleaching thermal stress, hereafter 'hotspots'. Following the methods of (Liu et al. 2013), we computed hotspots by subtracting the maximum mean month (MMM) from the current SST at each grid point. We computed the MMM by taking the mean temperature of each month during the period 1982-2014 (the first and last complete years which were available at the time of this analysis) and finding the month with the highest mean temperature, that is, the warmest month of the monthly climatology. We ran this computation individually for each grid point in latitude and longitude, with the MMM generally occurring in the boreal summer months in the Northern Hemisphere and the austral summer months in the Southern Hemisphere. From the calculated hotspots, DHW were then computed using the following criteria (included if the hotspot reached or exceeded MMM+1°C), as defined by (Liu et al. 2013), but modified to accommodate the use of daily data. For example, if the daily values of $SST – MMM$ are below zero for 11 weeks and then 0.2°C, 0.3°C, 0.5°C, 0.7°C, 0.9°C, 1.2°C, 1.3°C in the last week, only the values 1.2°C and 1.3°C would contribute to the DHW, for a total value of (1.2 + 1.3)/7, resulting in a DHW value of 0.36°C. The division by seven occurs due to the computation of DHW instead of degree-heating-days (DHD). To illustrate Time Lag, consider a point which has experienced significant heating 4 months prior to the sampling date. Here, latent effects may still be present, even though the current DHW may be zero. Therefore, in addition to current DHW, we return the previous maximum DHW and the time lag (in days) to it for inclusion in downstream analyses. To analyze a specific location and time, we return a number of additional output variables at each queried time and latitude/longitude (variables defined in Table 3.1; data accessible at https://www.CoralStress.org).

Calculation of effect sizes

We used the *metafor* package in R (R Development Core Team 2008, Viechtbauer 2010) to calculate the effect size Hedges' d (Hedges & Olkin 1985) for both percent bleaching and percent cover. We utilized Hedges' d because this commonly used effect size metric (Igulu et al. 2014, Przeslawski et al. 2015, McDevitt-Irwin et al. 2016) allows the inclusion of reported zero-values, which occurred frequently in our data set (e.g. in cases where there was zero bleaching prior to the El Niño).

(Equation 1): $d = ((Mean_1 - Mean_2)/(\left(\sqrt{(}(n_1 - 1)(s_1)^2) + \frac{(n_2-1)(s_2)^2)}{n_1+n_2-2}\right) * J)$ where $Mean_1$ is the mean percent cover or bleaching cover during or after the El Niño/La Niña event, $Mean_2$ is the mean percent cover or bleaching before the El Niño/La Niña event, s_1 and s_2 are the standard deviations, n_1 and n_2 are the sample sizes, and J is a correction for small sample size (Equation 2): $J = 1 - (3/(4(n_1 + n_2) - 1))$. Variance for Hedges' d is described by (Equation 3): $V_d = ((n_1 + n_2)/(n_1 * n_2)) + (d^2/2(n_1 + n_2)))$

Statistical analyses

We conducted all statistical analyses in R using the *metafor* package (Viechtbauer 2010). We first tested whether ENSO-associated warming increases coral bleaching or decreases coral cover by running each model without any moderator terms (i.e. random effects) to obtain the overall effect size. Here we used a random effects model, with individual estimates (e.g. by site or time point) nested within study number (i.e. publication) as the nested random effect, in order to account for heterogeneity amongst data points from within the same study and between studies, which can arise because of the consistent methodology, location, or species measured in an individual study. We used REML (restricted maximum-likelihood estimator) and we tested for within-group heterogeneity in the random effects model with the error heterogeneity estimate statistic (Q_E) (Hedges & Pigott 2004).

Next, to assess the factors influencing coral bleaching and coral mortality (measured as loss of coral cover), we used linear mixed-effects models with 'study' as the random effect and maximum DHW (MaxDHW), DHW time lag (i.e. time between maximum DHW and study measurement date; TimeLag), long-term temperature mean (SSTmean), and long-term temperature variance (calculated over the full 30-year AVHRR data set, SSTvar) as the moderators. Note that for coral bleaching we constructed two full models, one in which we simulated 'before' bleaching values for those studies missing this information and one in which we simulated 'before' bleaching values for the entire data set, as a sensitivity test of the effect of the 'before' simulation on our results. For each full model type, we used Akaike information criterion corrected for small sample size (AICc) to determine the best model by testing all possible combinations of moderators and all two-way interactions using the R package glmulti (Calcagno & de Mazancourt 2010). We also conducted reverse step-wise ANOVAs beginning with all possible combinations of moderators and continued simplifying the model until the minimum AICc was reached. For the step-wise ANOVAs we used MLE (maximum likelihood estimator) because it can be used to compare evidence weights (AICc) among models. In some cases, the top model had a similar AICc value as the next best models, we show these models in Appendix A Figures S3B and S3C. We also conducted a comparison of up to one year post-event against model results for the full two years post-event. For each moderator retained, we extracted the effect size and 95% confidence intervals from the final top model to determine significance at the different levels. To test for model fit, we used a pseudo-R^2 value calculated as follows:

$$\frac{\sum \sigma_S^2 - \sum \sigma_F^2}{\sum \sigma_S^2}$$ (Equation 4, generalized from Nakagawa & Schielzeth 2013),

where σ_S^2 is the variance component from the simple model (no moderators included), and σ_F^2 is the variance component from the full model (including all significant moderators). This pseudo-R^2 value shows how much more variance is accounted for in the full model, compared to the simple model without moderators, or how much better the fit is when moderators are taken into account. Although this is not a perfect metric for goodness of fit, it is the best method currently available to assess goodness of fit for our models.

To evaluate our data for publication bias, we evaluated our models in funnel plots, and determined the fail-safe number for each overall model (Rosenberg 2005). The fail-safe number represents the number of studies which would have to be added to the current meta-analysis in order to alter the significance of the overall effect size, with larger numbers providing support for the current result. Our fail-safe numbers for coral bleaching indicated that 118,994 papers for the fully simulated before values and 48,160 papers for partially simulated before values would need to be added in order to alter the statistical significance of the overall effect size. Similarly, for coral cover, 46,191 studies for coral cover would have to be added to alter the statistical significance of the overall effect size. These extremely large fail-safe numbers emphasize the strength of the relationship between El Niño/La Niña warming and coral decline.

Results and Discussion

El Niño heat stress

Comparisons of individual El Niño/La Niña thermal stress anomalies, using the consistent 33-year climatology in our new data product, reveal considerable differences in the overall intensity of heat stress during past El Niño events and in the locations of the maximum heat stress amongst these events (Figure 3.2 and Appendix A Figure S2). The El Niño events of 1982/1983 and 1997/1998, which were the largest prior to the 2015/2016 event (Philander 1983, Trenberth & Hoar 1997, Wilkinson & Hodgson 1999, McPhaden 1999, Goreau et al. 2000), show a typical El Niño pattern, with maximum heating occurring along the equator and the western coast of South America (Figure 3.2 and Appendix A Figure S2). In contrast, the Central Pacific El Niño or “Modoki”, with maximum heating along the equator near the dateline, can be seen clearly in the 2002/2003 and 2009/2010 El Niño events (Figure 3.2 and Appendix A Figure S2). Additionally, global maps of the maximum cumulative El Niño for each reef location illustrate stark differences in severity and areal impact of El Niño-associated heat stress (Figure 3.2).

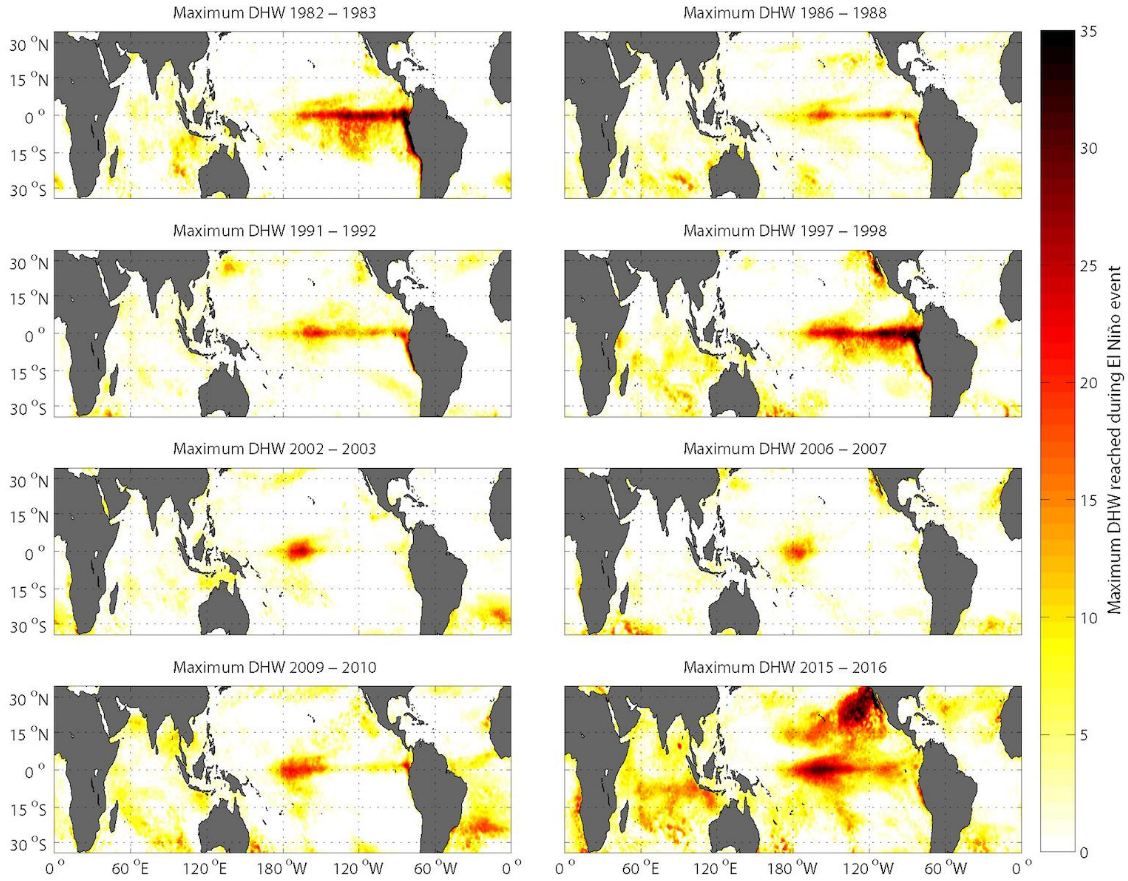


Figure 3.2. Maximum heat stress (DHW) for each reef location in the world (calculated at a 0.25° spatial resolution from AVHRR satellite data) during each of the eight El Niño events that occurred in the past 35 years.

Studies of El Niño/La Niña impacts on coral reefs

Our literature search identified a total of 773 unique publications, of which 36 fit all of our search criteria. From the original articles returned from the searches, only 144 articles addressed coral bleaching or cover losses in relation to ENSO-associated warming (Figure 3.1). Many of the initially-excluded studies were returned from our search but were irrelevant to the current meta-analysis, such as analyses of coral skeletal composition for reconstruction of historic climate. There is some overlap between coral bleaching papers (n = 50), and coral cover papers (n = 108) when split from relevant papers (n = 144), because a few studies (n = 7) provided data on both coral bleaching and coral cover. After all exclusion steps were complete, there were 5 coral bleaching studies which included "before bleaching" data, 15 coral bleaching studies which included data only during or after the bleaching event, and 28 coral cover studies which included all necessary attributes for analysis (Figure 3.1). In total, 6 studies reported on coral bleaching associated with ENSO, and 30 studies reported on coral cover losses associated with El Niño/La Niña (Figure 3.1). Our meta-analysis included a total of 453 data points globally (Figure 3.3), which we subset into coral bleaching (n = 251) and coral cover (n = 202). The majority of data points were taken from graphs (n = 158); however, data points were also obtained from tables or directly from the text (n = 72).

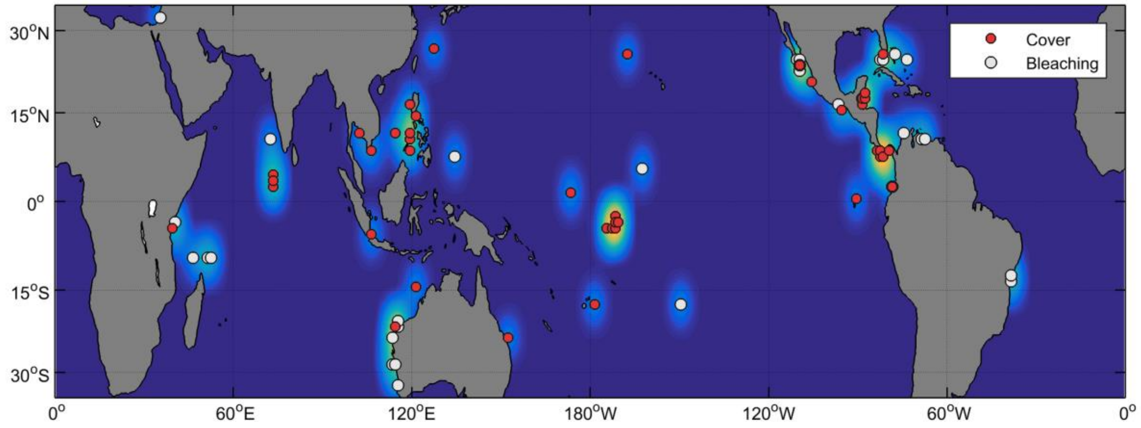


Figure 3.3. Study locations included in this global meta-analysis. Studies reporting changes in coral bleaching due to El Niño/La Niña warming are marked in white, and studies reporting changes in coral cover due to El Niño/La Niña warming are marked in red. The background color scale represents the number of data points that were extracted from each location. Data from non-El Niño/La Niña bleaching events, and from papers excluded from this meta-analysis are not included on this map.

The included studies employed a variety of survey types using both quadrat and transect methods, but in general these can be summarized into two categories: visual estimates and photo surveys. Included studies ranged in depth from 0.5 m to 40 m, although depth was often reported as a range nearly as large, hindering our ability to conduct any depth-specific analyses. We also were unable to examine taxonomic-specific effects of El Niño/La Niña-related bleaching analysis because even in the most commonly reported families (e.g. *Poritidae*, *Pocillloporidae*, *Acroporidae*) there were few (<20, generally <5-10) data points for each family. Additionally, most data points for each family were extracted from a maximum of 1-3 studies, which were often spatially clustered (e.g. *Pocilloporidae* on the Pacific coast of Central/South America). Similarly, we did not conduct analyses by biogeographic region or local sub-regions, as this is a broad-scale coral bleaching study focused on El Niño/La Niña-related warming events, and because we analyze underlying environmental factors (e.g. mean long-term temperature and variance) rather than coarse large-scale biogeographic regions. While we concede that factors such as depth and taxonomy are or may be important drivers of coral bleaching and cover changes during warming events, these specific analyses were not possible using currently available peer-reviewed research.

This meta-analysis focuses on peer-reviewed research on coral bleaching events which occur during El Niño and La Niña events. Although a great many other regional bleaching events have occurred outside of El Niño/La Niña events, the goal of this manuscript is to specifically investigate the influence of thermal stress on coral communities during El Niño and La Niña events. This may also, to some extent, limit the observed underlying environmental variability (e.g. by default our method excludes studies investigating bleaching due to cold water events, or point-source pollution). Additionally, if the "Web of Science" search is conducted similarly as above but excluding the terms (El Niño OR El Nino OR ENSO), a total of 8,686 potential papers are returned for inclusion compared to the 773 reviewed in the current study. While this would be an admirable meta-analysis, it is outside of the scope of the current study.

Table 3.2. Top model results for coral bleaching (including measured and simulated before-bleaching values) and coral cover loss (up to one year after maximum heat stress).

Model	Moderators (Top Model)	QM	df	QE	df
Bleaching	SSTmean, TimeLag:MaxDHW	7.5***	2	1652***	140
Cover	MaxDHW, SSTmean	21***	2	796***	153

QM is the test of moderators (i.e. whether the moderators explain a significant amount of variance in the model), and QE is the test for residual heterogeneity (unexplained variance) after accounting for all included variables (p-value < 0.001 noted with ***). MaxDHW is maximum DHW experienced by reef during the present El Niño event, SSTmean is the long-term mean temperature, and TimeLag is the time since maximum DHW occurred. A colon represents an interaction between two moderators. See Appendix A Figure S3 (b, c) for the top ten model results.

El Niño/La Niña effects on coral bleaching and coral cover

As expected, El Niño- and La Niña-related warming causes an increase in coral bleaching (Figure 3.4a), with maximum coral bleaching reaching 100% in some locations. The best model for the partially-simulated before bleaching data set both using glmulti and reverse stepwise ANOVAs included long-term mean temperature, SSTmean (pseudo-$R^2 = 0.058$, Table 3.2). Decreasing SSTmean lowered the effect of El Niño/La Niña heat stress on coral bleaching, with equatorial latitudes experiencing the most coral bleaching, and coral bleaching decreasing further away from the equator although this effect was small. We suggest that the most likely driver of this phenomenon is adaptation of corals to intra-annual temperature variability on higher latitude reefs (Donner 2011). Despite the fact that SSTmean accounts for a relatively small amount of variability (~6%, pseudo-$R^2 = 0.058$), it is still notable that this moderator appears to influence coral bleaching, given the variety of other factors that also contribute to patterns in coral bleaching. When variability of effect sizes between different sites/time points within a paper were taken into account, TimeLag was removed as a significant moderator. This is most likely due to the fact that we expect the effect size (i.e. how much bleaching occurred) to vary within a study in direct relation to time since maximum heating occurred. Consequently, taking this into account in our model construction essentially masks the true effect of TimeLag on coral bleaching by accounting for this in within-study variation.

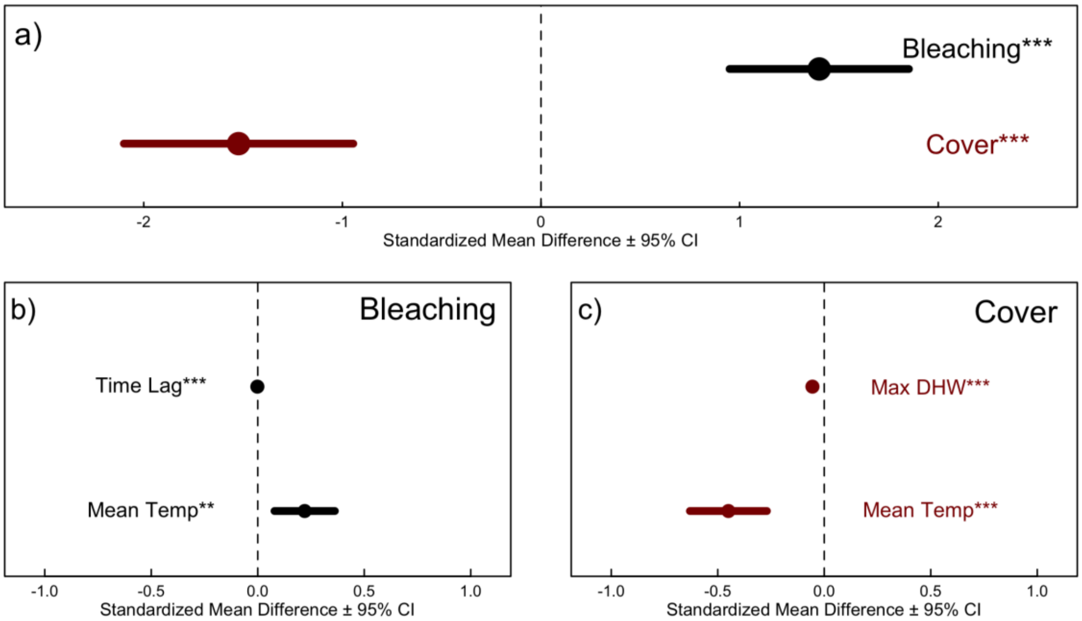


Figure 3.4. Effect size and moderators of top coral bleaching and coral cover models (p-value < 0.001 noted with ***, p-value <0.05 noted with *). a) Overall effect size (standardized mean difference ± 95% confidence intervals) for coral bleaching (black; including measured and simulated before-bleaching values) and coral cover loss (red; up to one year after maximum heat stress). El Niño/La Niña warming significantly increases coral bleaching and significant

decreases coral cover. Significant moderators in b) the coral bleaching model and c) the coral cover model. MaxDHW is maximum DHW experienced by reef during the present El Niño event, SSTmean is the long-term mean temperature, and TimeLag is the time since maximum DHW occurred. A colon represents an interaction between two moderators.

Overall, percent coral cover significantly decreased after an El Niño/La Niña event (Figure 3.4a). The maximum reduction of local reef cover among studies was 100%, which was due to taxa-specific losses estimated within studies (absolute loss across the entire coral community = 80.5%). The model moderators with the best consensus for explaining coral cover loss were MaxDHW and SSTmean (pseudo-R^2=0.047, Figure 3.4c, Table 3.2). As expected, coral cover loss increased as cumulative heat stress (MaxDHW) increased (Figure 3.4b). Long-term mean temperature is also a significant moderator, with increasing SSTmean related to decreases in coral cover. Only the top two consensus moderators were included in the main presentation of results (Figure 3.4, Table 3.2). Although three of four coral cover models agreed on these two moderators, one model that included the full two years of data (after the El Niño/La Niña peak, constructed with glmulti) also included a small, but statistically significant interaction between MaxDHW and TimeLag (Appendix A Figure S3a). The model including full two years of data (constructed with glmulti) did not include MaxDHW, although the other models (full two years of data constructed with glmulti, one year of data only and full two years of data constructed with reverse stepwise ANOVAs) did include MaxDHW as a significant moderator. Since SSTmean is a significant moderator in both bleaching and cover models, if we accept long-term mean temperature as a proxy for latitude, then low-latitude coral communities experience both higher bleaching and coral cover loss during El Niño/La Niña events (Figure 3.4) than corals at higher latitudes. However, the thermal gradient in coral cover loss we demonstrate in this meta-analysis (and the proposed corresponding decrease in coral loss with increasing latitude) is in contrast to a previously observed latitudinal gradient coral bleaching (increasing bleaching with increasing latitude) (Ulstrup et al. 2006). This lends support to the hypothesis of increased recovery at higher latitudes, where corals bleach more frequently, but are either acclimated or adapted to recover from bleaching stress.

Residual heterogeneity

Within all models, there was a significant degree of heterogeneity remaining after accounting for all significant moderators (Table 3.2). This suggests additional factors not included within these models have a significant influence upon coral bleaching and cover loss during ENSO. This may also be due to non-linearities in moderator-response interactions, which we were unable to test with the available data. With more data, or with a more constrained question (e.g. local- or regional-scale analyses), exploring non-linear models may be instructive for understanding residual heterogeneity not resolved with linear modelling. Factors not included within our models that could account for this heterogeneity include coral taxa, local adaptation, and coral depth, as well as a suite of abiotic factors including wind, upwelling and currents, and localized (i.e. meter to kilometer scale) thermal anomalies. The intensity and distribution of coral bleaching can be affected by the taxonomic composition of individual reefs, based on species-specific bleaching susceptibilities (Marshall & Baird 2000). Reef location, both depth and distance to shore, are also important factors determining the vulnerability of corals to thermal bleaching and subsequent mortality (Furby et al. 2013). Local acclimation and adaptation were not considered in our study, but almost certainly influence coral bleaching patterns as well (Guest et al. 2012). For example, local oceanographic conditions affect susceptibility to bleaching, as high water flow (McClanahan et al. 2005) and upwelling (D'Croz & Maté 2004) potentially limiting acclimation and decreasing coral tolerance to warming. Many additional factors contribute to heterogeneity in coral bleaching patterns, ranging from cloud cover (Mumby et al. 2001) and water quality (Wooldridge 2009, Wiedenmann et al. 2012), to basic coral biology and micro-complexity (Putnam et al. 2017). These factors are fundamentally important to understanding patterns in coral bleaching, survival, and resilience, and continued investigation and synthesis are encouraged as more data become available.

The novelty of the 2015-2016 El Niño event

Prior to 2015, the most extreme El Niño events observed to impact coral reefs globally occurred in 1982-83 and 1997-98. The 2015-2016 El Niño surpassed these events both in terms of ocean warming intensity and extent (Eakin et al. 2016), causing unprecedented ecological consequences worldwide. While areas affected by typical Eastern Pacific El Niño events (i.e. the coast of Central America) were still not affected worse than the massive El Niño in 1997-1998, the 2015-2016 El Niño now dominates tropical waters as the highest cumulative stress on corals globally on record (Figure 3.5). In fact, for several reefs in the Central Pacific region, the 2015-2016 El Niño exceeded the threshold "Not experienced by reefs as yet" (24 degree heating weeks) described by Hoegh-Guldberg merely 6 years ago (Hoegh-Guldberg 2011). As the return time between bleaching events decreases, we expect that the balance of long-term influence of coral resistance versus recovery from bleaching events will shift, making coral resistance incrementally more important than recovery (Côté & Darling 2010).

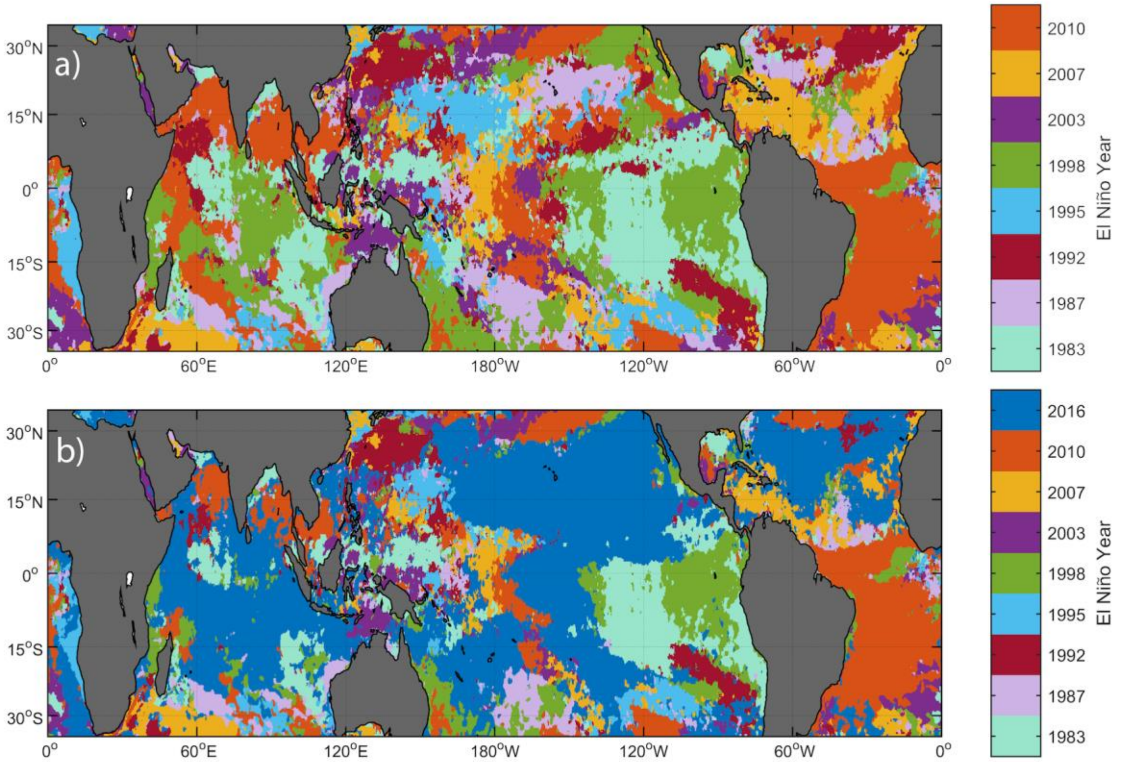


Figure 3.5. El Niño events with the greatest heat stress. Both figures show which El Niño event caused the greatest maximum DHW for each area. Note that this figure does not demonstrate bleaching response, only maximum cumulative heat stress per El Niño event. The events are color-coded by year. The 1997/1998 El Niño event (green) was the most severe event in the Eastern Pacific around the South American coast. a) All El Niño events from 1982-2010, showing how much heterogeneity there is in the geographic distribution of the most extreme heat stress. b) All El Niño events since 1982, including the 2015-2016 El Niño event, demonstrating the coral heat stress homogenization that occurred during this most recent El Niño/La Niña warming event.

Recommendations

Based on the outcomes of this systematic review of the published ENSO-warming related coral literature, we make several recommendations for future coral reef bleaching studies. First, researchers should include at least the following El Niño/La Niña warming parameters:

- Magnitude of warming: current local Degree Heating Week at the time of field sampling;
- Timing and trajectory of warming (e.g. include figure with temperature or DHW trajectory leading up to sampling time point);
- A history of bleaching events for the study location(s). We suggest including a supplementary figure of heat stress over time since 1982 (e.g. by extracting data from our new DHW data product). This would allow for examination of historic heat stress, as well as the potential for local acclimation and adaptation due to previous heat stress conditions.

Additionally, it is imperative that researchers present all study parameters including:

- Exact sampling dates for each location sampled;
- Exact GPS coordinates for each location sampled, including multiple GPS coordinates in cases where there is more than one study site;
- Sample size, at the smallest measured scale (e.g. samples per site);
- Sampling error (e.g. standard deviation or standard error);
- Any available before-impact data: coral cover, coral bleaching, including corresponding sampling errors, dates, and methods;
- Exact coral survey depth(s). Large depth ranges (e.g. 5-20 m depth), and even moderate ranges at critical depths (e.g. 2-10 m depth) obfuscate patterns in coral bleaching by greatly increasing unexplained variability in light exposure. If a study examines coral bleaching at different depths, this should be reported, with data and results specified by depth.

As well as study-specific taxonomic information including:

- Taxonomic composition of the surveyed corals, as well as species-specific coral responses to heat stress;
- Overall coral community response, in cases where the primary purpose of the study is to investigate single (or a few) coral species.

Finally, we encourage researchers to make their data and results fully open upon publication of their study. This includes but is not limited to: photo/video survey methods which can produce archives of coral reef status. Reproducibility, and consequently future synthesis work, would be enhanced by making images available online (through sites like CoralNet; Beijbom et al. 2015), and by making analyses transparent by providing data, code, and results (via sites like GitHub).

We found that only a subset of papers considered for this meta-analysis included both the temperature stress (i.e. either temperature anomaly at the time of sampling or cumulative DHW) and a specific time period of when the sampling took place. It will be much easier to identify patterns in bleaching and mortality if we can rectify the data we already have to a quantitative time frame of thermal stress. Additionally, we found that 20 out of 44 coral bleaching papers and 20 out of 61 coral cover papers did not include sample size, sampling error, or both. Finally, we note that long-term monitoring data sets are important, as they provide a baseline against which to compare changes to coral reef ecosystem structure and health, and we strongly support the development of such data sets. Building a mechanistic understanding of how local variability in baseline coral cover and bleaching changes during El Niño/La Niña warming will allow us to identify the processes that give rise to bright spots (Cinner et al. 2016) that foster coral reef resilience and recovery over the long term.

Conclusion

Understanding how El Niño/La Niña events impact coral reefs is crucial for developing strategies for coral reef conservation, which is important not only for biodiversity conservation, but also because tens of millions of people in over 100 countries rely on coral reefs for subsistence and to maintain their livelihoods (Salvat 1992). The additional benefits that coral reefs provide are extensive, including protection against wave action, provision of fish habitat (Friedlander & Parrish 1998), recreation and tourism, and aesthetic and cultural benefits (Spurgeon 1992, Moberg & Folke 1999, Ahmed et al. 2007). The resilience of these benefits is incrementally being eroded, as local stressors decrease baseline resilience (Wilkinson 1996, Souter & Lindén 2000) and climate change disables coral bleaching protection by shifting ocean warming trajectories on reefs from "protective" (trajectories that include a moderate amount of warming followed by a period of recovery before more intense heating instigates a bleaching event, essentially priming the corals to better respond to the heat), to "lethal" (trajectories that either spike rapidly and/or remain above bleaching thresholds) (Ainsworth et al. 2016). Our meta-analysis confirms that El Niño and La Niña-associated heat stress, as measured by maximum DHW, is a likely contributor to patterns of coral cover loss across the world's oceans. We also found that this trend is mediated by a temperature gradient in ENSO-associated coral cover loss, suggesting that higher latitude reefs may experience a smaller amount of El Niño and La Niña-associated decline compared to equatorial reefs. We show that there is a dearth of published studies reporting taxa-specific responses and changes in broad-scale ecosystem metrics to El Niño and La Niña, and we recommend that future studies should incorporate a broader range of resilience metrics in order to cope with measurement uncertainty and ecological surprise (Bellwood et al. 2004). Future syntheses of recent and emergent bleaching events will allow us to discover where reefs are doing better than expected, and to more accurately focus global research, management, and conservation efforts.

Chapter 4

Timing Matters: Survey timing during extended heat stress can influence perceptions of coral susceptibility to bleaching

Danielle C. Claar[1*], Julia K. Baum[1]

[1] Department of Biology, University of Victoria, Victoria, British Columbia, Canada

Published as: **Claar DC**, Baum JK (2018) Timing Matters: Survey timing during extended heat stress can influence perceptions of coral susceptibility to bleaching. *Coral Reefs* doi:10.1007/s00338-018-01756-7.

Abstract

The frequency and duration of episodic ocean warming events are increasing, threatening the integrity of coral reefs globally. Interspecific differences in susceptibility to heat stress result from variable capacities of corals to resist bleaching or to persist in a bleached state. During shorter bleaching events, stress responses occur rapidly and the 'window' for detecting bleaching is tightly constrained. However, during longer bleaching events, we argue that the timing of surveys can radically influence results, which need to be interpreted with care. For example, although 'heat resistant' corals may survive prolonged bleaching events, they have a greater chance of being recorded as having bleached because they can persist for longer in a bleached state. This could lead to erroneous conclusions about their vulnerability to heat stress compared with taxa that bleach and die rapidly. Therefore, as bleaching events lengthen, it is vital to consider not only temperature at the time of sampling, but also the accumulation of heat stress over the entire warming event. We present a simplified conceptual framework and an example from the Central Pacific to emphasize the importance of survey timing to perceived susceptibility of coral taxa to bleaching.

Main Text

Coral bleaching events are increasing in frequency and severity worldwide at an alarming rate (van Hooidonk et al. 2013, Heron et al. 2016). The corollary of this dramatic increase is that recovery intervals separating major bleaching events have diminished (Hughes et al. 2018). Additionally, coral bleaching events have recently shifted from heat stress exposures between 4 and 12°C-weeks on average (Hoegh-Guldberg 2011), to exceeding exposures of 24-35°C-weeks in some locations (Boyle et al. 2017, Brainard et al. 2018, Claar et al. 2018). Climate change models predict that these events will continue to increase this century (Cai et al. 2014). This underscores the importance of understanding how different coral species respond to episodic pulse heat-stress events, in order to determine which species and reefs will persist, and therefore how the structure and diversity of overall coral communities will be impacted over the coming decades.

Corals can exhibit marked intra- and inter-specific differences in their responses to heat stress events, with some colonies succumbing quickly to bleaching and death and others able to endure longer periods at elevated temperatures either in the fully pigmented or bleached state (Marshall & Baird 2000). Species-specific response differences, which may be attributed to coral life history traits (Darling et al. 2012, Madin et al. 2016) such as colony growth rate (Gates & Edmunds 1999) and investment in energy storage (Loya et al. 2001, Rodrigues & Grottoli 2007), have given rise to the concept of “winners” and “losers” (Loya et al. 2001). Because areas with thermally sensitive coral species tend to bleach more readily and more frequently than those with thermally tolerant coral species (Marshall & Baird 2000), species-specific responses can significantly alter the relative abundance of coral taxa at individual reefs toward thermally tolerant species, over the course of one or multiple bleaching events (Loya et al. 2001, Grottoli et al. 2014). Due in part to variability in coral species composition, the effect of pulse heat-stress events on coral reefs varies across scales, with susceptibility of corals varying amongst regions, islands, and reefs.

Typically, slow-growing mounding corals (e.g. massive *Porites*) are resistant to bleaching and are considered to be stress-tolerant (Loya et al. 2001, Darling et al. 2012). Conversely, fast-growing, branching coral species (e.g., *Pocillopora* and *Acropora*) are thermally sensitive and susceptible to bleaching (Loya et al. 2001, Putnam et al. 2012, Darling et al. 2012). Stress-tolerant corals are therefore generally “winners” and thermally sensitive species “losers” during thermal stress (Gleason 1993, Loya et al. 2001, Edwards et al. 2001, Hongo & Yamano 2013). However, recent monitoring studies (Chou et al. 2016), as well as rapid coral bleaching assessments in the Indo-Pacific Ocean during the 2015-2016 El Niño event have documented cases where this pattern appears to be reversed, with greater frequencies of bleaching observed in ‘stress-tolerant’ species, such as *Porites,* than in putatively thermally sensitive species. In this perspective piece, we examine the question: Why are “heat-resistant” corals sometimes observed to bleach more than “heat-sensitive” corals? We acknowledge that some seemingly counterintuitive observations in bleaching patterns of *Acropora* and *Porites* may reflect the capacity of these species for acclimation (Guest et al. 2012). We do not discount observed changes in bleaching frequency due to acclimation, and we agree that species-specific rates of acclimation and adaptation fundamentally impact bleaching resilience (Pandolfi et al. 2011). Instead, we propose an alternative survey-dependent mechanism to explain counterintuitive bleaching observations that does not depend upon coral acclimation or adaptation. We suggest that because coral species and colony-specific holobiont assemblages exhibit variable capacities to resist bleaching or to persist in a bleached state during prolonged heat stress events, the timing of bleaching surveys can influence perceptions of coral species' susceptibility to bleaching.

Species-specific bleaching responses

We frame our discussion around two evolutionarily and physiologically distinct coral taxa (i.e. one idealized heat tolerant, e.g. massive *Porites;* and one idealized heat sensitive, e.g. *Pocillopora*) for simplicity while recognizing that on natural reefs these strategies are more realistically represented by a gradient of potential responses. Heat-sensitive coral species may be able to retain their symbionts (or symbionts avoid being expelled; Silverstein et al. 2017) for a short time. However, once a sensitive coral starts to bleach, a combination of physiological factors (Putnam et al. 2017; including thin tissues and limited energy reserves, Loya et al. 2001 but see Lesser 2013), and a higher probability of containing thermally tolerant but potentially opportunistic symbionts (Putnam et al. 2012, Silverstein et al. 2017), mean that it can persist in the bleached state for only a limited amount of time before either recovering or dying (Baird & Marshall 2002, Jones 2008). Since thermally sensitive coral species are generally more likely to bleach, when a researcher surveys a reef during a short bleaching event, they would most likely predominantly observe bleaching of these sensitive species. In contrast, heat-tolerant species can generally resist bleaching for longer than thermally sensitive corals (Putnam et al. 2012). For example, in heat-tolerant massive *Porites*, this is due in part to a more evolutionarily-derived symbiosis between *Porites* and Symbiodiniaceae of the genus *Cladocopium* (C15; LaJeunesse 2005, LaJeunesse et al. 2018). Additionally, corals with thick tissues may be pre-conditioned to disturbances by environmentally-mediated priming, where internal (i.e. within-tissue) variability of physiochemical conditions allows microscale acclimation that prepares corals for external stress (Putnam et al. 2017). Furthermore, *Porites lobata* can increase heterotrophy and potentially dissolved organic carbon (DOC) uptake, which facilitates the maintenance of energy reserves during bleaching (Levas et al. 2013). These characteristics allow thick-tissued corals to resist, persist in, and recover from the bleached state over longer periods than thermally sensitive corals, making them generally less likely to bleach during ‘normal’ (i.e., short-term) thermal stress events. After heat stress subsides, thermally tolerant corals are generally able to recover (either fully, or with partial tissue loss), unless heat stress is extreme, although recovery may take longer than in thin-tissue corals (Gleason 1993). These taxa-specific bleaching patterns have been observed consistently on reefs around the globe, making recent observations of reversed bleaching dynamics appear conspicuous.

Bleaching observations during extended heat stress events are survey-timing dependent

With limited resources, local bleaching surveys and broad-scale monitoring efforts are often constrained to a single survey time point during the course of a heat stress event (Marshall & Baird 2000, Berkelmans et al. 2004, Zapata et al. 2010, Kelmo & Attrill 2013). Many coral bleaching studies also have been reactive, with sampling occurring only after the onset of widespread coral bleaching across the reef, at sites without any data on the ‘pre heat-stress’ coral community composition. Costs and complex logistics of ecological monitoring exacerbate these constraints in remote reef locations. When bleaching events are short-lived, it is likely that single surveys can accurately summarize the impacts of heat stress. However, as cool-season reprieves diminish (Heron et al. 2016) and bleaching events become longer, multiple monitoring surveys for each event likely will be needed to accurately quantify the impacts of heat stress. If multiple surveys during a bleaching event are not feasible due to logistical constraints, explicit consideration of survey timing with respect to heat stress is imperative so that the impact of bleaching can be accurately quantified and compared across studies and regions (Claar et al. 2018).

We now consider how a change in the timing of bleaching surveys could influence perceived species-specific bleaching responses during an extended heat stress event. We assume that monitoring in this example is reactive (i.e. all surveys occur after the onset of bleaching). This is often the case, even in repeatedly monitored sites, as researchers are interested in documenting bleaching patterns and prevalence and increase sampling efforts in response to a bleaching event. Before heat stress begins, we assume that effectively all existing corals are healthy, and negligible bleaching is observed (Figure 4.1). This assumption may not hold for reef ecosystems, since changes in coloration and minor bleaching (<25% of a coral colony) may occur due to seasonal variability (Gates 1990). This, along with other simplifying assumptions herein, should be carefully considered when applying this theoretical framework to real ecosystems. If researchers surveyed the reef at the onset of heat stress (Figure 4.1, time point A), they would document minimal bleaching of sensitive species (e.g. *Pocillopora;* depending on the dominant symbiont or local host/holobiont characteristics), as well as either some or no bleaching of thermally tolerant species (e.g. *Porites*). Observers would record no recent mortality at this time point. At the peak of heat stress (Figure 4.1, time point B), the prevalence of bleaching in thermally sensitive corals may appear very similar to the onset of heat stress (Figure 4.1, time point A). However, if time point *B* represents a significant amount of heat stress, the underlying symbiont community structure of affected corals may have already shifted. By this point, *Pocillopora* corals with thermally sensitive symbionts, genotypes, or phenotypes may have already started to die, while those with thermally tolerant symbionts, genotypes, or phenotypes may begin to bleach. If the reef was surveyed only at this time point one might conclude that thermally tolerant corals are as, or more, sensitive to heat stress than thermally sensitive ones since colonies of both types would be bleaching. By time point *C*, most heat stress has dissipated from the system (since a DHW of 0 indicates no accumulation of heat stress for 12 full weeks), and most thermally sensitive corals will have reached their end status; that is, if they are going to survive the event, they will have regained their symbionts, otherwise, they will have exhausted their energy reserves and died. Thermally tolerant coral species may still be bleached at time point *C*, relying on either heterotrophic feeding or energy reserves to maintain biological function. If the reef was surveyed only at this time point, it could lead to the incorrect conclusion that thermally tolerant coral species were more affected by the heat stress than thermally sensitive ones (i.e. if the thermally sensitive colonies that died are not recorded at all in surveys, and thermally tolerant colonies are recorded as being bleached). This scenario also highlights the importance of recording not only bleached corals, but also recent mortality. Finally, the outcome of the bleaching event (Figure 4.1, time point D) records either survival or mortality of each of sensitive and tolerant corals. Therefore, depending on the timepoint the survey take place (and whether recent mortality is recorded), results may appear counter to expectations from lab studies presented above.

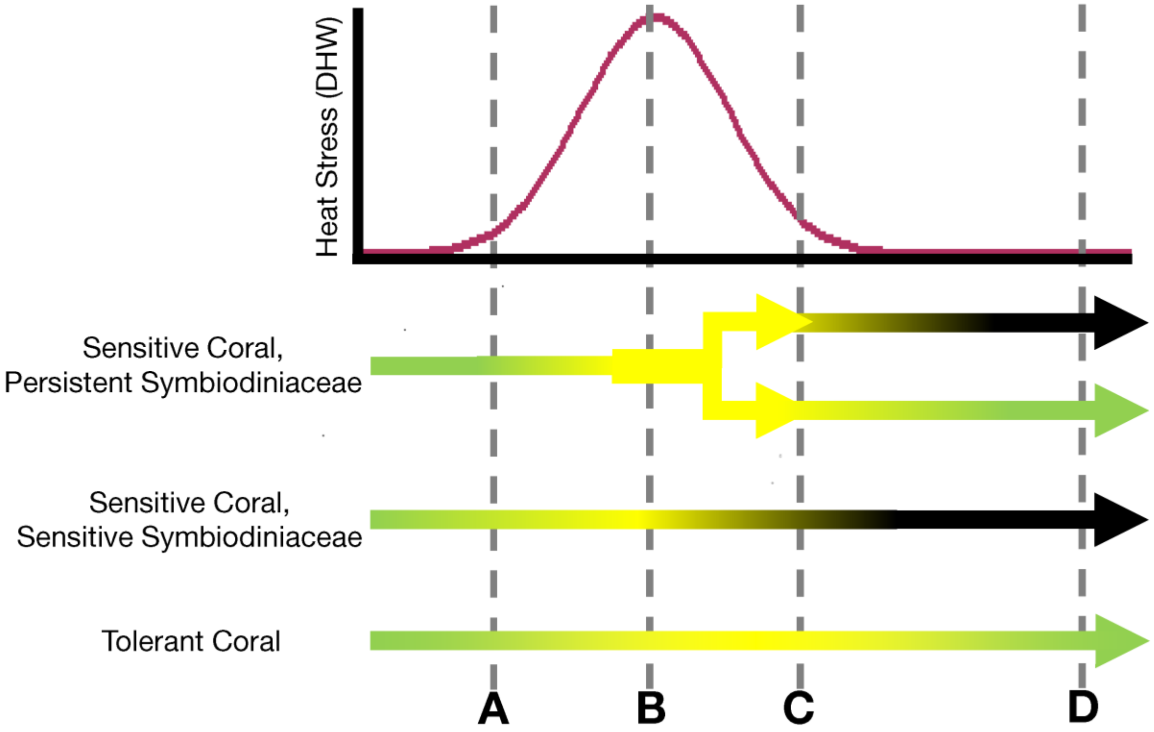


Figure 4.1 Potential trajectories of coral bleaching, survival or mortality for three model coral types over the course of a prolonged heat stress event (measured in degree heating weeks (DHW, °C-week)) : i) a sensitive coral (e.g. *Pocillopora*) in symbiosis with persistent Symbiodiniaceae (e.g. *Durusdinium*, previously *Symbiodinium* clade D), ii) a sensitive coral (e.g. *Pocillopora*) in symbiosis with a less persistent Symbiodiniaceae (e.g. *Cladocopium*, previously *Symbiodinium* clade C), and iii) a thermally tolerant coral (e.g. *Porites lobata* or *Platygyra daedalea*). Trajectories are color-coded as follows: green represents a healthy, unbleached coral; yellow represents a bleached coral colony; black represents a colony that died. Letters (A-D) denote four potential sampling time points during the heat stress event.

The importance of survey timing was also emphasized by a previous study in which repetitive bleaching surveys were conducted during the 1997/1998 El Niño (surveyed 6, 10, 14, 20, 28, and 40 weeks after bleaching began; Baird & Marshall 2002).

This study found that six weeks after bleaching began, when all living *Acropora* were bleached and massive corals (e.g. *Porites*, *Platygyra*) were only nominally affected, that 70% of the previously abundant *Acropora hyacinthus* population were already dead. Between six and ten weeks after bleaching began, more *Acropora* died, but others had completely recovered. Within this same period, bleaching increased across the community and more than half of the massive corals became severely bleached. Fourteen weeks after bleaching began (when temperatures dropped to the seasonal average), *Acropora* had reached their end status (i.e. fully recovered, or dead), but many massive colonies remained bleached for several months after. Without baseline data, the loss of a large proportion of *Acropora* colonies could have been missed. Furthermore, if sampling had only taken place at any one of these timepoints, the conclusions drawn would not have shown the complete picture of coral species-specific responses during this bleaching event. For example, at 10 or 14 weeks into the event, one might have erroneously concluded that *Acropora* were not affected but that massive corals were. By surveying bleaching at multiple time points during this event, the authors accurately captured the dynamics of bleaching and mortality and provided a foundation for future work.

Specific example - Kiritimati Island during the 2015-2016 El Niño

A more recent example of the importance of survey timing comes from a study of coral bleaching, mortality, and survival during extended heat stress on Kiritimati atoll over the course of the 2015-2016 El Niño event, we observed what at first appeared to be counterintuitive species-specific bleaching patterns (Figure 4.2). We note that this example is unusual compared to previous bleaching events, in that this is the first record of a year-long continuous heat stress event. While not representative of most coral bleaching events to date, the extended nature of this heat stress event underscores the importance of bleaching survey timing, as heat stress events continue to lengthen under climate change. After two months of heat stress (reaching DHW = 11.8°C-week;

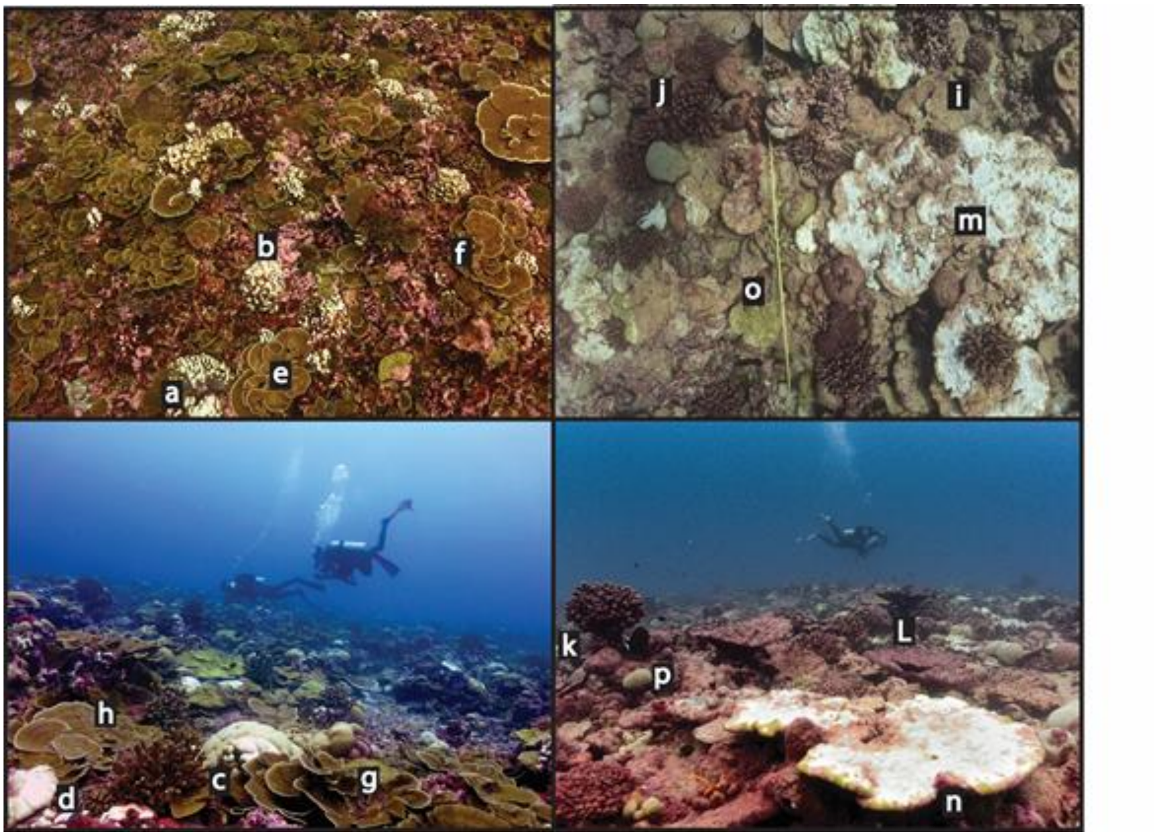


Figure 4.2 Example images from the 2015-2016 El Niño bleaching event on Kiritimati Island. This extended bleaching event lasted for over ten months and reached 24.7°C-weeks (Chapter 8). Left panels: Two months after heat stress began, July 2015 (~11.8°C-weeks), showing bleaching massive corals (a, b: *Goniastrea stelligera*; c: *Dipsastraea speciosa*, *Astreopora* sp.; d: *Porites lobata*) adjacent to apparently healthy branching and plating corals (e-h: *Montipora aequituberculata*); Right panels: Near the end of the bleaching event (nine months after heat stress began, March 2016) showing dead branching and plating corals (i: *M. aequituberculata*, j-k: *Pocillopora grandis*, l: *Acropora* sp.), and bleaching (m, n: *P. lobata*) and recovering/apparently healthy (o: *P. lobata*, p: *Platygyra daedalea*) massive corals.

approximately halfway between Figure 4.1 time points A and B), many heat-tolerant corals (e.g. *Platygyra daedalea*, *Porites lobata*, *Dipsastrea matthaii*) were mostly or fully bleached, while thermally sensitive corals (e.g. *Pocillopora eydouxi* and *Montipora aequituberculata*) still appeared fully pigmented. If we had surveyed this atoll's reefs only on this one occasion, we might have concluded that Kiritimati has extraordinarily resistant *Pocillopora* and *Montipora* (or extraordinarily sensitive Merulinids, e.g. *Platygyra* and *Dipsastrea*). However, when we returned after ten months of heat stress (peak DHW = 24.7°C-week; sampling just before Figure 1 time point C), we found nearly the opposite pattern: mass mortality of the thermally sensitive species and many colonies of thermally tolerant species that had either persisted in the bleached state or recovered to full pigmentation (Figure 2). These patterns (with example photos in Figure 2) were observed at many sites around the atoll. Subsequent sampling in late 2016 and 2017 revealed that indeed many colonies of these thermally tolerant species had survived the event.

This study also underscored the importance of sampling before a bleaching event for accurate documentation of coral mortality and changes to overall coral community composition. For example, large table *Acropora* were present at several of our sites prior to the heat stress event. While these *Acropora* were variably bleached two months into the heat stress event (adjacent colonies exhibited all states between full "healthy" coloration and severe bleaching), they were all dead after ten months of heat stress. Since it is difficult to determine a coral's "time of death" from a skeleton (as turf and/or macroalgae are quick to overgrow exposed coral skeleton), it is vital to have quantitative pre-bleaching estimates of taxa-specific cover. These baseline measurements are particularly important during prolonged bleaching events, because as the duration and severity of warming increases, the possibility of local extirpation of entire coral taxa also increases, erasing knowledge of the historical state. Long-term coral reef monitoring programs, such as that of the National Science Foundation's Long-term Ecological Research (LTER) site at Moorea, French Polynesia (e.g. Adam et al. 2011, Holbrook et al. 2018), NOAA's Pacific Reef Assessment and monitoring program (Kenyon et al. 2006, Brainard et al. 2018) and the AIMS Long Term Monitoring Program (LTMP) on the Great Barrier Reef (e.g. Sweatman et al. 2004), are of critical importance in this regard. These extensive data sets record baseline dynamics against which responses to acute disturbances can be compared, providing vital insights into underlying biological and ecological mechanisms of community change.

We suggest that counterintuitive observations in species-specific bleaching patterns may be emerging due to a mismatch between the frequency of ecological bleaching surveys and the fundamental shift in the severity of thermal stressors facing coral reefs today. As the temporal extent of bleaching events expands, timing of surveys will increasingly influence observed trends in species-specific bleaching. In conclusion, we propose that timing of coral bleaching monitoring influences observations and species-specific conclusions. If unaccounted for, the timing of coral monitoring will become a more commonly confounding factor in bleaching studies, as pulse heat stress events become prolonged and more intense on a global scale. We recommend that, if at all possible, repeat surveys should be conducted throughout the course of long bleaching events, as well as following recovery. At minimum, we recommend researchers report the amount of accumulated heat stress at the time of sampling (DHW) compared to the overall event (i.e. at what point in the timeline of the overall heat stress event the survey was conducted), and explicitly consider this factor when interpreting the results of their bleaching surveys. Additionally, recent coral morality should be recorded in the field when possible and compared to baseline coral abundances to ensure that changes outside of the bleaching survey are not missed. Ideally, long-term surveys can establish accurate baselines before bleaching events and can be used to detect overall changes in response to episodic thermal stress. Careful consideration of survey timing will minimize spurious conclusions and maximize the amount of knowledge extracted from coral bleaching studies.

Chapter 5 Chronic disturbance modulates symbiont beta diversity on coral reefs

Danielle C. Claar[1*], Kristina L. Tietjen[1], Kieran D. Cox[1,2], Ruth D. Gates[3], Julia K. Baum[1,3]

1 Department of Biology, University of Victoria, Victoria, BC, V8W 2Y2, Canada
2 Hakai Institute, Calvert Island, BC, Canada
3 Hawaii Institute of Marine Biology, Kaneohe, HI 96744, USA

In prep as: **Claar DC,** Tietjen KL, Cox KD, Gates RD, Baum JK (*in prep*) Chronic disturbance modulates symbiont beta diversity on coral reefs. *MEPS.*

Abstract

Chronic disturbance can alter marine communities, threatening ecological interactions including the foundational symbiosis between reef-building corals and *Symbiodinium*. *Symbiodinium* are photosynthetic endosymbionts necessary for coral survival, but they can also be free-living in the environment. With bleaching events in which corals lose their symbionts becoming increasingly frequent, and most corals acquiring *Symbiodinium* from the environment each generation, free-living populations represent important pools from which corals draw their symbionts. Yet, little is known about the diversity of free-living *Symbiodinium* or how disturbances alter these communities. To determine how chronic (i.e. local human disturbance) and pulse (i.e. a storm event) stress influence *Symbiodinium* communities, we sampled three ecological compartments - sediment, water, and coral - at two levels of chronic disturbance, three times over nine months. We found that chronic stress was consistently associated with higher symbiont beta diversity (i.e. variability amongst assemblages). We also found a change in community composition (i.e. turnover) due to chronic disturbance in all compartments except one coral species. Pulse stress exhibited some influence on

symbiont beta diversity, but the effect was inconsistent. This suggests that the effects of chronic stress are more prominent than temporal variability during a pulse stress event for shaping these symbiont communities.

Introduction

Disturbances, both natural and anthropogenic, can alter diversity and cause stress on ecosystem structure and function. Stress can manifest as chronic disturbance (i.e. long-term or press disturbance) or pulse disturbance (i.e. short-term or acute disturbances events) (Bender et al. 1984, Novak et al. 2016). Both types of disturbance can alter not only alpha diversity (i.e., species diversity within a community; e.g. Hobbs & Huenneke 1992, Cayuela et al. 2006) but also beta diversity (i.e., differences in species composition among communities (Anderson et al. 2011, Clavel et al. 2011, Magurran et al. 2015, Socolar et al. 2016), which has recently emerged as a sensitive indicator of ecosystem change under stress (Dornelas et al. 2014, McGill et al. 2015). Beta diversity metrics can be split into two working definitions: ‘turnover’ can be defined as compositional changes of species assemblages across a gradient, while ‘variation’ can be defined as the amount of variability among species assemblages within each community (Anderson et al. 2011). When communities are analyzed in a multivariate context, ‘turnover’ represents multivariate location, while ‘variation’ represents multivariate spread (beta dispersion). A growing body of literature has shown that at large scales, disturbances often cause a decrease in beta diversity, leading to ‘biotic homogenization’ (i.e. communities become simplified and more similar to one another; (McKinney & Lockwood 1999, Olden & Poff 2004, Karp et al. 2012, Iacarella et al. 2018). At microecological scales, however, emerging evidence suggests that disturbance may have the opposite effect, resulting in increased beta diversity (e.g. Zaneveld et al. 2016, McDevitt-Irwin et al. 2017; reviewed in Zaneveld et al. 2017). This effect has been attributed to the ‘Anna Karenina’ principle, wherein stress causes an increase in stochastic changes (i.e. variation) and community dispersion within a microbiome, as opposed to directional changes (i.e. turnover) in community structure (Zaneveld et al. 2017).

Understanding how disturbances alter the diversity of coral reefs is of high priority, given the biological and socioeconomic importance of these diverse tropical ecosystems (Costanza et al. 1997, Bellwood et al. 2004), and one of the most critical and susceptible components of these ecosystems is the symbiosis between scleractinian corals and the diverse dinoflagellate genus *Symbiodinium*. These coral-algal symbioses are an important component of coral health, but sensitive to chronic stressors, such as poor water quality and eutrophication (Wiedenmann et al. 2012, Cunning & Baker 2013, Vega Thurber et al. 2014, Browne et al. 2015). There is significant functional diversity within *Symbiodinium* (Stat et al. 2008), and local conditions such as natural environmental variability and chronic human disturbance act as filters to limit the types of *Symbiodinium* available for coral symbiosis (Quigley et al. 2017). Therefore, local conditions are important drivers that set the baseline and potential scope of corals’ ability to respond to pulse stress events. Thermal gradients have a strong influence on *Symbiodinium* biogeography, but chronic local stressors may also influence these patterns (Tonk et al. 2013, Kennedy et al. 2016). When the symbiosis between corals and *Symbiodinium* breaks down due to environmental stress, corals bleach (Brown 1997). Bleaching events are now occurring globally at an alarming rate, exacerbated by global warming and chronic local stressors such as fishing, pollution, and eutrophication (Hoegh-Guldberg 1999, Hughes et al. 2003, Hughes, Anderson, et al. 2018).

Since 80-85% of all scleractinian (reef building) coral species must uptake symbionts anew from the environment each generation (Richmond & Hunter 1990, Baird et al. 2009), *Symbiodinium* in the environment are vital for the initiation of new symbioses with coral larvae and newly settled recruits. *Symbiodinium* in the environment may also provide a source for adult corals to uptake new *Symbiodinium* after disturbance and bleaching (LaJeunesse et al. 2010, Boulotte et al. 2016), but the frequency and ecological relevance of this uptake is contested (Little et al. 2004, Coffroth et al. 2010). *Symbiodinium* are found in several environmental "compartments", including sediment, the water column and as epiphytes on macroalgae and seagrass (Littman et al. 2008, Manning & Gates 2008, Hirose et al. 2008, Takabayashi et al. 2012, Yamashita & Koike 2013, Sweet 2014, Granados-Cifuentes et al. 2015). *Symbiodinium* found in the environment ("free-living *Symbiodinium*") can be divided into two categories: 1) *Symbiodinium* that do not participate in symbioses and only live separate from other organisms are referred to as "exclusively free-living *Symbiodinium*"; and 2) symbiotic *Symbiodinium*, which are typically associated with invertebrate hosts but can be transient in the environment, are known as "transiently free-living *Symbiodinium"* (Coffroth et al. 2006, Yamashita & Koike 2013, Nitschke et al. 2016). It is likely that transiently free-living *Symbiodinium* do not persist perpetually outside host corals, but rather that these populations are ephemeral and consistently replenished (Thornhill et al. 2017). Therefore, the diversity and stability of free-living *Symbiodinium* communities are likely subject to environmental fluctuations (Pochon et al. 2010, Huang et al. 2013).

Although free-living *Symbiodinium* provide a pool of potentially viable symbionts for coral uptake, little is known about how these *Symbiodinium* communities vary in response to chronic stressors (which often vary across locations) and pulse stressors (which typically are of short-duration and hence require measurement over time). Initial research showed that free-living *Symbiodinium* abundance can be spatially variable at local scales, with cells preferentially aggregating in reef habitats (Littman et al. 2008). Additional research showed that free-living *Symbiodinium* communities may vary over space (km-scale), but can remain consistent over time (~17 months; Sweet 2014), and that thermal variability may drive differences in *Symbiodinium* beta diversity (Cunning et al. 2015) . Nonetheless, significant questions remain about the variability of *Symbiodinium* communities across distinct environmental compartments, and how chronic and pulse disturbances influence them, including: 1) Does chronic stress change the beta diversity of free-living *Symbiodinium* communities? 2) Can pulse physical disturbance events instigate ecologically meaningful changes in *Symbiodinium* beta diversity, thereby altering the availability of certain symbiont types for uptake into symbiosis? and 3) Are the impacts of chronic and pulse disturbances consistent across coral species, and other environmental compartments?

In this study, we investigated how chronic and acute disturbance alters the beta diversity of *Symbiodinium* communities. First, we examined the influence of chronic local disturbance (fishing pressure, decreased water quality, pier infrastructure) by sampling free-living symbionts in the water and sediment, and three dominant coral species at two sites with different disturbance intensities (medium and very high). We hypothesized that, unlike for macro-organisms, these disturbances would increase observed *Symbiodinium* beta diversity in both coral-associated and free-living symbiont communities, causing turnover and increased variation. Second, we tested whether and how a natural pulse disturbance, namely a major winter swell event, altered *Symbiodinium* communities across compartments. Since seasonality is minimal at our equatorial field site, such storm waves generated in the western Pacific may be one of the main drivers of intra-annual temporal variability in *Symbiodinium* exchange among compartments in this region. Storm wave events can lead to mechanical homogenization of the water column and suspension of the benthos, as well as releasing *Symbiodinium* from corals through coral injury and death and may provide important opportunities for exchange among these compartments. We hypothesized that physical disturbance caused by a major storm swell event would homogenize *Symbiodinium* communities between free-living *Symbiodinium* pools, thus decreasing beta diversity, but that it would not significantly alter coral-associated *Symbiodinium* communities. Understanding the variability of *Symbiodinium* communities in response to multiple stressors can provide insights into how coral symbioses interact with their dynamic environment, and hence how these critical symbioses may be re-established following disturbance events.

Methods

Study design

We collected coral, water, and sediment samples from four sites on the fore reef (all at 10-12 m depth; Fig. 1) of Kiritimati (Christmas Island, Republic of Kiribati), a large atoll in the central equatorial Pacific (01°52′N 157°24′W) in August 2014, January 2015, and May 2015. Two of the sample sites (VH1 and VH2) experience very high human disturbance in the form of localized fishing pressure, sedimentation and pollution due to pier infrastructure near VH2, and localized sewage runoff from adjacent villages, while the other two sites (M1 and M2) experience a moderate level of human disturbance, primarily from fishing (Figure 5.1; Walsh 2011, Watson et al. 2016). Large swell from a major storm that hit the leeward (western) side of the atoll in January 2015 significantly impacted the reefs at all four sites. We quantified the intensity of storm waves during this time using NOAA's Multi-grid WaveWatch III Hindcast (Appendix B Supp. Material and Appendix B Fig. S1). We collected a small subset of coral samples in the days immediately preceding the storm disturbance but were unable to collect water or sediment samples before the arrival of the storm waves. Water, sediment, and coral samples were collected from all four sites immediately after the waves subsided enough to allow diving, but while the water was still well-mixed and turbid from the storm.

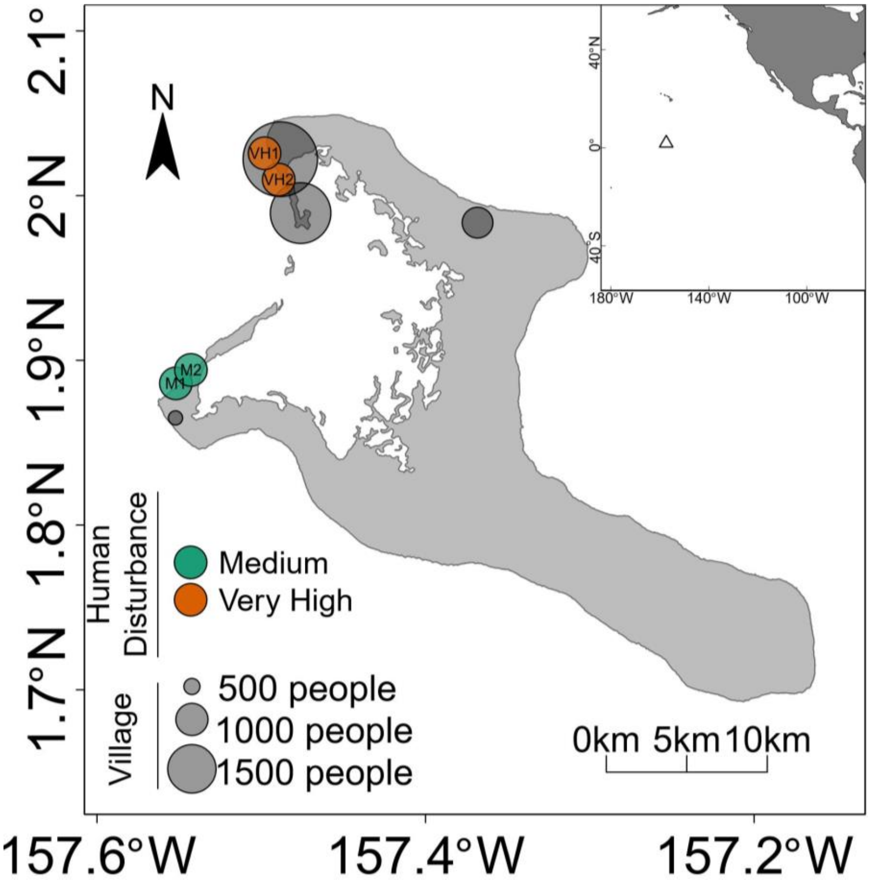


Figure 5.1 Sample sites on Kiritimati, showing locations with medium (M1 and M2) and very high (VH1 and VH2) local human disturbance. Inset shows Kiritimati's location in the central equatorial Pacific Ocean (open triangle).

Sample collection and processing

To quantify *Symbiodinium* sequence identities, we collected coral tissue samples from *Pocillopora grandis* (n = 70; previously *Pocillopora eydouxi*), *Montipora aequituberculata* (n = 78), and *Porites lobata* (n = 73), along with water (n = 39) and sediment (n = 58) samples (see Appendix B Table S1 for sample size by site). We sampled coral colonies along a 60 m transect at each site, using a small metal chisel to biopsy the coral tissue (<1 cm^3). Coral samples were transferred to sterile sample bags, and then stored in a cooler until laboratory processing began. We collected water samples (2.98-10.95 L seawater per sample; Appendix B Table S2) within 1 m of the reef surface, with the goal of collecting four samples per site during each field season. We collected sediment samples (2-7 mL per sample; Appendix B Table S3) along the same transect as the coral colonies (i.e. <1 m distance from the transect tape), sampling the top layer of sediment within 3 cm of the water-sediment interface, which was generally loose and well-mixed.

During processing, we cleaned coral biopsies using filtered freshwater to remove as much skeleton as possible, and approximately 50 µL of coral tissue was preserved in 400 µL of guanidinium buffer (50% w/v guanidinium isothiocyanate; 50 mM Tris pH 7.6; 10 µM EDTA; 4.2% w/v sarkosyl; 2.1% v/v-mercaptoethanol). All samples were stored at 4°C until DNA extraction. To process the sediment samples, we resuspended each sample in 350-400 mL filtered seawater and filtered them using a two-step process. First, the resuspended sediments were gravity filtered through a 20 µm pore size nylon mesh filter, and the resulting solids were discarded. Secondly, the filtrate was passed through a Whatman Nucleopore track-etched 5 µm pore size filter. Each 5 µm filter and its contents was preserved in a 1.5 µL centrifuge tube with 400 µL of guanidinium buffer, and the filtrate was discarded. We filtered the water samples using the same two-step filtering process, and then stored them in the same way as the sediment samples. Quantities of water and sediment filtered for each sample are listed in Appendix B Tables S3 and S4.

DNA was extracted from all samples using a modified guanidinium-based extraction protocol (Supplementary Methods; Stat et al. 2009, Cunning et al. 2015). Samples were prepared for ITS-2 amplicon sequencing on the Illumina MiSeq platform (see Supplementary Methods).

Bioinformatics

All code for this bioinformatics pipeline is available on GitHub (https://github.com/baumlab/ Claar_et_al_2019_KI_Compartment). Illumina-Utils (Bokulich et al. 2013, Eren et al. 2013) was used to filter raw fastq reads (iu-filter-quality-bokulich) and merge paired-end sequences (iu-merge-pairs, max-mismatch = 3 bases between reads per sequence). After initial quality filtering, we processed all sequences, generated operational taxonomic units (OTUs) by clustering at 97% sequence similarity within each sample, and identified *Symbiodinium* types following the procedure in (Cunning et al. 2017). Resulting OTU tables, taxonomic information, and sample metadata were analyzed using the *phyloseq* package (McMurdie & Holmes 2013) implemented in R (R Development Core Team 2008). OTUs were removed if they were observed fewer than 5 times in the entire dataset (removed n = 150 of 504 initial OTUs; removed n = 424 sequences, or <0.0001% of all sequences), and samples were removed if they were comprised of fewer than 100 sequences (removed n = 20 of 338 samples). A total of 318 samples were successfully amplified and sequenced (coral n = 221; sediment n = 58; water n = 39; Appendix B Table S1), yielding a final dataset consisting of 4,166,542 sequences. Finally, a phylogenetic tree (similar to Putnam et al. 2012) was built by separating *Symbiodinium* sequences by clade identity. Sequences were aligned within each clade separately (align_seqs.py from QIIME; Caporaso et al. 2010) using the muscle program (Edgar 2004). A distance matrix was created based on nr28s-rDNA distances (Pochon & Gates 2010) and within-clade alignments, and *upgma* (R package *phangorn* v.2.2.0; Schliep 2011) was used to build a representative phylogenetic tree that was incorporated into the *phyloseq* object.

The genus *Symbiodinium* is highly diverse (Rowan & Powers 1992), and is thus divided into nine 'clades' (named clades A - I) which are further divided into many subclades. OTUs are study-specific, but each OTU can be mapped back to a documented *Symbiodinium* subclade. Since OTUs are a finer taxonomic level than subclades, there can be one or many OTUs detected within a subclade present in a sample or study. Understanding OTU presence and abundance provides insight into the sequence diversity within samples and among compartments, while aggregating at the subclade level provides information that is easily compared across studies. Thus, we provide results for both levels of taxonomic organization.

Statistics and visualization

We used multivariate statistics and visualization to examine variability in *Symbiodinium* beta diversity: 1) between sites with distinct chronic disturbance levels (for each individual compartment), and 2) over time (i.e. across a pulse disturbance), for each compartment and disturbance level. For each question, we evaluated changes in symbiont community composition, first evaluating community structure, by assessing the multivariate location of *Symbiodinium* sequence assemblages, using a permutational multivariate analysis of variance (*adonis* function in the R package vegan; Oksanen 2017). Second, we evaluated variation by quantifying changes in multivariate dispersion (i.e. beta dispersion) using PERMDISP (*betadisper* function; *vegan*). To display the beta dispersion (multivariate dispersion) of *Symbiodinium* communities at each sampling time point, we conducted PCoA analysis with the *betadisper* function (in *vegan*), using weighted UniFrac distances. Weighted UniFrac is a phylogenetically informed beta diversity distance metric that is useful for examining differences in community structure in environmental samples (Lozupone et al. 2011). Furthermore, we conducted an indicator species analysis using the package *indicspecies* (De Cáceres 2013) to determine whether certain *Symbiodinium* taxa were specifically associated with different compartments, and the stability of these associations. We used the *Eulerr* package in R (Larsson 2018) to create Venn diagrams to visualize shared *Symbiodinium* taxa among compartments and over space and time. We utilized the *iNEXT* package to quantify and visualize taxa accumulation curves in order to visualize the relationship between sampling depth and number of OTUs observed (Chao et al. 2014; Appendix B Figs. S4, S5, S6). Finally, *ggplot2* (Wickham 2016) was used for visualizing our results. All analyses were conducted in R version 3.4.3, and all code is available at https://github.com/baumlab/Claar_et_al_2019_KI_Compartment.

Results

Symbiodinium sequence diversity varied substantially across the three distinct compartments sampled (coral, water, sediment; Figure 5.2A, C-E), as well as amongst the three coral species (Figure 5.2B). In total, we detected 351 operational taxonomic units (OTUs; coral, n = 213; sediment, n = 124; water, n = 94), which mapped to 58 *Symbiodinium* subclades (Appendix B Fig. S3) from six of the nine known *Symbiodinium* clades (A, C, D, F, G, I; Appendix B Fig. S2; Appendix B Table S4). Despite occurring in close proximity on the reef, less than 6% (n=20) of the *Symbiodinium* OTUs were found in all three ecological compartments; a further 11.4% were found in two compartments (Figure 5.2A). Almost half of all *Symbiodinium* OTUs (n=171) were found uniquely in the coral compartment; twenty-one percent (n=75) were found uniquely in sediment, and 13% (n=45) occurred only in water (Figure 5.2A). Very few *Symbiodinium* OTUs (5.6% of all coral-associated taxa) were shared amongst the three coral species (Figure 5.2B; Appendix B Table S5). *P. grandis* had the most unique OTUs (n = 82; 38.5% of all coral-associated taxa), followed by *M. aequituberculata* (n = 56; 26.3% of all coral-associated taxa), and *P. lobata* (n = 41; 19.2% of all coral-associated taxa) (Figure 5.2B). Compared to the two free living compartments, *P. grandis* had more unique OTUs than either sediment or water (Figure 5.2C); it was also the only coral species with unique *Symbiodinium* subclades that were not represented in either of the other two coral species (Appendix B Table S6). *M. aequituberculata* had fewer unique OTUs than sediment but more than water (Figure 5.2D), and *P. lobata* had fewer unique OTUs than either sediment or water (Figure 5.2E).

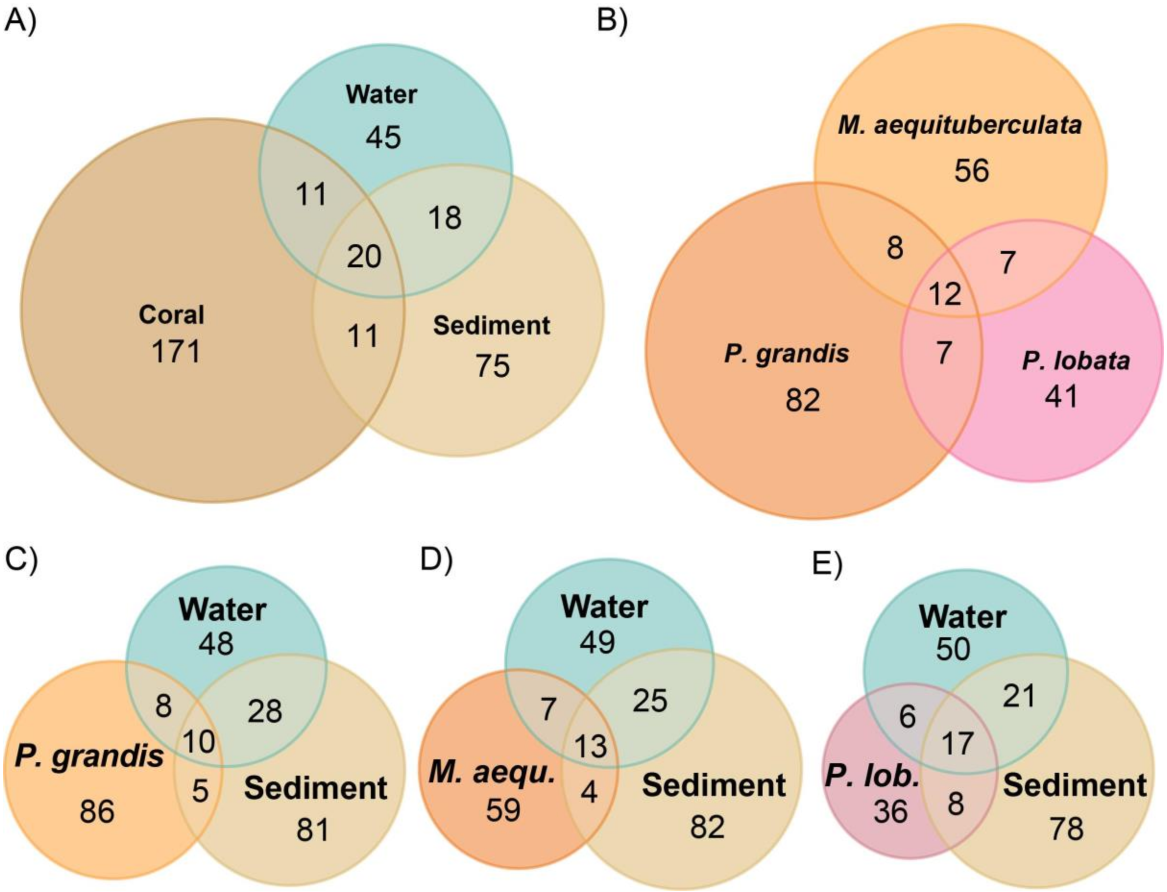


Figure 5.2 *Symbiodinium* denovo OTUs (at 97% similarity) at all sites and time points combined a) in each ecological compartment (i.e. coral, sediment, water), and b) in each coral species (i.e. *P. grandis, M. aequituberculata* [*M. aequ.*], *P. lobata* [*P. lob.*]). Venn diagram shows the number of *Symbiodinium* OTUs present in each sample type, as well as the amount of overlap between and among sample types.

Chronic local disturbance

There were significant differences in beta diversity between symbiont communities exposed to medium and very high chronic disturbance levels for all compartments, except for the coral *P. lobata* (Figure 5.3). Multivariate location, a metric for turnover, was significantly different between disturbance levels for *P. grandis* (F = 11.3, $R^2 = 0.14$, p = 0.001; Figure 5.3A), *M. aequituberculata* (F = 2.8, $R^2 = 0.04$, p = 0.022; Figure 5.3B), sediment (F = 12.6, $R^2 = 0.18$, p = 0.001; Figure 5.3D), and water

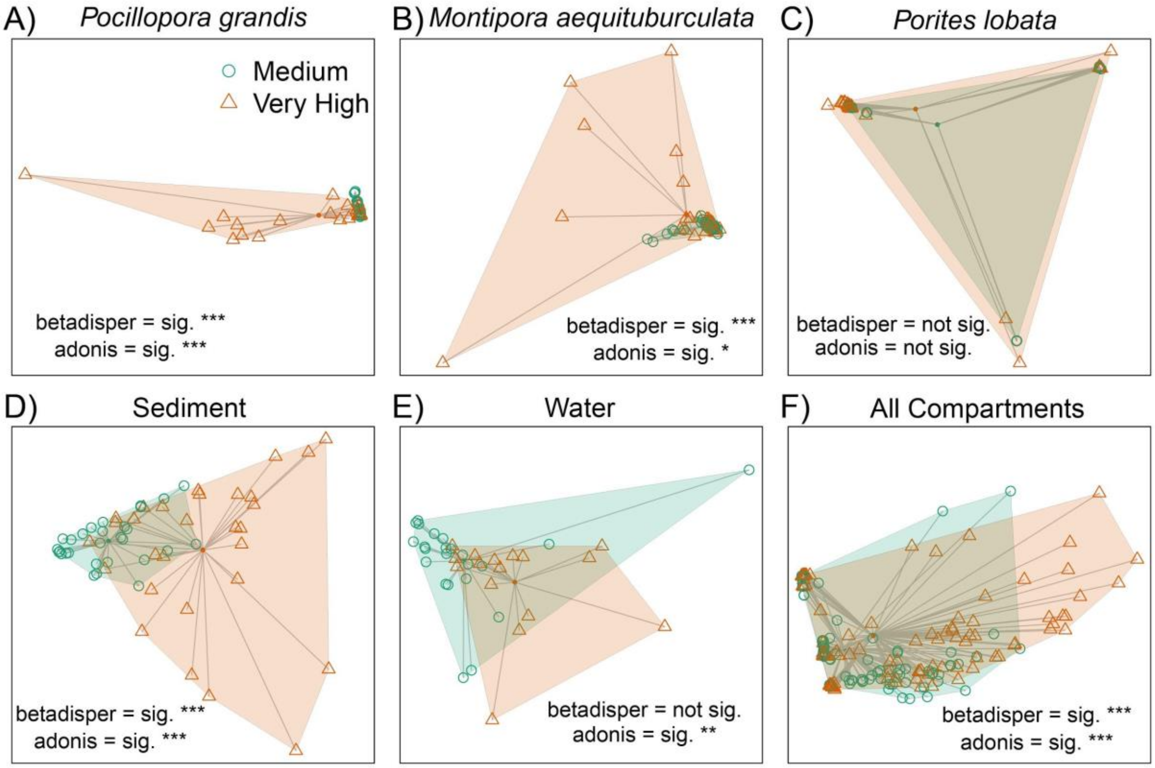


Figure 5.3 Multivariate ordination (PCoA) of *Symbiodinium* communities associated with coral (A-C), free-living (D, E) samples, and all samples combined (F). Points indicate individual samples (connected to the centroid point in the center), color indicates human disturbance level, and shaded areas indicate boundaries of observed community structure. F and p-values represent statistics from the beta dispersion analysis. Significance level of each test is denoted by *** < 0.001, ** < 0.01, * < 0.05.

(F = 3.4, $R^2 = 0.08$, $p = 0.019$; Figure 5.3E), but not for *P. lobata* ($p > 0.05$; Figure 5.3C). Beta-dispersion, a measure of multivariate spread indicative of the variation component of beta diversity, was greater in the very high disturbance sites than in the medium disturbance sites for *P. grandis* (F = 35.8, $p < 0.001$; Figure 5.3A), *M. aequituberculata* (F = 12.0, $p < 0.001$; Figure 5.3B), and the sediment (F = 14.1, $p < 0.001$; Figure 5.3D), but was not significantly different for *P. lobata* or water ($p > 0.05$; Figure 5.3C, E). Interestingly, *P. grandis* also showed the most dramatic change in clade-level

Symbiodinium community structure with chronic disturbance (Appendix B Fig. S3). *P. grandis* at medium disturbance sites were primarily dominated by clade C, with zero or very low abundances of clade D, while at the very high disturbance level, clade D was present in several samples at sequence abundances >20%. This suggests that, compared to the other two coral species, *P. grandis* exhibit more flexible symbioses across all analyzed taxonomic levels.

Pulse disturbance overlaid on two levels of chronic disturbance

The pulse disturbance had a variable influence on *Symbiodinium* beta diversity, depending on the compartment and the underlying level of chronic disturbance. In *P. grandis* and the water column, community structure and beta dispersion (i.e. variation) differed significantly over time at the medium disturbance sites, but not at the very high disturbance sites (Table 5.1; Figure 5.4A, B, I, J). In *M. aequituberculata* and sediment, the opposite occurred, with evidence of a change in community structure and variation over time at the very high disturbance sites, but not at the medium disturbance sites (Table 5.1; Figure 5.4E, F, G, H). Beta diversity metrics for *P. lobata* colonies did not change over time in either disturbance treatment (Table 5.1; Figure 5.4C, D).

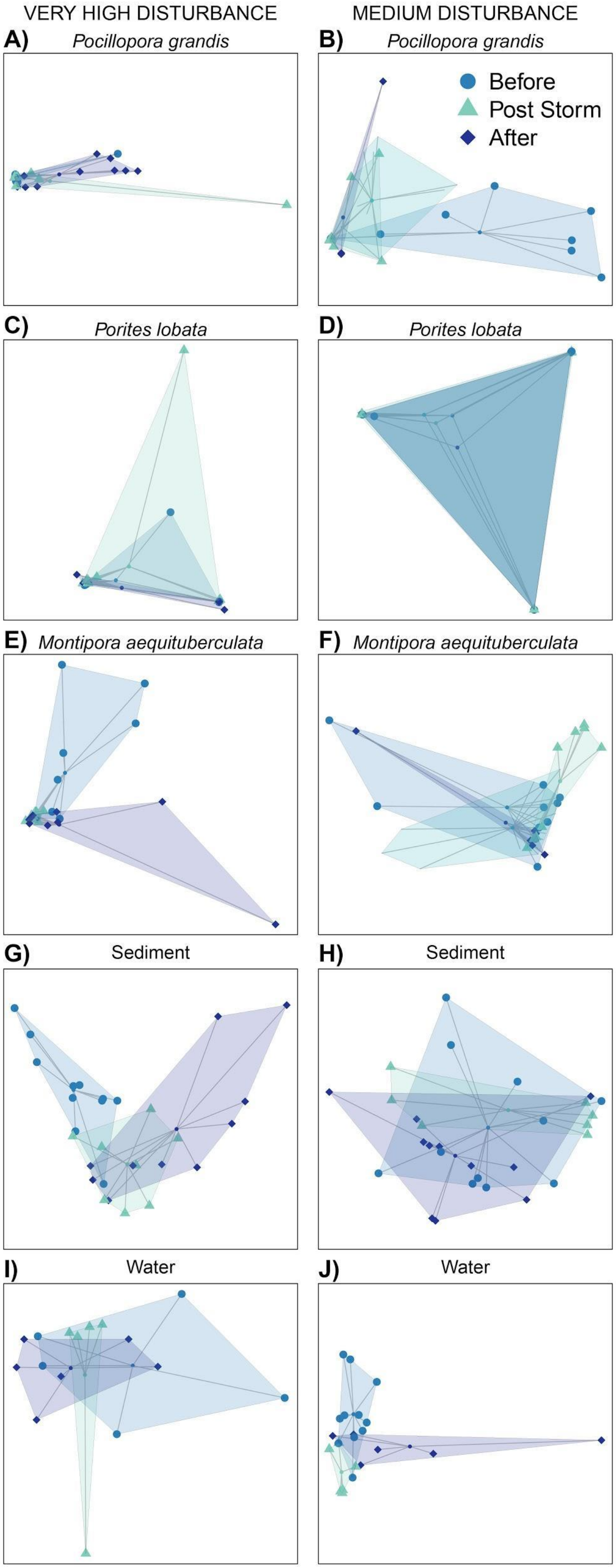


Figure 5.4 Multivariate ordination (PCoA) of *Symbiodinium* communities associated with coral (A-F), sediment (G, H), and water (I, J) samples. Points indicate individual samples (connected to the centroid point in the center), color indicates sampling time point, and shaded areas indicate boundaries of observed community structure.

Table 5.1 Changes in symbiont beta diversity over time, with compartments subjected to a pulse disturbance. Beta dispersion (PERMDISP) and multivariate location (PERMANOVA) over time for each *Symbiodinium* compartment. Shaded boxes indicate non-significant model results.

	Very High Disturbance		**Medium Disturbance**	
	Multivariate location	**Beta dispersion**	**Multivariate location**	**Beta dispersion**
P. grandis	$p > 0.05$	$p > 0.05$	$F = 4.2$ $R^2 = 0.27$ $p = 0.001$	$F = 8.2$ $p < 0.001$
M. aequituberculata	$F = 3.2$ $R^2 = 0.16$ $p = 0.006$	$F = 3.5$ $p = 0.04$	$p > 0.05$	$p > 0.05$
P. lobata	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
Sediment	$F = 5.1$ $R^2 = 0.28$ $p = 0.001$	$F = 4.9$ $p = 0.016$	$p > 0.05$	$p > 0.05$
Water	$p > 0.05$	$p > 0.05$	$F = 3.7$ $R^2 = 0.27$ $p = 0.001$	$F = 5.3$ $p = 0.014$

Despite some observed differences in symbiont beta diversity across the three sampling time points (Table 5.1; Figure 5.4), much of the symbiont community remained consistent within each compartment over the course of this pulse disturbance (Figure 5.5). For example, nearly half of all subclades present in each compartment were observed during all three time points (coral = 46%, sediment = 41%, water = 46%; Figure 5.5). Only a few subclades were found in only two time points (up to 15% in any one compartment), and a moderate number of subclades were observed during only one time point (coral = 29%, sediment = 44%, water = 38%). Known coral associates (e.g. *Symbiodinium* C31, C42, C15, C1, C3, D1) were present at all time points in water and sediment. Additionally, *Symbiodinium* subclades A5 and I4 are present throughout the sediment time series but were only present in the water column during the storm event.

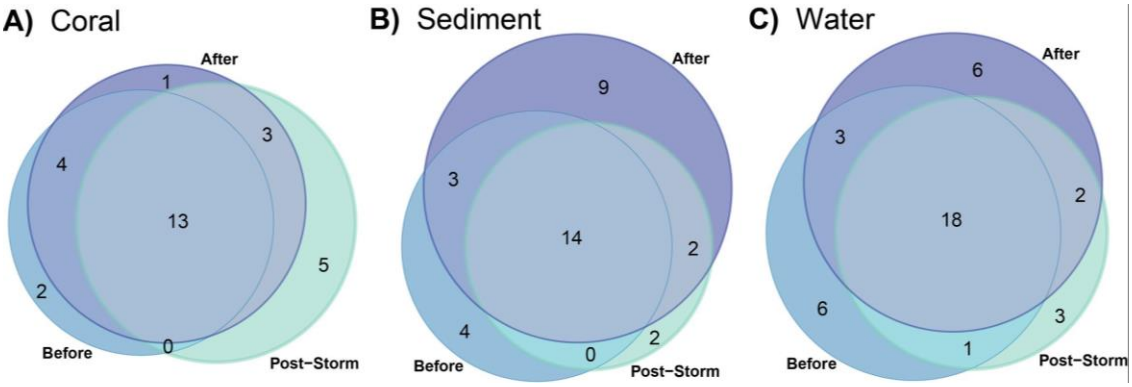


Figure 5.5 Consistency of *Symbiodinium* subclades in each compartment before (August 2014), immediately post-storm (January 2015), and after (May 2015) the pulse disturbance caused by the winter storm wave event. Venn diagrams show the number of *Symbiodinium* subclades present during each time point, as well as the amount of overlap between and amongst the time points.

Discussion

Beta diversity increases at a high level of human disturbance

A fundamental ecological question is whether local disturbance increases beta diversity by allowing for habitat expansion by a diversity of potentially opportunistic symbionts (in accordance with the Anna Karenina principle), or whether it decreases beta diversity due to environmental filtering or biotic homogenization. Thus far, the former mechanism appears to be more common in microbial systems, with increased beta diversity with disturbance has been documented in multiple ecosystems including soil bacteria in the tropics (Lee-Cruz et al. 2013, de Carvalho et al. 2016), animal microbiomes (Zaneveld et al. 2017), and coral microbiomes (Zaneveld et al. 2016). Our study provides further evidence of the Anna Karenina principle, and supports our hypothesis that local human disturbance is associated with consistent increases in beta diversity (specifically, beta dispersion) across *Symbiodinium* communities in most ecological compartments. This means that there was an increase in the number of potential *Symbiodinium* community compositions, and that individual samples were more different than one another at highly disturbed sites compared to moderately disturbed sites. In some systems, biotic homogenization is considered a negative result of anthropogenic stress, but in this specific case, increased diversity may negatively impact the stability of coral symbioses.

Contrary to our second hypothesis, despite some variability among sampling time points, we did not detect consistent changes in the *Symbiodinium* communities of different compartments over time (Figure 5.4, Table 5.1). However, there was some variability over time, raising questions about whether these changes are due to stochasticity in *Symbiodinium* populations, or whether site-specific environmental drivers might be at play. For example, *P. grandis* had variable *Symbiodinium* community structure over time at moderately disturbed sites, but not at highly disturbed sites, while *M. aequituberculata* exhibited the opposite pattern (Figure 5.4, Table 5.1). Consistent with previous results, we found that *Porites* symbioses are highly specific (Figure 5.4, Table 5.1), dominated by *Symbiodinium* C15 (and C15-like OTUs), and stable across both time and space (Putnam et al. 2012, Cunning et al. 2017). One potentially interesting temporal observation is that *Symbiodinium* I4, commonly associated with benthic forams, was found in seawater only during the storm time point and could potentially represent mixing or breakage of benthic forams during mechanical disturbance. Coral associates were found consistently in the environment, which is different than a previous study (Pochon et al. 2010) that did not find *Symbiodinium* subclades C1, C1d, C3, C15, or C31 in water or sediment samples. Detecting these subclades in the environment is notable because sediment is important for *Symbiodinium* uptake and the initiation of coral symbiosis (Cumbo et al. 2018), and symbiont uptake is greater in the presence of sediment compared to seawater only (Adams et al. 2009, Nitschke et al. 2016).

Similarity and overlap between *Symbiodinium* communities in different compartments

Although there has been a recent increase in the use of next-generation sequencing techniques for quantifying *in hospite Symbiodinium* communities (Quigley et al. 2014, Green et al. 2014, Arif et al. 2014), the use of these methods for other *Symbiodinium* compartments is still limited (but see Quigley et al. 2017, Cunning et al. 2017). We add to this nascent literature here, expanding our understanding of spatial and temporal variability in *Symbiodinium* communities, and how they vary under the influence of multiple stressors. In contrast with two recent studies on the Great Barrier Reef (GBR) that found four times more unique taxa in sediments than in juvenile corals (Quigley et al. 2017) and two times more unique taxa in water and sediment than in adult corals (Ziegler et al. 2018), we found 1.7-fold more unique taxa (OTUs at 97%) in adult corals than in sediment, and 1.2-fold more unique taxa in adult corals than in water and sediment combined. From the host perspective, it is possible that the difference in the number of taxa in coral and sediments between these studies was due to the focal coral species and life stages sampled in each study. However, we view this as unlikely because although Quigley et al. (2017) sampled juveniles of two closely related Acroporids (*A.*

tenuis and *A. millepora*), Ziegler et al. (2018) sampled a range of adults from endosymbiotic taxa that encompassed more diversity than our study. Conversely, from the symbiont perspective, it is possible that there are fewer free-living *Symbiodinium* taxa on Kiritimati compared to the Great Barrier Reef, due to the isolation of this remote atoll. While symbiotic *Symbiodinium* could potentially arrive on Kiritimati in association with coral larvae with long planktonic larval durations, connectivity of free-living *Symbiodinium* from reefs several hundred to thousands of kilometers away may be limited (Treml et al. 2008, Baums et al. 2014). This idea is supported by previous research demonstrating that *Symbiodinium* tend to disperse locally (Fitt & Trench 1983, Kirk et al. 2009), are negatively buoyant (Howells et al. 2009), and are competent in the water column for only approximately seven days (Nitschke 2015), while coral larval competency can be one month or more (Harii et al. 2010, Connolly & Baird 2010).

Despite the discrepancy between symbiotic and free-living *Symbiodinium* diversity observed between these studies, the proportion of taxa shared between coral and sediment was similar (10.1% of taxa excluding those found only in water in our study, compared to 10.6% of taxa shared between sediment and juvenile corals in (Quigley et al. 2017). However, both of these studies found different proportions of shared taxa than Ziegler et al. (2018), which found that half of all environmental *Symbiodinium* OTUs were also found in symbiosis, although this may in part be due the fact that they surveyed symbioses of not only scleractinian corals but also soft corals and Foraminifera. Our research also agrees with previous findings showing that certain subclades are not found in symbiosis, but are instead exclusively free-living (e.g., *Symbiodinium* subclades A2 and F1; LaJeunesse 2001, Thornhill et al. 2017). Future research on *Symbiodinium* connectivity and diversity on a variety of reefs across a broad geographic range may help to reveal how environmental and biological drivers shape patterns of *Symbiodinium* survival and persistence in both symbiotic and free-living environments on a reef.

Although we consider all *Symbiodinium* found in the sediment to be free-living (either transiently or exclusively), some *Symbiodinium* also occur in symbiosis with benthic Foraminifera (e.g., *Symbiodinium* C91) that may be captured during sediment collection. It is possible that even after pre-filtering at 20 µm (which should remove all living symbiotic benthic forams), mechanical damage could have broken open forams and released endosymbiotic algae that were then detected as part of the sediment-associated free-living community. We expect that this represents a small amount of the sequences extracted from the sediment, but future work could focus on comparisons between endosymbiotic *Symbiodinium* in forams and free-living *Symbiodinium* from the same sediment samples. Interestingly, a previous study showed that foram assemblages on Kiritimati have changed in response to chronic human disturbance over the past 40 years, towards heterotrophic rather than symbiotic assemblages (Carilli & Walsh 2012). Additionally, foram-associated *Symbiodinium* may participate in driving the evolution of *Symbiodinium* (Pochon et al. 2006), so further research on the exchange of symbionts among foraminiferans, sediment, and corals may reveal ecological and evolutionary connectedness among these major *Symbiodinium* compartments.

***Symbiodinium* community overlap among coral species**

Although overall *Symbiodinium* community structure varied amongst the three coral species, there was overlap in the presence of *Symbiodinium* subclades amongst the three species which may provide reservoirs of symbiosis-ready *Symbiodinium* cells. In this study, we focused on three coral species common on Kiritimati reefs that are direct transmitters of *Symbiodinium* (*Symbiodinium* propagules are passed from parent colonies to offspring, limiting the necessity for these species to uptake *Symbiodinium* from the environment) (Kojis & Quinn 1981, Willis et al. 1985, Baird et al. 2009). However, recent research has shown that *Symbiodinium* uptake by larvae may still be important, even for direct transmitters (Byler et al. 2013, Quigley et al. 2018). Additionally, since there is substantial overlap in symbiont communities among these three phylogenetically disparate coral species, it is likely that there is also overlap with other species in the coral community that rely primarily on horizontal uptake of symbionts each generation.

Potential implications of increased *Symbiodinium* beta diversity, and future research

Measuring changes in *Symbiodinium* communities at varying levels of local human disturbance is an important aspect of understanding coral reef resilience, since coral reproduction and recruitment is a vital part of coral recovery after severe bleaching events and *Symbiodinium* uptake by juvenile corals is bipartite (i.e., selective within the available pool of symbionts; Quigley et al. 2017). There is currently no empirical evidence demonstrating whether increased beta diversity in *Symbiodinium* meta-communities is beneficial or harmful to coral health and survival. We provide evidence that *Symbiodinium* beta diversity is indeed higher at very high levels of local disturbance, and posit two hypotheses regarding the implications of this increased community variability: increased beta diversity could potentially be beneficial, because it may offer more combinations of symbionts to the coral host and thus may increase the potential for adaptation, or conversely, increased beta diversity could be harmful, because it may allow for the proliferation of more opportunistic *Symbiodinium* assemblages that stray towards parasitism by inadequately provisioning their coral hosts (Baker et al. 2018).

Additional research is necessary to better understand the dynamic linkages among ecological compartments, and to determine how disturbance drives variation in *Symbiodinium* community structure within each compartment. Our results suggest that beta dispersion metrics of sediment *Symbiodinium* communities are potentially useful as indicators of water quality or reef degradation. The use of sediment could be beneficial because it may limit the need to sample corals for this purpose. However, the filtering method we utilized for this study was extremely time consuming, which limits utility for rapid assessments in the field. Additionally, this approach warrants further research to verify whether this pattern is consistent across other reef environments in different geographic regions. Future research investigating this idea would need to quantify differences in *Symbiodinium* community structure across the spectrum of local human disturbance at many different reef locations and for more coral taxa. While our study found no consistent change amongst compartments across three sampling dates, assessment of seasonal changes in *Symbiodinium* community structure would necessitate additional studies including increased sampling (i.e. multiple years during the same month). Additional time series studies are warranted in order to determine whether observed community changes are stochastic, seasonal, or driven by other cyclic or irregular environmental or biological drivers. In conclusion, chronic disturbance appears to increase beta diversity consistently across compartments, while pulse physical disturbance does not consistently alter *Symbiodinium* beta diversity. If increased beta diversity is consistent in locations with high levels of local human impact, empirical and modelling studies should address whether increased *Symbiodinium* community variability bolsters or erodes a reef’s capacity for resilience to future stressors.

Chapter 6

Biogeography of coral-associated Symbiodiniaceae and bacterial communities across a local human disturbance gradient

Danielle C. Claar[1], Jamie McDevitt-Irwin[1,2], Melissa Garren[3], Rebecca Vega Thurber[4], Ruth D. Gates[5], Julia K. Baum[1]

[1] University of Victoria, Department of Biology, British Columbia, Canada
[2] Stanford University, Department of Biology, 371 Serra Mall, Stanford, CA, 94305 USA
[3] California State University Monterey Bay, School of Natural Sciences,
100 Campus Center, Seaside, CA, 93955, USA
[4] Oregon State University, Department of Microbiology, 454 Nash Hall, Corvallis, OR, 97331, USA
[5] University of Hawai`i, Hawai`i Institute of Marine Biology, 46-007 Lilipuna Rd, HI, 96744, USA

Abstract

Coral-associated bacteria and endosymbiotic algae (Symbiodiniaceae spp.) are both vitally important for the biological function of corals. Despite their importance, little is known about the co-occurrence of these organisms within corals, how the abundance and diversity of these communities vary across different coral species, or how they are impacted by anthropogenic disturbances. Here, we quantified relative abundance and diversity of coral-associated Symbiodiniaceae and bacterial communities across a gradient of local human disturbance (n = 11 sites on Kiritimati (Christmas) atoll) in the central equatorial Pacific. We sampled 479 colonies from seven coral species that encompass a range of life history traits and found that very high chronic human disturbance was associated with higher microbial alpha and beta diversity for both Symbiodiniaceae and bacteria. When all coral species were considered together, we also found there was concordance between the bacterial and Symbiodiniaceae community structure, suggesting both Symbiodiniaceae and bacteria respond similarly to environmental change. However, when host species were considered separately, there

was only concordance between Symbiodiniaceae and bacterial communities for two massive species, *Hydnophora microconos* and *Favites pentagona*, implying that concordance at the coral community level may be due to species-specific coral associations. Furthermore, the presence of rare Symbiodiniaceae (genera *Symbiodinium sensu stricto, Durusdinium*, and *Gerakladium*, formerly *Symbiodinium* clades A, D, and G, respectively) was associated with differential abundances of several bacterial taxa which may be indicative of changes in the symbiotic state, although this requires further research. These results suggest that Symbiodiniaceae and bacterial communities are both affected at high levels of chronic disturbance, and that there are links between these communities at an island scale.

Introduction

Coral-associated communities of microorganisms are vitally important for the growth, reproduction, and resilience of corals (Ainsworth et al. 2010, 2015, Bourne et al. 2016, Peixoto et al. 2017). Corals function as ‘meta-organisms’, associating with a diverse array of Symbiodiniaceae (prev. *Symbiodinium;* LaJeunesse et al. 2018) (dinoflagellates), bacteria, archaea, viruses, and other single-celled eukaryotic organisms (Rosenberg et al. 2007). Despite burgeoning research on their individual roles (Ainsworth et al. 2010, Claar et al. 2017, Putnam et al. 2017), the interactions between corals, bacteria, and Symbiodiniaceae are still not well understood.

Understanding the dynamics among the constituents of the coral meta-organism is vital in the face of increasing threats to coral reefs by a suite of anthropogenic stressors that operate at local and global scales (Hoegh-Guldberg et al. 2007, Hughes et al. 2018).

Globally, climate change is impacting corals through gradually increasing stress (e.g. ocean warming and acidification), and through pulse heat stress events (e.g. El Niño) (Ainsworth et al. 2016, Heron et al. 2016, Claar et al. 2018). Local stressors can decrease coral reef health by eroding resilience capacity over time, making them more sensitive to climate stressors over the long term (Zaneveld et al. 2016). Stress can weaken the coral meta-organism by harming single components within it (e.g. by increasing pathogenic bacteria within the microbial community; Vega Thurber et al. 2009), by impacting interactions among components (e.g. by decoupling coral-algal symbioses; Brown 1997), or both. These impacts increase the probability of coral bleaching (Wiedenmann et al. 2012), disease (Vega Thurber et al. 2014), and consequently mortality. In this manuscript, we investigate the responses of two vital groups of microorganisms residing within corals, Symbiodiniaceae and bacteria, to varying levels of local disturbance.

A vital component of the coral meta-organism is the Symbiodiniaceae, single-celled endosymbiotic dinoflagellates that provide their host with photosynthetic metabolites in exchange for inorganic nutrients and a relatively stable symbiotic environment. Symbiodiniaceae is divided into seven defined genera, which are each further divided into numerous subclades (LaJeunesse et al. 2018). Some Symbiodiniaceae subclades (e.g. *Cladocopium* C15 and related) are more evolutionarily derived, and thus provide their host with a greater proportion of their photosynthetic products. Conversely, other Symbiodiniaceae subclades are more opportunistic and flexible, leading to selfish symbiotic interactions that can deviate towards parasitism (Baker et al. 2018). The sensitivity of coral symbioses to environmental stress is dependent on both the identity and abundance of their Symbiodiniaceae partners (Cunning & Baker 2013). Nutrient enrichment can destabilize Symbiodiniaceae communities and increase symbiont abundance, making corals more susceptible to bleaching and disease (Wiedenmann et al. 2012, Cunning & Baker 2013, Vega Thurber et al. 2014).

The coral microbiome is comprised of a diverse range of microbes, including bacteria, Archaea, fungi, and viruses (Knowlton & Rohwer 2003, Ainsworth et al. 2010). The most thoroughly studied members of the coral microbiome are the bacteria, which may act both in beneficial roles (Lesser et al. 2004), or as coral pathogens (Ben-Haim et al. 1999). Thermal stress can cause shifts in bacterial community structure (Littman et al. 2010), and increase the prevalence of opportunistic pathogens (Bourne et al. 2008, Ainsworth & Hoegh-Guldberg 2009). It can also increase coral production of dimethylsulfoniopropionate (DMSP), which elicits a chemotactic response in pathogenic bacteria (e.g. *Vibrio*) and guides them towards heat-stressed corals (Garren et al. 2014). Local human disturbance (e.g. pollution, excess nutrients) can increase alpha and beta diversity of microbial communities (McDevitt-Irwin et al. *accepted*; Vega Thurber et al. 2009). A variety of stressors can cause increases in the abundance of microbial genes involved in virulence and stress resistance, as well as a shift from healthy microbial communities to unhealthy communities often found in association with diseased corals (Vega Thurber et al. 2009). Additionally, anthropogenic stressors generally increase coral-associated microbial taxonomic richness and abundance of opportunistic microbial taxa while decreasing the abundance of a purported coral microbial symbiont (Endozoicomonas) (McDevitt-Irwin et al. 2017).

Stress often increases beta diversity in many microbial communities. This stress response has been dubbed the Anna Karenina Principle, which states that, “all happy families [microbiomes] look alike; each unhappy family [microbiome] is unhappy in its own way" (Zaneveld et al. 2017). This suggests that microbial communities within multiple individuals of the same taxa or environment will be more dissimilar from one another under stress than they are under healthy or baseline conditions (i.e. stress causes increased microbial beta diversity) (Zaneveld et al. 2017). Since changes in microbial community composition can influence the overall health of a coral, determining how these changes relate to Symbiodiniaceae within corals is important for understanding coral resilience under stress.

Since both Symbiodiniaceae and the microbiome are crucial components of the coral meta-organism, it is important to understand interactions between them. Research to date has not found any evidence of bacterial presence within the symbiosome (i.e. the membrane-bound organelle within corals that houses Symbiodiniaceae cells; (Yellowlees et al. 2008, Emerich & Krishnan 2014)) (Venn et al. 2009b, Pernice et al. 2012), but despite the lack of immediate contact between Symbiodiniaceae and bacteria, the transfer of metabolic products among coral, Symbiodiniaceae, and bacteria are likely important to symbiotic interactions (Ainsworth et al. 2015). For example, *Pocillopora damicornis* larvae provide their Symbiodiniaceae with nitrogen acquired from bacteria (Ceh et al. 2013). In a coral model system (*Aiptasia*), distinct bacterial communities associated with symbiotic and aposymbiotic states with direct links to sulfur- and nitrogen-cycling bacterial abundance (Röthig et al. 2016). In coral tissue, bacterial diazotrophs fix nitrogen that *Symbiodiniaceae* communities need to grow and reproduce (Rädecker et al. 2015), and the abundance of nitrogen-fixing gamma proteobacteria has been associated with the abundance of Symbiodiniaceae (Olson et al. 2009).

Recent studies also provide evidence that the interactions between these symbiotic partners can change under stress. For example, in *Acropora tenuis* juveniles, thermal stress caused a dramatic shift in the microbial community structure of corals dominated by *Durusdinium* (previously *Symbiodinium* clade D), but no observable changes in the microbial communities of corals dominated by *Cladocopium* C1 (previously *Symbiodinium* subclade C1) (Littman et al. 2010). Furthermore, during coral bleaching (the breakdown of the symbiosis between corals and Symbiodiniaceae), coral microbiomes may shift from healthy communities towards opportunistic taxa such as *Vibrio* (Bourne et al. 2008). Additionally, environmental adaptation of corals likely involves multiple members of the meta-organism (e.g. coral, microbes, Symbiodiniaceae; van Oppen et al. 2018). Although their interactions are important, the relationships between Symbiodiniaceae and bacteria may not be easily discernible, because interactions may be masked by disproportionate variability within the microbiome compared to algal symbionts (Chen et al. 2011). Alternatively, extrinsic factors such as habitat or location may be more influential to the coral microbiome than intrinsic factors such as host genotype or Symbiodiniaceae taxa (Pantos et al. 2015). Furthermore, co-occurrence (i.e. the simultaneous presence of two different microbial taxa in one coral sample) and concordance (i.e. similarity in multidimensional microbial community shapes and response to external forcing) may be difficult to detect, and co-occurrence does not necessarily imply interactions between taxa (Freilich et al. 2018). Regardless, discovering associations between these important symbiotic partners and potential parasites and pathogens may provide insights into species-specific mechanisms of resilience from a coral-meta-organismal view.

To evaluate coral-associated Symbiodiniaceae and bacterial communities under stress, we examined paired coral-associated Symbiodiniaceae and microbe communities across a spatial gradient of local human disturbance on Kiritimati atoll (Christmas Island, Republic of Kiribati, 01°52′N 157°24′W; Walsh 2011, Watson et al. 2016) in seven different coral species that span a range of life history strategies. Within this natural experimental context, we tested two major hypotheses: First, we hypothesized that alpha and beta diversity of both Symbiodiniaceae and microbial communities would increase across the disturbance gradient (McDevitt-Irwin et al. 2017, Zaneveld et al. 2017). Second, we hypothesized that microbial communities would be more sensitive to disturbance, and exhibit more site-to-site variability, than Symbiodiniaceae communities, however, we expected that the human disturbance gradient would still drive broad concordance between Symbiodiniaceae and microbial communities due to coral species-specific associations within both communities.

Methods

Study design and sampling

To investigate the effects of human disturbance on the diversity and structure of the coral holobiont, we sampled coral colonies at eleven fore reef sites (all 10-12 m depth) spanning the local disturbance gradient on Kiritimati (Fig. S1). Sites in the *very high* human disturbance category experience localized fishing pressure, sedimentation and pollution associated with pier infrastructure near VH2, and localized sewage runoff from adjacent villages, while moderately disturbed sites experience human disturbance primarily caused by fishing (Figure 5.1; Walsh 2011, Watson et al. 2016). *Low* and *very low* disturbance sites experience intermittent fishing pressure but are generally protected from most local disturbance. Sites were sampled in August 2014. We also included Symbiodiniaceae samples from January 2015, to add an additional human disturbance level for our analysis which was not sampled in August 2014 due to logistical constraints. This is unlikely to influence Symbiodiniaceae results, since Symbiodiniaceae communities within corals on Kiritimati do not appear to change seasonally (Chapter 5). These sites span a gradient of local human disturbance, which has been quantified based on human population densities, fishing pressure and infrastructure, with sites divided into five disturbance categories, termed *very low, low, medium, high,* and *very high* (Appendix C, Fig. S1; Walsh 2011, Watson et al. 2016). All sampling occurred during "normal" non-warming conditions, prior to the onset of heat stress caused by the 2015-2016 El Niño event.

At each site, we sampled approximately 8-12 colonies of each of three focal species *Montipora aequituberculata* (n_{TOTAL} = 124), *Pocillopora grandis* (previously *Pocillopora eydouxi;* n_{TOTAL} = 116))*, and Porites lobata* (n_{TOTAL} = 101), plus four additional less abundant species: *Hydnophora microconos* (n_{TOTAL} = 49), *Platygyra daedalea* (n_{TOTAL} = 42)*, Favites pentagona* (n_{TOTAL} = 29), and *Favia matthaii* (n_{TOTAL} = 18). These coral species represent a range of symbiotic flexibility (Putnam et al. 2012) (Appendix C Table S1).

Coral biopsies (<1 cm^3) were collected using a small steel chisel. Samples were stored on ice in individual sterile sample bags until processing. Coral biopsies were manually cleaned to remove as much skeleton as possible and subsampled into two separate tubes. Approximately 50 µL of coral tissue was rinsed with filtered fresh water and preserved in 400 µL of guanidinium buffer and stored at 4°C until DNA extraction for ITS-2 sequencing preparation. For 16S sample preparation, another 50 µL aliquot of coral tissue was immediately frozen at -20°C in the field and subsequently stored at -80°C.

A total of 479 coral colonies were sampled and processed for Symbiodiniaceae identification (Appendix C Table S1), and a total of 241 coral colonies were sampled and processed for microbe identification (Appendix C Table S2). Due to logistical constraints, not every coral colony has data available for both Symbiodiniaceae and bacteria. However, to increase sample size for individual Symbiodiniaceae and bacteria analyses (i.e. alpha and beta diversity), we used all available samples, even if a corresponding sample was not available for the other community. A subset of coral colonies (n = 220) was successfully collected and amplified for both Symbiodiniaceae and microbe communities, and only this subset of samples was used for all comparisons between the two communities (Appendix C Table S3).

Amplicon sequencing

For Symbiodiniaceae identification, DNA was extracted from guanidinium-preserved samples using a modified guanidinium-based extraction protocol (Stat et al. 2009, Cunning, Silverstein, et al. 2015, Chapter 7). Extracted DNA was cleaned following the standard protocol for Zymo Genomic DNA Clean and Concentrator^{TM}-25 kits (Catalog Nos. D4064 & D4065). Samples were prepared for ITS-2 amplicon sequencing and sequenced on the Illumina MiSeq platform using 2x300 paired-end read chemistry. Raw paired-end sequence reads were pre-filtered using the iu-filter-quality-bokulich script (Bokulich et al. 2013), and then merged using the iu-merge-pairs script with a maximum mismatch of three bases between the forward and reverse reads (both scripts from Illumina-Utils; Eren et al. 2013). After pre-filtering and merging, chimeric sequences were removed, primers were trimmed, each sample was clustered using 97% within-sample OTU clustering, and resulting OTUs were collapsed at 100% identity across samples (as in Cunning et al. 2017). Sequences were aligned using the Needleman-Wunsch global alignment algorithm (R, R Development Core Team 2008; Biostrings package, Pagès et al. 2017), and Symbiodiniaceae sequences were identified against a reference database (as in Cunning et al. 2015, 2017; reference database is archived, along with full bioinformatic pipeline at https://github.com/baumlab/Claar_etal_2019_Symbio_Bacteria). The phyloseq package (McMurdie & Holmes 2013) in R was used to store and analyze OTU tables, taxonomic information, and sample metadata. The phyloseq object was further filtered to remove OTUs observed <5 times in the entire data set (n = 234 taxa removed and n = 519 kept), and then the phyloseq object was filtered to remove samples with low sequence abundances (<100 sequences; n = 13 samples removed, and n = 483 samples kept). Next, a Symbiodiniaceae phylogenetic tree was constructed by aligning sequences from each genus separately using align_seqs.py from QIIME (Caporaso et al. 2010) using the program muscle (Edgar 2004). After sequences were aligned within each genus, a distance matrix was created using nr28s-rDNA distances (divergence of the D1–D3 region of the 28S; Pochon and Gates 2010, Putnam et al. 2012) to describe between-genera distances. Using upgma (R package phangorn (Schliep 2011) v.2.2.0), a phylogenetic tree was created and imported into the phyloseq object before statistical analysis.

For bacteria DNA extraction, library preparation, and sequencing was conducted by the Earth Microbiome Project (EMP) following their standard protocols (http://www.earthmicrobiome.org/protocols-and-standards/16s; Caporaso et al. 2011). 16S rRNA amplification was conducted using the EMP's 515fbc/806r primers (Caporaso et al. 2012). Sequence data were filtered and trimmed to 90 bp, and singletons were removed using the software Deblur (Amir et al. 2017). Deblur is a sub-operational-taxonomic-unit (sOTU) approach that uses error profiles to obtain single-nucleotide resolution clusters. Deblur also includes denovo chimera filtering using UCHIME as implemented by VSEARCH (Amir et al. 2017). sOTU assignments were done using the 16S rRNA database Greengenes 13.8 (McDonald et al. 2012). Chloroplast and mitochondrial sOTUs were removed prior to downstream analysis. Pipeline details and OTU tables are available on GitHub (https://github.com/baumlab/Claar_etal_2019_Symbio_Bacteria)

All downstream analyses were completed in R (Version 3.4.3, R Development Core Team 2008) using the package *phyloseq* (McMurdie & Holmes 2013). Code for all analyses is located on GitHub (https://github.com/baumlab/Claar_etal_2019_Symbio_Bacteria).

Alpha diversity

To estimate alpha diversity, we rarified each sample to an equal level of sequences (n = 800) and calculated the Shannon diversity index for each sample. We rarified to 800 sequences to strike a balance between having enough sequences for downstream analysis, while including as many samples as possible (since samples with fewer than the cutoff are discarded). We chose the Shannon Index due to its suitability in situations where rare species are as important as abundant species (Morris et al. 2014). To quantify differences in alpha diversity among human disturbance levels and coral species, we then fitted a linear model with the Shannon Index as the response variable and performed backwards stepwise ANalyses Of VAriance (ANOVAs) to determine the best model fit using the Akaike Information Criterion corrected for small sample size (AICc). Next, we used the R package *lsmeans* to extract the least squares means for the best model to test for significant differences among the levels of the included categorical variables (Lenth 2016).

Beta diversity

We consider two components of beta diversity: 1) community structure, defined as a difference in multivariate location or structure among sample groups, and 2) variation (dispersion), defined as multivariate spread within each sample group. To quantify microbial beta diversity, we transformed all sample counts to proportions and calculated Bray-Curtis dissimilarity distances. To quantify Symbiodiniaceae beta diversity, we transformed all OTU counts to proportions and calculated both Bray-Curtis and weighted UniFrac dissimilarity. We chose Bray-Curtis for comparability with bacterial samples, and weighted UniFrac because this metric takes into account phylogenetic distance among clades, which is likely more important than differences between OTUs. We assessed community structure using permutational ANOVAs (PERMANOVAs), implemented using the *adonis* function in the R package *vegan* (Oksanen 2017). For community structure, our models included coral species, as well as site nested within human disturbance category. We then assessed the variation component of beta diversity using PERMDISP, which tests the homogeneity of multivariate dispersions within groups (implemented as function *betadisper* in R package *vegan* (Oksanen 2017). Since analyses with *betadisper* can only include one grouping variable, we included only human disturbance category as a predictor in beta diversity variation analyses. We conducted this test for each data set (ITS2/Symbiodiniaceae and 16S/bacteria) among coral species and then among local human disturbance categories.

Concordance of *Symbiodinium* and microbial communities

Concordance represents similarity in multivariate community shape between two communities across locations or sampling units and can indicate co-occurrence or similar responses of both communities in response to environmental drivers. To test for concordance between community structures of Symbiodiniaceae and microbes, we used Procrustes analysis (functions *procrustes* and *protest* in the R package *vegan*; Oksanen 2017), using all coral colonies for which we had both Symbiodiniaceae and bacterial sequence data (n = 220). Procrustes (Gower 1975) is a symmetric canonical analysis method that enables the comparison of multidimensional community shape between two communities. Procrustes analysis works by comparing two community matrices, one 'target matrix' that is kept constant, and another 'rotated matrix' which is translated to superimpose the centroids of both matrices, scaled so dispersion is equal, and then rotated to make the matrices match as well as possible. Here, the Symbiodiniaceae community is the target matrix, and the bacterial community is the rotated matrix. A significant procrustes result indicates that the two matrices exhibit consistent multivariate community changes (i.e. similar multivariate "shape") in response to environmental drivers. That is, either both Symbiodiniaceae and bacterial communities are responding similarly to external forcing, or the bacterial community (the rotated matrix) is changing in response to the Symbiodiniaceae community (the target matrix). We chose Procrustes analysis over Mantel tests because 1) Procrustes has increased statistical power compared to Mantel (Peres-Neto & Jackson 2001); 2) Procrustes can be used to compare multiple data matrices (Lisboa et al. 2014); and 3) Procrustes is particularly good when the compared matrices (communities) are equally applicable as explanatory and response variables (Lisboa et al. 2014). We conducted Procrustes analysis for all coral species combined, as well as for each coral species individually.

Symbiodiniaceae genera and changes in the microbiome

We quantified microbiome members that may be associated with Symbiodiniaceae genus presence by testing for differential abundance of OTUs using DESeq2 (Love et al. 2014). We chose to investigate Symbiodiniaceae genera, as they are a conservative indicator of symbiotic flexibility or invasibility at the coral colony level. First, we determined the presence of Symbiodiniaceae (genera *Symbiodinium, Cladocopium, Durusdinium, Fugacium,* and *Gerakladium*) for each microbial sample (i.e. Yes/No; *Symbiodinium* 15/205, *Cladocopium* 220/0, *Durusdinium* 159/61, *Fugacium* 2/218, *Gerakladium* 24/196). Since all samples contained *Cladocopium*, and the total number of samples with *Fugacium* was extremely low (n = 2), we did not calculate differential microbial abundances in association with these *Symbiodiniaceae* genera. For symbionts *Symbiodinium, Durusdinium*, and *Gerakladium*, we used DESeq2 to calculate differential bacterial abundances of all samples combined. Increased abundance of a bacterial OTU indicates that there is more of that OTU detected if that Symbiodiniaceae genus is present. DESeq2 is able to flag and remove outlier values when sample size is greater than six samples per group and thus minimizes false discovery rates. Therefore, we also used DESeq2 to analyze differential abundance of the bacterial microbiome for individual coral species that met this criterion (*Symbiodinium*: *P. daedalea*; *Durusdinium*: *M. aequituberculata*, *P. lobata, H. microconos*; *Gerakladium*: *M. aequituberculata*).

Results

Symbiodiniaceae sequence diversity

Symbiodiniaceae 97% OTU alpha diversity was significantly different among coral species and human disturbance categories ($F = 23.3$, adj $R^2 = 0.43$, $p < 0.001$; Figure 6.1A). Across all coral species, Symbiodiniaceae alpha diversity was higher at *very high* human disturbance compared to all of the other four human disturbance categories (all $p < 0.001$; Figure 6.1B). Among coral species, Symbiodiniaceae alpha diversity ranged from *H. microconos* (lowest), followed by *F. matthaii, F. pentagona, P. daedalea*, *P. lobata*, *P. grandis*, and finally *M. aequituberculata* (highest) (Figure 6.1C).

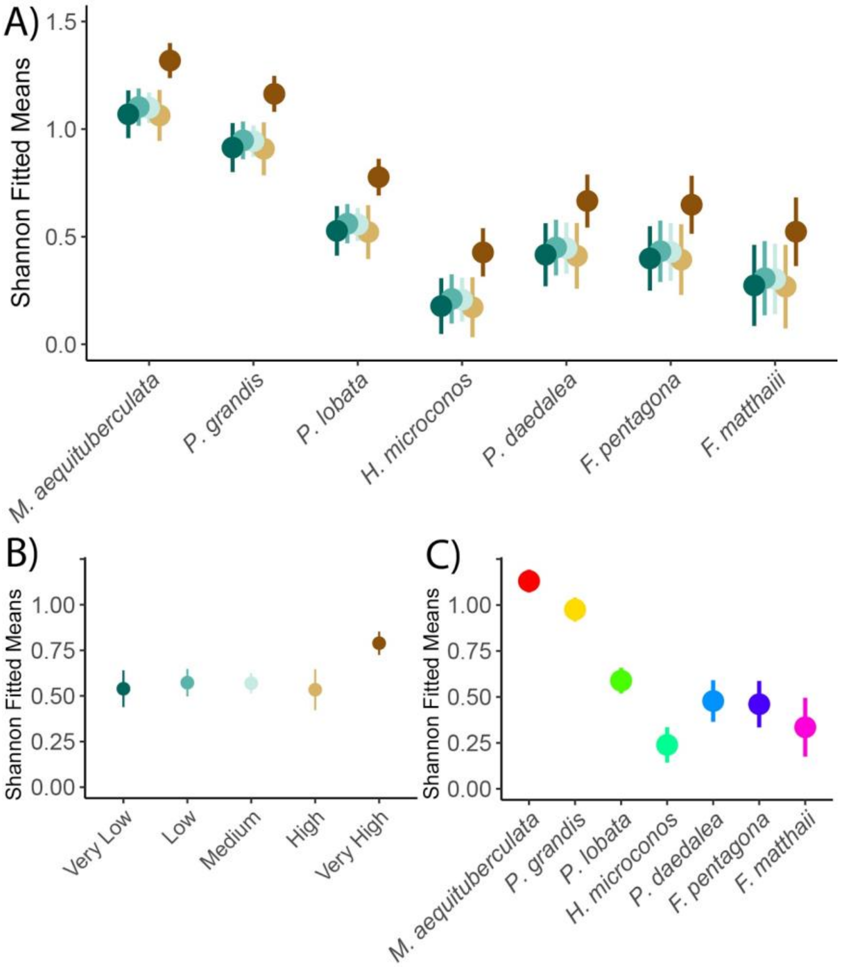


Figure 6.1. Coral-associated *Symbiodinium* OTU alpha diversity; A) by coral species and chronic disturbance category, human disturbance increases from left to right within each coral species, see 1B for color legend by human disturbance level B) by chronic disturbance category (all species combined), and C) by coral species (all sites combined). Coral species in A and C are ordered by overall sample size.

The variation component of Symbiodiniaceae beta diversity was significantly different among human disturbance categories (F = 63, df = 4, $p < 0.001$; Figure 6.2A, Appendix C Table S4), with significantly higher beta diversity at *very high* human disturbance than any of the other disturbance categories (all $p < 0.001$; Figure 6.2A, Appendix C Table S4). Beta diversity variation was also significantly different among coral species (F = 122, df = 6, $p < 0.001$; Figure 6.2B, Appendix C Table S4). When each coral species was analyzed separately, there was also significantly more variation at *very high* disturbance compared to the other disturbance levels for *P. grandis* (F = 27, df = 4, $p < 0.001$), *H. microconos* (F = 39, df = 4, $p < 0.001$), and *F. matthaii* (F = 55, df = 2, $p < 0.001$) (Appendix C Fig S2). *P. daedalea* was less consistent across the disturbance gradient, with significantly more variation at *low* disturbance compared to *high* disturbance (Appendix C Table S3, Appendix C Fig S2).

Symbiodiniaceae community structure was also significantly different among human disturbance categories (F = 28, df = 4, $R^2 = 0.11$, $p < 0.001$; *adonis* from R package *vegan*), and coral species (F = 59, df = 7, $R^2 = 0.41$, $p < 0.001$). There was also a significant interaction of human disturbance category and site (F = 3.4, df = 6, $R^2 = 0.02$, $p < 0.001$), which demonstrates that there is some variability among sites within each disturbance category.

When each coral species was analyzed separately, community structure was significantly different among disturbance categories for *P. grandis* (F = 12, df = 4, $R^2 = 0.3$, $p < 0.001$), *H. microconos* (F = 39, df = 4, $R^2 = 0.40$, $p < 0.001$), *F. pentagona* (F = 36, df = 4, $R^2 = 0.85$, $p < 0.001$), and *F. matthaii* (F = 7.9, df = 3, $R^2 = 0.27$, $p = 0.016$) (Appendix C Fig S3). There was also a significant interaction between disturbance category and site for *M. aequituberculata* (F = 2.4, df = 6, $R^2 = 0.11$, $p = 0.012$), *H. microconos* (F =32, df = 6, $R^2 = 0.50$, $p < 0.001$), *P. daedalea* (F = 6.5, df = 4, $R^2 = 0.43$, $p < 0.001$), and *F. matthaii* (F = 6.4, df = 4, $R^2 = 0.56$, $p = 0.016$).

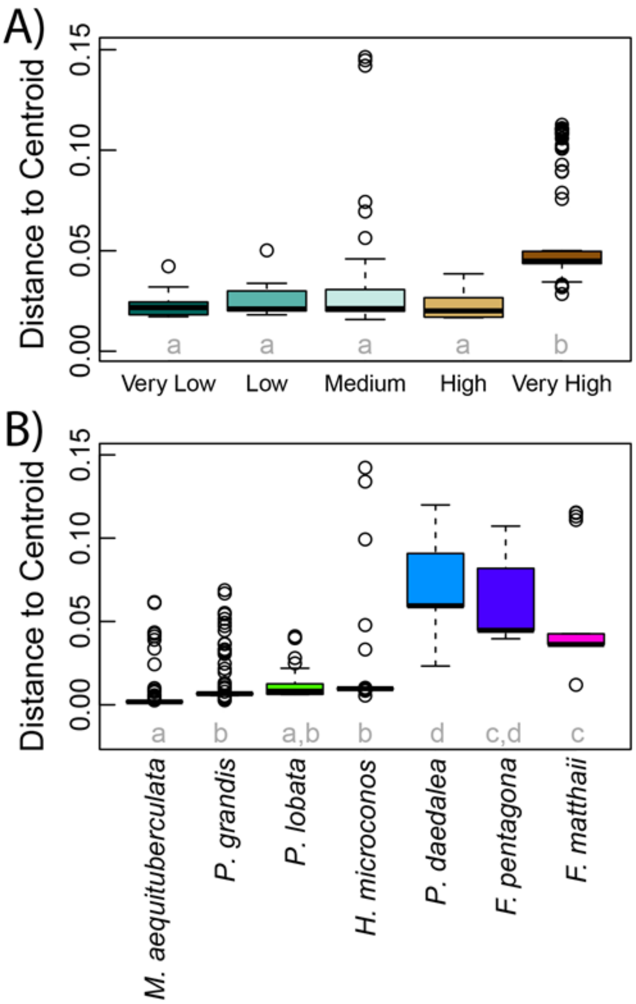


Figure 6.2 Coral-associated Symbiodiniaceae beta diversity variation (beta dispersion); A) by chronic disturbance category (all species combined), and B) by coral species (all sites combined). Significant groupings and differences among species are shown with lower-case letters.

Coral-associated bacterial diversity

As with Symbiodiniaceae, bacterial alpha diversity was significantly different among coral species and human disturbance categories (F = 5.2 adj $R^2 = 0.22$, $p < 0.001$; Figure 3A). Microbial alpha diversity was significantly higher at *very high* disturbance sites than at *medium* ($p = 0.012$) or *low* disturbance ($p=0.002$) (Figure 6.3B). Bacterial alpha diversity (measured using the Shannon Index) was lowest in *H. microconos* and highest in *P. lobata* (Figure 6.3C).

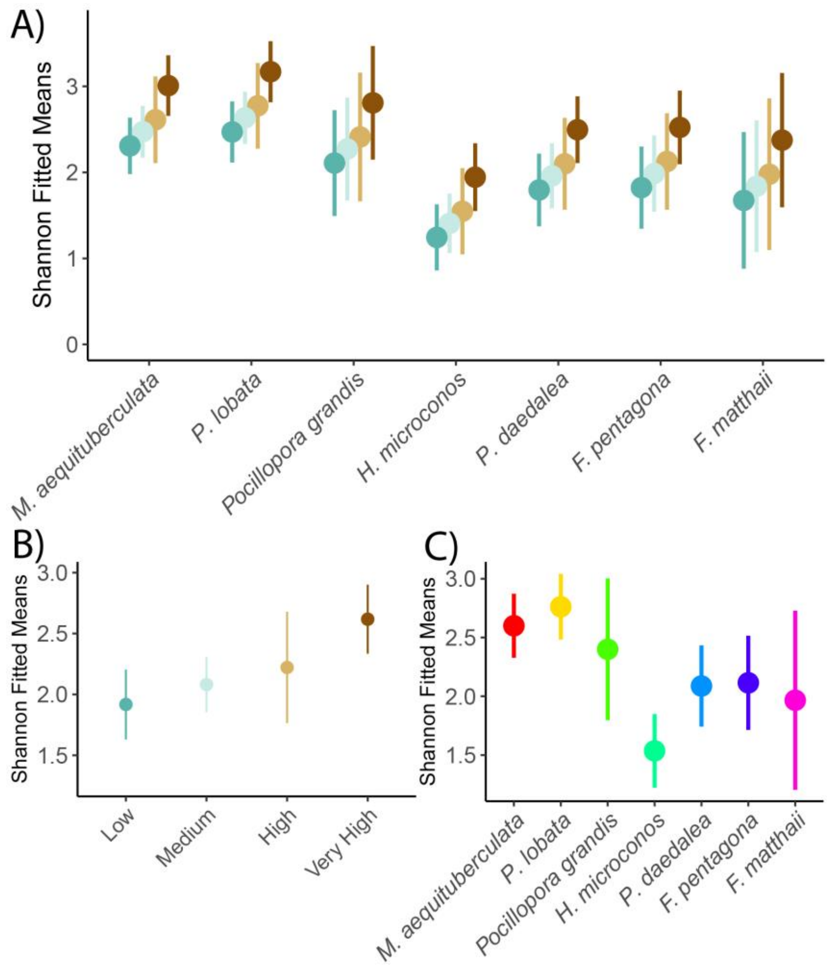


Figure 6.3. Coral-associated microbial alpha diversity; A) by coral species and chronic disturbance category, human disturbance increases from left to right within each coral species, see 1B for color legend by human disturbance level, B) by chronic disturbance category (all species combined), and C) by coral species (all sites combined), each color represents a single coral species.

The variation component of bacterial beta diversity was significantly different among human disturbance categories (F = 5.5, df = 3, p = 0.001; Figure 6.4A, Table S5), with significantly lower beta diversity at *high* than at *very high* (p < 0.001), *medium* (p = 0.01), and *low* human disturbance (p = 0.007). Beta diversity variation was also significantly different among coral species (F = 7.9, df = 6, p < 0.001; Figure 6.4B, Appendix C Table S5). However, when each species was analyzed separately, there were no significant differences in beta diversity among human disturbance categories (Appendix C Fig. S4).

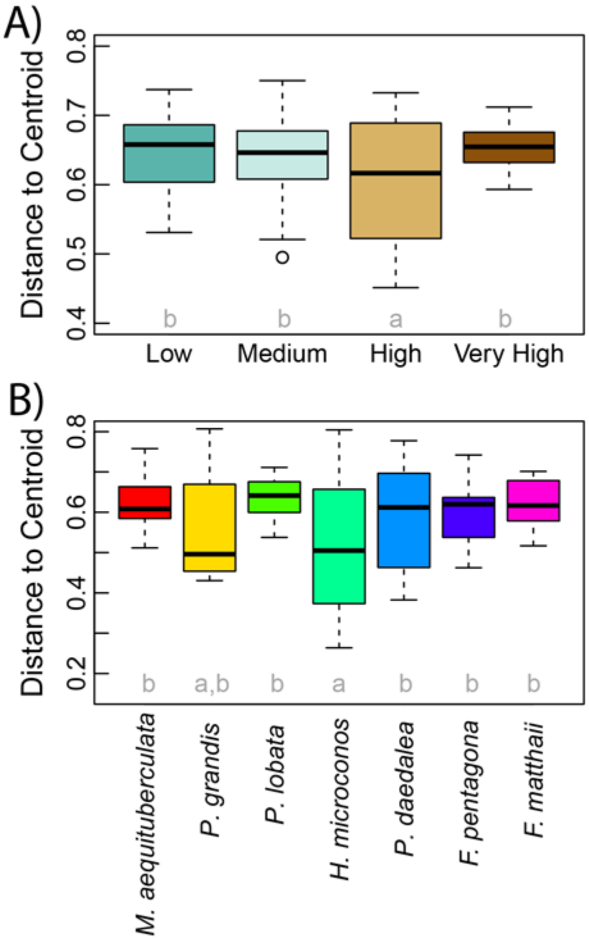


Figure 6.4 Coral-associated microbial beta diversity variation (beta dispersion); A) by chronic disturbance category (all species combined), and B) by coral species (all sites combined). Significant groupings and differences among species are shown with lower-case letters.

Community structure was significantly different among human disturbance categories (F = 2.8, df = 3, $R^2 = 0.028$, $p < 0.001$; *adonis* from R package *vegan*), and coral species (F = 4.3, df = 10, $R^2 = 0.14$, $p < 0.001$), and there was a significant interaction of human disturbance category and site (F = 2.1, df = 5, $R^2 = 0.035$, p <

0.001). When each coral species was analyzed separately, community structure was significantly different among disturbance categories for *M. aequituberculata* (F = 1.9, df = 3, $R^2 = 0.08$, $p < 0.001$), *P. lobata* (F = 1.6, df = 3, $R^2 = 0.08$, $p < 0.001$), *F. pentagona* (F = 1.6, df = 2, $R^2 = 0.16$, $p = 0.05$), and *F. matthaii* (F = 2.0, df = 1, $R^2 = 0.19$, $p = 0.027$; Appendix C Fig S5). There was also a significant interaction between disturbance category and site for *M. aequituberculata* (F = 1.6, df = 5, $R^2 = 0.11$, $p < 0.001$) and *P. lobata* (F = 1.6, $R^2 = 0.13$, $p < 0.001$).

Concordance between Symbiodiniaceae and bacterial communities

When all coral species were analyzed together, there was significant concordance (i.e. similarity in multivariate shape or community structure) between Symbiodiniaceae and microbial communities across the disturbance gradient (Procrustes $m^2 = 0.81$, $p < 0.001$; Figure 6.5A). Since this concordance was likely due to coral species-specific microbial and Symbiodiniaceae communities (i.e. apparently similar responses within coral species, Figure 6.5A), we next conducted a Procrustes analysis for each coral species individually, and found significant concordance for *H. microconos* ($m^2 = 0.91$, $p = 0.024$; Figure 6.5B) and *F. pentagona* ($m^2 = 0.82$, $p = 0.05$; Figure 6.5C) but not for any of the other coral species.

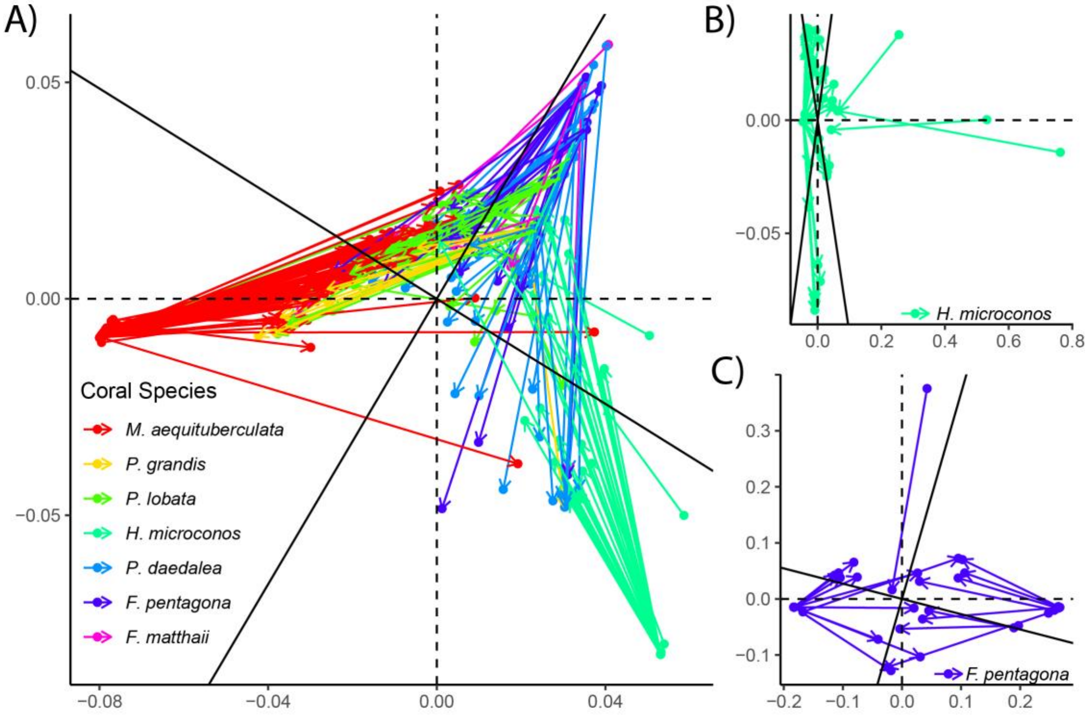


Figure 6.5 Procrustes plots for A) all coral species (Procrustes $m^2 = 0.81$, $p < 0.001$), B) *Hydnophora microconos,* and C) *Favites pentagona*. Each coral colony is represented by two points, connected by an arrow; the arrow starts at the Symbiodiniaceae community and points toward the bacterial community. All coral colonies that had samples for both Symbiodiniaceae and bacteria are included in these plots.

Changes in microbial communities related to Symbiodiniaceae taxa presence

Symbiodinium sensu stricto (previously *Symbiodinium* clade A) was present at all disturbance categories, and we found that across all coral species the presence of *Symbiodinium sensu stricto* in colonies was associated with a decreased abundance of eight bacterial orders from four phyla (Proteobacteria, Chlamydiae, Firmicutes, and Bacteroidetes; Deseq2 analysis; Figure 6.6). This included families Endozoicomonaceae (order Oceanospirillales), Rhodobacteraceae (order Rhodobacterales), Rhizobiaceae (order Rhizobiales), Streptococcacaeae (order Lactobacillales), Flavobacteriaceae (order

Flavobacterales), Simkaniaceae (order Chlamydiales), and Legionellaceae (order Legionellales). *Platygyra daedalea* was the only individual coral species with a sufficient number of colonies to conduct this analysis for *Symbiodinium sensu stricto*. When *P. daedalea* corals were considered alone, *Symbiodinium sensu stricto* was instead associated with increased abundance of four alphaproteobacteria orders (Rhodobacterales, Rickettsiales, and two unnamed orders; Appendix C, Fig S6) and no decreased abundances.

Durusdinium (previously *Symbiodinium* clade D) was present at all disturbance categories, and the presence of *Durusdinium* was associated with an increased abundance of one bacterial OTU from the order Rhizobiales, and one bacterial OTU from the family Simkaniaceae (order Chlamydiales; Figure 6.6). However, when subsets of individual coral species were analyzed using DESeq2 for *M. aequituberculata*, *P. lobata*, and *H. microconos*, only a single significantly differentially abundant OTU in the presence of *Durusdinium* was found for *P. lobata* colonies. In *P. lobata*, the presence of *Durusdinium* was associated with increased abundance of a Deltaproteobacteria taxon (order Desulfobacterales, family Desulfobulbaceae). *Gerakladium* (previously *Symbiodinium* clade G) was present in coral colonies at each disturbance category. In the presence of *Gerakladium*, several bacterial OTUs had decreased abundance, including OTUs within the families Symkaniaceae (Chlamydiales), Clostridiaceae (Clostridiales), Pseudoaltermonoadaceae (Vibrionales), Streptococcaceae (Lactobacillales), and the order Kiloniellales (Figure 6.6). *Montipora aequituberculata* was the only coral species with enough samples for a subset analysis of *Gerakladium* presence. This species had two differentially abundant OTUs: an Alphaproteobacteria (order Rhodobacterales, family

Rhodobacteraceae), and a Deltaproteobacteria (order Spirobacilliales) (Appendix C Fig S6).

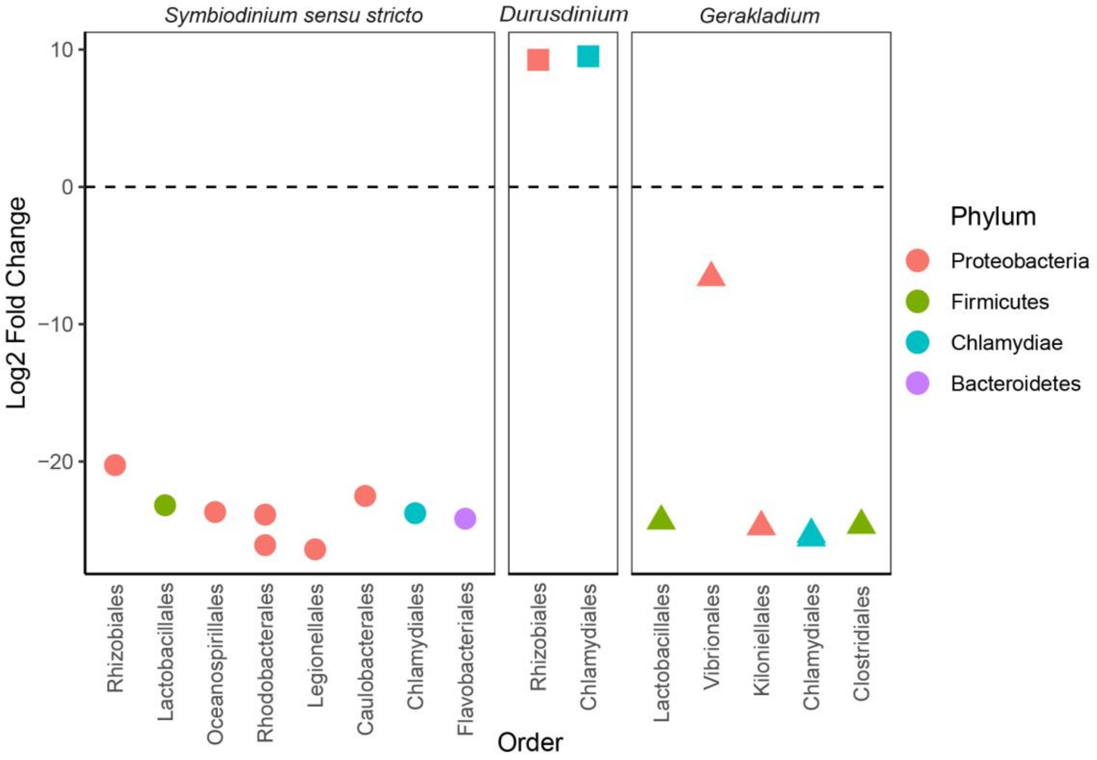


Figure 6.6 Differential abundance of microbial OTUs in the presence of *Symbiodinium sensu stricto* (previously clade A), *Durusdinium* (previously clade D), and *Gerakladium* (previously clade G) *Symbiodiniaceae*. All coral colonies that had samples for both Symbiodiniaceae and bacterial communities are included in this figure.

Discussion

Overall, the Symbiodiniaceae and bacterial communities varied among coral species and spatially across the disturbance gradient in terms of diversity and concordance. Chronic disturbance increased the alpha and beta diversity of both Symbiodiniaceae and bacterial communities. When all samples were considered together, there was a correlation in community structure (i.e. concordance) between the

Symbiodiniaceae and bacterial communities. However, when samples were subset by coral species, there was only concordance between and Symbiodiniaceae and bacterial communities for *H. microconos* and *F. pentagona*. When rare Symbiodiniaceae clades were present in corals, there were significant changes in the abundance of some microbial OTUs. Specifically, when clade D was present there was a significant decrease in one nitrogen-fixing OTU, and when clade G was present there was an increase in a variety of potentially symbiotic or pathogenic bacterial OTUs.

Chronic disturbance and diversity of the coral symbiome

We found that alpha and beta diversity of both *Symbiodinium* and microbial communities varied across the disturbance gradient with increased diversity at *very high* disturbance. Diversity did not increase linearly across the disturbance gradient for Symbiodiniaceae communities, but instead was fairly similar at low to moderate levels of disturbance and significantly higher at *very high* disturbance. However, diversity did increase fairly linearly across the disturbance gradient for bacterial communities. This suggests that for Symbiodiniaceae, there may be a tipping point (Walker et al. 2004, Hodgson et al. 2015), below which chronic disturbance is not high enough to influence diversity, but above which it alters symbiont and microbial diversity. Conversely, bacterial communities appear to be incrementally influenced by increasing local disturbance. It is possible that for microbial communities, increased diversity could be mediated by the presence of increased turf algae and macroalgae with increased disturbance. This may be due to localized hypoxia caused by an increase in microbial activity caused by algal release of dissolved organic carbon (Barott et al. 2009, Rohwer et al. 2010) or the presence of allelochemicals produced by algae, which can harm corals (Morrow et al. 2011). Additionally, direct transfer of algae-associated microbes to corals through contact (Nugues et al. 2004, Thurber et al. 2012) may instigate changes in microbial community structure at chronically disturbed sites. Alternatively, for both microbial and *Symbiodinium* communities, very high disturbance may inhibit holobiont control of associated microorganisms, allowing invasion by opportunistic and pathogenic taxa under intense chronic stress (Vega Thurber et al. 2009, Littman et al. 2011, Krediet et al. 2013).

At the coral taxa-specific level, we found that alpha diversity was generally related to coral life history. For Symbiodiniaceae alpha diversity, stress-tolerant massive coral species (i.e. *P. lobata*, *H. microconos, F. pentagona*, *P. daedalea,* and *F. matthaii*) had lower diversity than the generalist and competitive species (i.e. *M. aequituberculata*, *P. grandis).* There were fewer differences in microbial alpha diversity by coral species, with significant differences only between the lowest diversity species (*H. microconos*) and the two highest diversity species (*M. aequituberculata*, *P. lobata*). The high alpha diversity of *P. lobata* microbial communities was unexpected, since this coral species has previously been found to have a relatively low-diversity microbiome (McDevitt-Irwin et al. *accepted*).

For Symbiodiniaceae, the relationship between coral life history and beta diversity was less clear cut. First, *M. aequituberculata* and *P. lobata* had no significant difference in their variation (i.e. beta dispersion), even though *M. aequituberculata* is a generalist, and *P. lobata* is stress tolerant. Contrary to our expectations, *M. aequituberculata* had the lowest beta diversity variation of all species, while stress tolerant massive corals *P. daedalea* and *F. pentagona* had the highest beta diversity (Figure 6.2B). This is likely because *M. aequituberculata* tended to be dominated by only one Symbiodiniaceae type (C31; n = 121 of 124), whereas *P. daedalea* and *F. pentagona* could be dominated by four Symbiodiniaceae types (C15, C1232, C3, or D1 and C1232, C3, C31 or D1, respectively). For microbial communities, *M. aequituberculata* had the highest beta diversity variation, as we expected, while *H. microconos* and *P. lobata* (both massive corals) had the lowest and second lowest beta diversity, respectively (Figure 6.4B). The remaining species had similar beta diversity, with fewer differences among species than we expected. For example, microbial beta diversity of *P. grandis* (a competitive species) was no different than most of the massive coral species (excluding *H. microconos*, which it was significantly higher than). It appears that in these coral species, specificity and flexibility are not tightly correlated between microbial and Symbiodiniaceae communities.

Concordance between Symbiodiniaceae and microbe communities

The community structures of Symbiodiniaceae and bacteria were concordant (i.e. their multidimensional shapes matched up) at an island scale (i.e. all disturbance categories considered together). However, since Symbiodiniaceae communities are strongly structured by coral species (e.g. several coral species are dominated by just one subclade of Symbiodiniaceae), it seemed likely that the similarity in population structure at this scale was due to coral species-specific Symbiodiniaceae and microbial communities. Therefore, we next tested for concordance between Symbiodiniaceae and microbial community structure for each coral species separately. This revealed that individual coral colonies of only two coral species showed concordance between their Symbiodiniaceae and microbe communities (i.e. *H. microconos*, and *F. pentagona*), which could suggest that these communities respond similarly to external forcings such as local human disturbance. We anticipated at least some degree of concordance between Symbiodiniaceae and microbe communities across the disturbance gradient because corals with derived symbioses may be better at host control and partner feedback and thus have more stable microorganismal communities. Conversely, we expected that corals that are susceptible to one type of opportunist may be more susceptible to other opportunist taxa. However, the remainder of the coral species did not show similar structure between the two communities across the disturbance gradient, which may be due to a high level of variability within microbial communities compared to Symbiodiniaceae. For example, *M. aequituberculata* has a relatively stable Symbiodiniaceae community dominated by subclade C31, but a highly variable microbial community (Figure 6.3C, Figure 6.4B). Therefore, while external forcing may have instigated changes within the microbial community of this coral species, the *Symbiodiniaceae* community remained stable. Alternatively, the lack of concordance of the other coral species may be due to an Anna Karenina Principle-type response, where susceptibility to opportunistic taxa leads to stochastic changes in coral-associated taxa (Zaneveld et al. 2017). That is, if a coral was susceptible to one opportunistic taxon, it could be susceptible to a variety of other opportunistic taxa, leading to a variable response and a lack of community concordance.

Changes in microbial communities related to Symbiodiniaceae taxa presence

Since we found some concordance between Symbiodiniaceae and microbes at a community level, we used DESeq2 to examine if the presence of rare, low-abundance

Symbiodiniaceae genera was associated with differential abundance of some microbial taxa. At the coral community level, the presence of *Durusdinium* was associated with increased abundance of one OTU each from the bacterial orders Rhizobiales and Chlamydiales. Some members of Rhizobiales are nitrogen-fixing Alphaproteobacteria that in terrestrial environments are important symbionts for plant roots, however, other taxa within this order can be pathogenic (Carvalho et al. 2010). Although Rhizobiales have consistently been found within the healthy coral microbiome, and may be important nitrogen fixers within the coral holobiont (Lema et al. 2012, Garcia et al. 2016), this order also tends to be over-represented in stressed corals (McDevitt-Irwin et al. 2017). Consequently, the observed increase of Rhizobiales in the presence of *Durusdinium* may be indicative of stress within the holobiont. The implication of increased abundance of a Chlamydiales OTU is unclear, since their role in coral-associated microbial communities is still largely unknown (Goldsmith et al. 2018). This bacterial order includes obligate intracellular species that are well known pathogens of vertebrates (Everett et al. 1999), but is now recognized to be widely present in the environment (Wagner & Horn 2006). In corals, Chlamydiales may act as inter- or extra-cellular symbionts, but their role in these environments is still poorly understood (Work & Aeby 2014, Apprill et al. 2016). Further research is needed to determine whether these bacteria-Symbiodiniaceae interactions are consistent in other locations, and to evaluate the functional roles of these bacterial taxa.

At the coral community level, the presence of *Gerakladium* was associated with decreased abundance of multiple members from three phyla and five orders of bacteria, some of which are considered beneficial to the coral meta-organism. For example, the OTUs within Pseudoalteromonadaceae, which may produce antimicrobial compounds to protect the coral against pathogens (Thøgersen et al. 2016). Additionally, members of Kiloniellales are denitrifiers that are typically associated with healthy coral tissue (Imhoff & Wiese 2014, Soffer et al. 2015). Decreased abundance of these bacterial taxa could indicate a degraded microbiome in the presence of *Gerakladium*. However, a concomitant decrease of multiple potentially harmful or pathogenic bacteria was also found, potentially indicating a healthy microbiome in the presence of *Gerakladium*. This included a potential coral pathogen (Streptococcaceae, Kellogg et al. 2014, Roder et al. 2014), and a bacterial order that typically increases under stress (Flavobacteriales, McDevitt-Irwin et al. 2017). Additional research is necessary to disentangle whether and how *Gerakladium* is associated with functional changes in the coral microbiome.

In conclusion, we found that both Symbiodiniaceae and bacterial communities vary across an island-scale chronic human disturbance gradient, with the most significant changes in community structure occurring at very high disturbance levels. This expands on previous work (McDevitt-Irwin et al. *accepted*) by including samples from seven coral species across multiple levels of disturbance. There was significant concordance between Symbiodiniaceae and bacterial communities at an island scale. However, this was likely due to coral species-specific communities, since when each coral species was analyzed separately, only two massive coral species exhibited concordance between their Symbiodiniaceae and bacterial communities. Differential abundance analysis revealed that rare Symbiodiniaceae clades were associated with changes in bacterial community structure, and some distinct microbial OTUs. Further research is needed to determine whether these differences are consistent and functionally relevant at larger scales. As local and global stressors continue to increase, a better understanding of the connections between the microbial components of the coral meta-organism will provide novel insights in the mechanisms underlying coral resilience.

Chapter 7
Coral resilience to unprecedented heat stress

Authors: Danielle C. Claar[1*], Kristina L. Tietjen[1], Ross Cunning[2], Andrew C. Baker[2], Ruth D. Gates[3], Julia K. Baum[1,3]

[1]Department of Biology, University of Victoria, PO Box 1700 Station CSC, Victoria, BC, V8W 2Y2, Canada
[2]Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149, USA
[3]Hawaii Institute of Marine Biology, 46-007b Lilipuna Road, Kaneohe, HI 96744, USA.

Abstract

Coral survival through increasingly frequent and severe heat stress events is a critical factor governing the persistence of reef ecosystems under climate change. However, prospects for survival through prolonged events have seemed bleak: recovery from bleaching has only been documented after temperatures returned to normal, and evidence that local management measures protect corals is equivocal. Strikingly, we found corals that recovered from globally unprecedented thermal stress, experienced during the 2015-2016 El Niño, while still at elevated temperatures. Only corals protected from local stressors exhibited this capacity. Protected corals had distinct pre-bleaching algal symbiont communities, endured bleaching, and then recovered through proliferation of different symbionts. By illuminating connections between local protection and climate change resilience, these findings reveal unanticipated potential for coral survival in the Anthropocene.

One Sentence Summary: When protected from local stressors, some corals can regain symbionts and recover from bleaching while still at elevated temperatures.

Main Text

Climate change-amplified pulse heat stress events pose an imminent threat to the world's coral reefs (Hoegh-Guldberg et al. 2007, Ainsworth et al. 2016, Heron et al. 2016, Hughes et al. 2018). Heat stress disrupts the vital association between corals and their photosynthetic algal symbionts (*Symbiodinium spp.*), causing corals to bleach (Brown 1997). Whereas small doses of thermal stress can protect corals by inducing thermal tolerance (Ainsworth et al. 2016), extreme or long-lasting thermal anomalies typically lead to widespread mortality because physiologically compromised bleached corals cannot meet their energy demands, and succumb to starvation or disease (Douglas 2003). Thermal anomalies of this type have become increasingly common in recent decades (Hughes et al. 2018), culminating in the 2015-2016 El Niño, which triggered the worst global bleaching and mass mortality event on record (Eakin et al. 2016). Thus, while gradual ocean warming and acidification threaten the persistence of coral reefs this century (Hoegh-Guldberg et al. 2007), pulse warming events are already driving catastrophic coral loss across the world's oceans (Hughes et al. 2018). Global climate models predict these events will increase in frequency and intensity, such that annual severe bleaching will become the norm for reefs in many parts of the world this century (van Hooidonk et al. 2016).

With intervals between recurrent bleaching events now insufficient for coral ecosystem recovery (Hughes et al. 2018), there is an urgent need to develop targeted interventions that ensure coral populations can withstand and survive frequent heat stress events (Anthony et al. 2017). Current reef conservation efforts often focus on marine protected areas and other measures to reduce the local stressors upon which climate change is superimposed, based on the premise that doing so will enhance coral resistance to additional stressors, including heat stress. But whereas local management measures can significantly influence reef recovery rates following bleaching events (Mumby & Harborne 2010), the mechanistic link between local protection and climate change resistance has remained unclear. At the organismal level, the ability of corals to host different *Symbiodinium* may be one critical element of coral resilience. Different *Symbiodinium* types vary in their capacity to withstand heat stress, and extensive manipulative experiments have shown that corals that change their symbionts during recovery from bleaching can become more heat tolerant as a result (Buddemeier & Fautin 1993, Glynn et al. 2001, Baker et al. 2004, Berkelmans & van Oppen 2006, Cunning, Silverstein, et al. 2015). However, open questions remain about the ecological trade-offs of hosting variable symbiont communities, and the effects of local protection on symbiosis maintenance and recovery from thermal stress in natural coral populations.

We capitalized on the globally unprecedented heat-stress event that unfolded in the central equatorial Pacific Ocean during the 2015-2016 El Niño to test mechanisms of coral resilience to climate change. We tracked the fate of individual coral colonies throughout the El Niño at Kiritimati (Christmas Island, Republic of Kiribati, 01°52′N 157°24′W), the epicenter of this event (Appendix D Figure S1). Thermal anomalies rapidly exceeded NOAA's Coral Reef Watch (CRW) Bleaching Alert Level 1 and Alert Level 2 thresholds, reaching an unprecedented level (Hoegh-Guldberg 2011) of accumulated heat stress (25.5 Degree Heating Weeks (DHW)) over an extended bleaching event (Figure 7.1A; Appendix D Figure S2), and causing mass coral mortality around the atoll (Harvey 2016). This extreme thermal anomaly was overlaid on Kiritimati's strong gradient of chronic local human disturbance (Figure 7.2A; Appendix

D Figure S3), creating an ecosystem-scale natural factorial experiment that we leveraged to test the potential contribution of local protection to climate change resilience. We monitored bleaching, mortality, and algal symbiont communities in 80 tagged colonies of the common shallow water brain coral *Platygyra daedalea* (Scleractinia; Merulinidae) at fourteen fore reef sites (10-12 m depth) spanning Kiritimati's local disturbance gradient (Figure 7.2B), over the course of the El Niño (Figure 7.1A, B). We then assessed the relationship between coral survival, local human disturbance, pre-bleaching *Symbiodinium* community structure and the timing and nature of *Symbiodinium* community shifts during the El Niño and interpreted our findings in light of the unanticipated abilities of some corals to not only survive ten months of continuous heat stress, but to recover while still subjected to this stress.

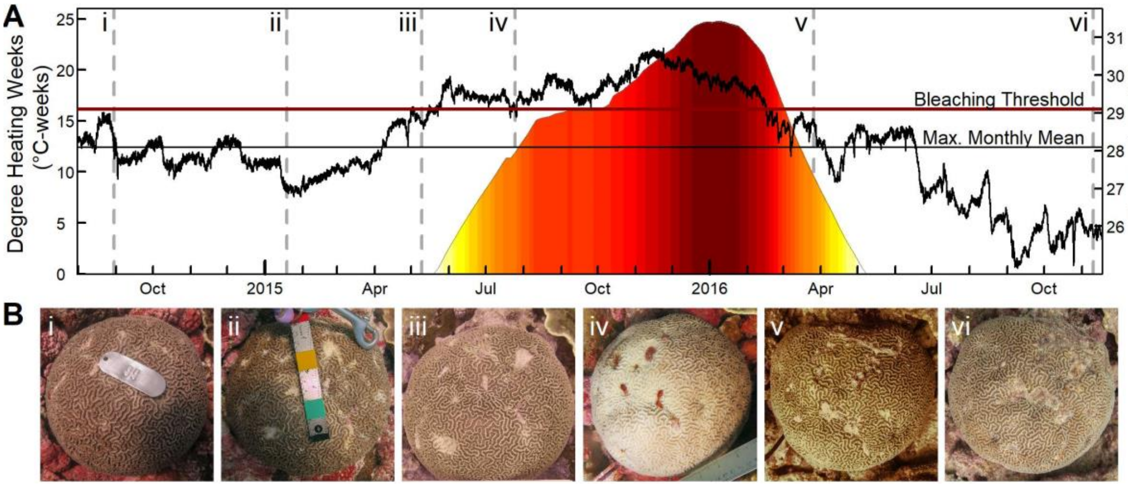


Figure 7.1. Thermal stress and bleaching response of corals at the epicenter of the 2015-2016 El Niño. A. *In situ* temperature on Kiritimati (black), maximum monthly mean and bleaching threshold (black and red lines; right axis). Shading shows cumulative heat stress on Kiritimati, as degree heating weeks (DHW; left axis) according to the following thermal thresholds: NOAA CRW Bleaching Alert 1 (4 DHW; yellow) and 2 (8 DHW; orange), 'mass coral mortality' (Hoegh-Guldberg 2011) (12 DHW; red), 'not experienced by reefs' (Hoegh-

Guldberg 2011) (24 DHW; maroon). B. A single tagged colony of *Platygyra daedalea* photographed at time points (i) to (vi) from panel a. illustrating an unusual pattern of bleaching and recovery: initially healthy (i-iii); bleached after two months (iv) and ‘recovered’ after ten months (v) of heat stress; still alive six months post-heat stress (vi). We observed similar bleaching and recovery patterns in several other coral species, although survival was variable (Appendix C Figure S4).

Widespread coral bleaching and mortality occurred at all sites, but corals situated at sites with very high levels of local human disturbance had lower survivorship than corals in areas of lower disturbance, despite experiencing the same thermal stress (Figure 7.2C; Appendix D Figure S2). We investigated if this response to local disturbance was related to *Symbiodinium* community structure using high-throughput (Illumina MiSeq) sequencing and 97% within-sample clustering of *Symbiodinium* ITS2 (phylogenetic tree showing closest related sequence(s) in Appendix D Figure S5) to identify the symbionts in each tagged colony. Corals had distinct pre-bleaching *Symbiodinium* communities depending on their level of exposure to local human disturbance, with clade D dominance in areas of very high disturbance, where subsequent survival was low, and clade C dominance in all less impacted areas, where survival was high (Figure 7.2C; Appendix D Figure S7). In contrast with previous studies that have found higher survivorship of D-dominated corals during less extreme heat-stress events (Glynn et al. 2001, Jones et al. 2008), and no relationship between local protection and coral bleaching response (Darling et al. 2010, Hughes et al. 2017), our findings suggest that C-dominated corals may be better prepared to survive prolonged periods in the bleached state, and that one mechanism by which protection from local human stressors can enhance coral resistance to heat stress is by selecting for these symbiont communities.

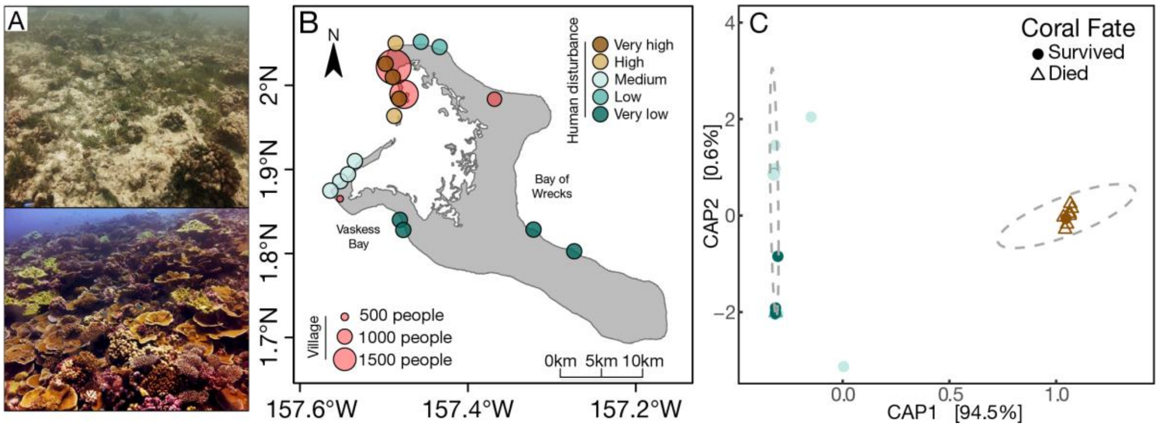


Figure 7.2. Coral bleaching response under different levels of chronic local stress. A. Examples of reef states before the 2015-2016 El Niño at very high (upper panel) and very low (lower panel) disturbance sites. B. Levels of chronic local disturbance at reef sites on Kiritimati with tagged coral colonies. C. Constrained ordination plot of 36 *Symbiodinium* communities from 29 individual *Platygyra* colonies sampled prior to the El Niño, showing two distinct groups distinguished by local disturbance intensity (F = 188, p = 0.001). Ellipses show separation of colonies that survived the bleaching event ("Survived", left side of plot) and those that did not ("Died", right side of plot), as a function of their level of local disturbance (colors as in panel B). Values in square brackets show percent variation explained by each constrained axis.

The current paradigm of coral bleaching is that, following a heat stress event, there is a window for recovery during which, if warming has ceased and conditions have returned to normal, a coral may regain its symbionts (Putnam et al. 2017). Remarkably, however, we observed coral colonies ten months into the bleaching event at Kiritimati's less disturbed sites that had regained their pigmentation, indicating that they had recovered their symbionts while still at elevated temperatures (Figure 7.1B; Appendix D S4). To corroborate this visual evidence of recovery, we used qPCR assays to quantify changes in overall algal symbiont abundance and dominant symbiont identity as a function of the timing of bleaching and recovery in tagged coral colonies (Figure 7.3,

Appendix D S6). Corals at the less disturbed sites that survived prolonged heat stress had a baseline symbiont-to-host (S:H) cell ratio of 0.037 ± 0.010 (mean ± s.e.; time point iii), which dropped to 0.008 ± 0.003 (mean ± s.e.) while bleached (time point iv), but then returned to pre-bleaching levels (0.045 ± 0.006 mean ± s.e.) by late-March 2016 (time point v) (Figure 7.3) while temperatures were above the maximum monthly mean temperature and cumulative heat stress was greater than 10 DHWs (Figure 7.1B, Figure 7.3).

The recovery of corals at protected sites also involved a near-complete shift in the relative abundance of symbiont types, including those that were initially rare. Although highly abundant symbionts drive interaction outcomes across a range of symbiotic taxa, rare types may be unstable (Coffroth et al. 2010) and are sometimes disregarded as

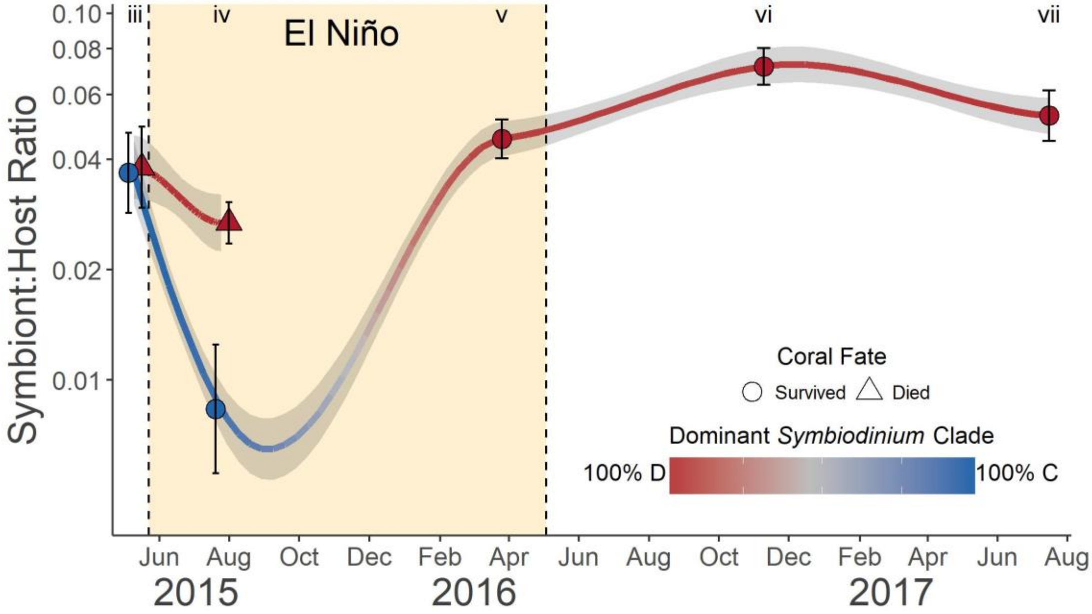


Figure 7.3. Changes in *Symbiodinium* relative abundance and identity, from clade C to clade D dominance, during the 2015-2016 El Niño. Mean symbiont:host cell ratios at different time points of the event for tracked *Platygyra daedalea* coral colonies that survived (circles) or died (triangles). Fitted lines show potential trajectories between sampled time points, and color indicates dominant *Symbiodinium* clade in tracked colonies. Bleaching is indicated by a low cell ratio density (e.g. 0.01). Shaded area between dashed vertical lines corresponds to DHW > 0 from Fig. 7.1.

having minimal functional significance (Lee et al. 2016). We found that D1/D1a *Symbiodinium* types, which were extremely rare (0.05% ± 0.03%, mean ± s.e.) or undetectable in protected coral colonies before the bleaching event, increased enormously during recovery in surviving corals (98.8% ± 1.05%, mean ± s.e.), driving a shift from clade C to clade D dominance (Appendix D Figure S7). Newly dominant symbionts in these corals were the same type that had originally dominated in the corals that died at the highly disturbed sites. These results support recent studies suggesting that rare species can be disproportionately important in maintaining functional processes during environmental change, and in some cases play a critical role in mediating host survival (Silverstein et al. 2015, Boulotte et al. 2016).

Shifts in favor of *Symbiodinium* clade D have been associated with increases in heat tolerance and improved coral survival (Glynn et al. 2001, Baker et al. 2004, Berkelmans & van Oppen 2006, McClanahan et al. 2014, Silverstein et al. 2015), and indeed we found *Platygyra* corals, which initially contained clade C symbionts, that recovered with clade D symbionts and ultimately survived. However, *Platygyra* at highly disturbed sites that started with putatively thermotolerant clade D symbionts ultimately fared worse during this period of prolonged heat stress than their counterparts on less impacted reefs. This negative outcome may reflect physiological tradeoffs, such as lower contribution of energy from clade D symbionts to their coral hosts (Cantin et al. 2009), leaving little capacity for the coral holobiont to buffer subsequent environmental stress. We cannot, however, discount the possibility that environmental differences characteristic of highly disturbed sites, such as increased dissolved organic carbon (DOC) and microbial activity (Dinsdale et al. 2008), may also have reduced the ability of these corals to survive in the bleached state. Regardless, it appears that shifts to thermotolerant symbionts can be critical in mediating coral survival, and local protection can promote these shifts by reducing mortality while bleached. In the long-term, however, harboring thermotolerant clade D symbionts may be suboptimal, and chronic declines in reef condition that cause shifts in favor of these symbionts might reduce the capacity for corals to survive during prolonged heat stress that exceeds their bleaching tolerance. Corals at protected sites that have shifted to clade D as a short-term survival strategy may therefore need to shift back to clade C symbionts (e.g., Thornhill et al. 2006) if they are to survive a subsequent prolonged thermal anomaly.

Overall, our study illustrates that some corals can survive extremely prolonged heat stress and reveals a previously unrecognized connection between local protection, algal symbiont communities, and coral survivorship. By tracking individual coral colonies throughout an extreme heat stress event, we have linked macro- and micro-ecological processes on reefs and demonstrated that the capacity for coral resilience to climate change can be tangibly related to local reef protection. Our findings lay the foundation for investigating the prevalence of this mechanism across coral communities, understanding which types of management measures are most effective for inducing this response, and testing if corals have the capacity to maintain successful symbioses and shift symbionts frequently enough to survive repeated bleaching. The future of coral reef ecosystems depends not only on large-scale mitigation of global greenhouse gas emissions, but also on our ability to build from these unanticipated findings and develop targeted management interventions that enhance coral resilience to repetitive warming events.

Chapter 8 Conclusion

Coral resilience is vitally important for the persistence of coral reefs over the next century as oceans warm and bleaching events become more frequent (Hughes et al. 2018). Resilience can be incrementally eroded or catastrophically eliminated by repeated heat stress and by local disturbance (such as eutrophication and pollution). However, corals have a diverse set of resilience mechanisms to respond to stressors in their environment (McClanahan et al. 2012), including host responses and shifts in their associated *Symbiodinium* and microbial communities. Corals host a diverse community of *Symbiodinium* and other microorganisms, that range from symbionts to parasites and pathogens (Ben-Haim et al. 1999, Baker et al. 2018), and environmental adaptation involves multiple members of the coral metaorganism (van Oppen et al. 2018). Therefore, determining how changes in these communities erode or promote resilience is of paramount importance to understanding coral persistence over the next century. For example, empirically measuring *Symbiodinium* sequence diversity can be used as a tool to identify reefs that may have a strong potential for resilience to human stressors (Baskett et al. 2009). Despite the foundational importance of coral symbioses to reefs worldwide, much is still unknown about how multiple local and global stressors alter the resilience of these partnerships.

In this doctoral thesis, I used satellite-based oceanography measurements, next generation sequencing, and ecological surveys to quantify interactions between coral symbioses and stress (both local and global), with implications for coral resilience. I first synthesized the literature on coral symbiosis in Chapter 2. Rapid advancements have taken place in this field since this manuscript was submitted for publication in 2015, which I discuss in more detail below. I conducted a global meta-analysis for Chapter 3, in which I showed that the influence of El Niño on coral bleaching and mortality is substantial, and that reefs in warmer locations may be more sensitive to warming than cooler locations (Figure 3.4). Chapter 4 is a perspectives piece focusing on the importance of timing of bleaching surveys, which has implications for future studies as bleaching events become longer and more severe. Focusing at an island-scale for Chapters 5 through 7, I showed that local human disturbance 1) increases *Symbiodinium* beta diversity in corals, sediment, and the water column (Figure 5.3), 2) increases both *Symbiodinium* and microbial alpha and beta diversity across a range of coral species (Figure 6.2, Figure 6.3), and 3) shifts *Symbiodinium* communities towards more opportunistic taxa (Figure 7.2, Figure 7.3). These findings suggest that local disturbance can shape coral, *Symbiodinium,* and microbial communities on reefs, and that mitigating local impacts may help to improve their resilience capacity. The 2015/2016 El Niño event was extreme (Figure 3.5) and caused heat stress that was globally unprecedented since at least the 1800s (Lough et al. 2018). This event caused catastrophic coral mortality at my focal field site, Kiritimati, but it also revealed a new mechanism of coral persistence, namely that, depending on their *Symbiodinium* communities, some corals can recover from bleaching before heat stress subsides (Figure 7.1). Natural experiments like the 2015/2016 El Niño allow us to better understand coral resilience under stress, and these results emphasize that environmental and baseline data are of the utmost importance to explaining underlying ecological mechanisms.

Advances, limitations, and future directions

The field of coral symbiosis research is rapidly evolving, and significant changes have taken place even since the beginning of my PhD program. Draft genomes of multiple *Symbiodinium* clades have been published, and comparative analyses among these and previously published draft *Symbiodinium* genomes have allowed for the identification of genes associated with coral symbiosis (Liu et al. 2018). A significant manuscript regarding *Symbiodinium* evolution and taxonomy was just published, providing evidence that modern scleractinian corals and dinoflagellates within the family Symbiodiniaceae diversified together during the middle Jurassic Period (LaJeunesse et al. 2018). This revises our understanding of the origin of this family from the Paleocene or Eocene (50-65 mya) to the Mesozoic (140-200 mya). The authors also revised Symbiodiniaceae from a single genus (*Symbiodinium*) with multiple clades (A-I) to approximately fifteen new genera within this family, including seven newly named genera that coincide with previous *Symbiodinium* clades (LaJeunesse et al. 2018). The previous *Symbiodinium* clade A retains that genus name, while other clades have been renamed (i.e. clade B, *Breviolum*; clade C, *Cladocopium*; clade D, *Durusdinium*; clade E, *Effrenium*; clade F, *Fugacium*; clade G, *Gerakladium*) (LaJeunesse et al. 2018). Since two of my PhD chapters are already published, and three more have already been submitted for publication, I have retained the previous clade terminology with the exception of Chapters 4 and 6. In future work, I intend to consistently incorporate these new naming conventions, and I am keen to see the field move forward with this new knowledge.

As our ecological and evolutionary knowledge within this field is growing, so too are the tools we use to address them. Amplicon sequencing is not without its problems, and several assumptions are necessary to use these data. For example, one must assume that extraction, amplification, and sequencing are not biased towards or against certain taxa. While I have no evidence to show that there was bias in these steps during my sampling processing, these biases can be insidious and difficult to detect. Despite these potential biases, amplicon sequences have been used to successfully identify ecological patterns in coral symbioses (e.g. Quigley et al. 2014, Boulotte et al. 2016, Cunning et al. 2017). High-throughput amplicon sequencing can be interpreted as relative abundances of observed taxa; however this is limited by assumptions regarding amplification and sequencing success for each individual taxon. Additionally, in Chapter 7, I conducted quantitative PCR (qPCR) at the *Symbiodinium* clade level in conjunction with amplicon sequencing and found that clade abundances within a coral colony were comparable between both methods. The development of additional *Symbiodinium* subclade-level qPCR assays would be necessary to further test this assumption at a finer taxonomic scale. The available methods to analyze these data are also progressing; from 97% OTU clustering towards amplicon sequence variants (ASV) which allow for finer resolution (Callahan et al. 2017), and also to oligotyping and minimum entropy decomposition (MED) (Eren et al. 2013, Eren et al. 2015). As these tools continue to be explored and evolve, our understanding of coral symbiosis ecology will grow and evolve as well.

In the global meta-analysis (Chapter 3), I found that qualitative bleaching records and inconsistent statistical reporting severely limited synthesis of previously published data. I provided a reporting checklist for new bleaching studies, in the hopes that more thorough data inclusion will allow for more informative syntheses in the future. Unfortunately, because of these limitations, I was unable to parse our results by coral taxa or other abiotic factors (e.g. reef depth, upwelling, currents). These factors likely influence variability in coral bleaching and mortality at a global scale and should be investigated as more comprehensive data sets become available.

The 2015/2016 El Niño event was extreme, and it will likely take several years for the results of studies from individual reefs and regions to be published, although initial research has shown a catastrophic breakdown of coral assemblages in response to this event (e.g. this event transformed 29% of the 3,863 individual reefs of the Great Barrier Reef; Hughes et al. 2018). Once these data are available, it will be interesting to compare coral responses during this event to research on previous El Niño events. Specifically, by using the same data analysis steps I used in the global meta-analysis (Chapter 3), it will be possible to quantify whether and how reef responses to pulse warming have changed over the past few decades.

I found intriguing patterns and mechanisms of coral symbioses across a human disturbance gradient on the world’s largest atoll. Namely, local human disturbance increases *Symbiodinium* beta diversity in corals, sediment, and the water column (Chapter 5), increases microbial alpha and beta diversity across a range of coral species (Chapter 6), and shifts *Symbiodinium* communities towards more opportunistic taxa (Chapter 7). Specifically, I also found that local human disturbance fundamentally changes coral symbiotic communities, making them more vulnerable to bleaching and mortality (Chapter 7). Conversely, I found that even moderate alleviation of local human disturbance, can protect corals by maintaining healthy symbiotic communities (Chapter

7). I found this potential resilience mechanism in one species of coral on Kiritimati, and additional research is necessary to test whether these patterns and mechanisms are present in other coral species and locations. Future research should also follow up on these findings by investigating similar questions in other biogeographic regions or across broader spatial scales. Although scaling up will likely introduce additional confounding factors, expansion of these research questions to a regional scale, while carefully accounting for local influences, is a promising avenue of future research.

Another important avenue of research is continuing to investigate the function of coral-associated microbes. Except for a handful of bacterial genera (e.g. *Vibrio*, *Endozocomonas*, Ben Haim 1999, Ben Haim 2003, Kushmaro et al 2001, Yang et al 2003, Ding et al 2016), very little is known about the fine-scale taxonomy and function of coral-associated microbes. This knowledge gap can be a major roadblock to understanding interactions between coral and their microbial communities. For example, in Chapter 7, I found that within the coral *Platygyra daedalea*, the presence of clade D *Symbiodinium* was associated with increased abundance of an operational taxonomic unit (OTU) of Rhizobiales. Members of this family may be pathogenic (Carvalho et al. 2010) or beneficial (as nitrogen fixers; Lema et al. 2012, Garcia et al. 2016). They can be found in abundance in healthy corals (Lema et al. 2012) but may also be over-represented in stressed corals (McDevitt-Irwin et al. 2017). Consequently, it is unclear whether this increased abundance of Rhizobiales in the presence of clade D *Symbiodinium* is a slightly different variation of a healthy microbiome, or an indicator of stress caused by local disturbance. Many microbial taxa have even less known about them, and additional research is needed to elucidate the implications of changing microbial communities.

While logistically difficult and time consuming, tagging and tracking individual coral colonies was essential for the results and conclusions of these studies. Quantifying symbioses before the 2015/2016 El Niño allowed us to confirm baseline community structure and to identify shifts in dominant *Symbiodinium* types coincident with local human disturbance and pulse warming. Coral tracking efforts on Kiritimati are ongoing; some corals now have four years of symbiosis samples (August 2014 – July 2018). Additional tagging efforts are taking place across the globe, including in Hawaii (e.g. Cunning et al. 2016), Panama (e.g. Neal et al. 2017), the US Virgin Islands (e.g. Edmunds 2007, 2017). There are still many intriguing questions that can be asked and answered with tagged coral samples, including investigating how coral life history shapes symbiont diversity dynamics during and after an extreme warming event, and whether local disturbance influences post-warming changes in symbiotic community structure.

Conclusion

Local management strategies are relevant, even on severely stressed reefs (Mumby et al. 2013) and under climate change, and managing for resilient coral reefs in the context of synergistic stressors is one of the greatest conservation challenges of our time (Knowlton & Jackson 2008). This research on spatial and temporal dynamics of reef resilience mechanisms, and specifically the resistance and recovery of coral-*Symbiodinium* partnerships, provides baseline data to inform conservation strategies that may help corals survive impending climate change stressors. For example, my research supports previous research showing that local protection is indeed important (Wooldridge & Done 2009, Wooldridge 2009, Mumby & Harborne 2010). Therefore, local stressors must be addressed simultaneously with global climate change solutions to bolster coral reef resilience. A better understanding of coral-*Symbiodinium* dynamics at the island scale will aid in reef conservation, supporting the design and implementation of management strategies for resilient reefs.

Bibliography

Abrego DD, Willis BL, Oppen MJH van (2012) Impact of light and temperature on the uptake of algal symbionts by coral juveniles. PLoS ONE 7:e50311

Adam TC, Schmitt RJ, Holbrook SJ, Brooks AJ, Edmunds PJ, Carpenter RC, Bernardi G (2011) Herbivory, connectivity, and ecosystem resilience: Response of a coral reef to a large-scale perturbation. PLoS ONE 6:e23717

Adams LM, Cumbo VR, Takabayashi M (2009) Exposure to sediment enhances primary acquisition of *Symbiodinium* by asymbiotic coral larvae. Marine Ecology Progress Series 377:149–156

Aeby GS, Kenyon JC, Maragos JE, Potts DC (2003) First record of mass coral bleaching in the Northwestern Hawaiian Islands. Coral Reefs 22:256

Ahmed M, Umali GM, Chong CK, Rull MF, Garcia MC (2007) Valuing recreational and conservation benefits of coral reefs - The case of Bolinao, Philippines. Ocean & Coastal Management 50:103–118

Ainsworth TD, Heron SF, Ortiz JC, Mumby PJ, Grech A, Ogawa D, Eakin CM, Leggat W (2016) Climate change disables coral bleaching protection on the Great Barrier Reef. Science 352:338–342

Ainsworth TD, Hoegh-Guldberg O (2009) Bacterial communities closely associated with coral tissues vary under experimental and natural reef conditions and thermal stress. Aquatic Biology 4:289–296

Ainsworth T, Krause L, Bridge T, Torda G, Raina J-B, Zakrzewski M, Gates RD, Padilla-Gamiño JL, Spalding HL, Smith C, Woolsey ES, Bourne DG, Bongaerts P, Hoegh-Guldberg O, Leggat W (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. The ISME Journal 9:2261

Ainsworth TD, Thurber RV, Gates RD (2010) The future of coral reefs: a microbial perspective. Trends in Ecology & Evolution 25:233–240

Allen MR, Barros VR, Broome J, Cramer W, Christ R (2014) IPCC fifth assessment synthesis report-climate change 2014 synthesis report. IPCC

Amir A, McDonald D, Navas-Molina JA, Kopylova E, Morton JT, Zech Xu Z, Kightley EP, Thompson LR, Hyde ER, Gonzalez A, Knight R (2017) Deblur rapidly resolves single-nucleotide community sequence patterns. mSystems 2:e00191-16

Anderson MJ, Crist TO, Chase JM, Vellend M, Inouye BD, Freestone AL, Sanders NJ, Cornell H V, Comita LS, Davies KF, Harrison SP, Kraft NJB, Stegen JC, Swenson NG (2011) Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecology Letters 14:19–28

Anthony K, Bay LK, Costanza R, Firn J, Gunn J, Harrison P, Heyward A, Lundgren P, Mead D, Moore T, Mumby PJ, Oppen MJH van, Robertson J, Runge MC, Suggett DJ, Schaffelke B, Wachenfeld D, Walshe T (2017) New interventions are needed to save coral reefs. Nature Ecology & Evolution 1:1420–1422

Apprill A, Weber LG, Santoro AE (2016) Distinguishing between microbial habitats unravels ecological complexity in coral microbiomes. mSystems 1:e00143-16

Arif C, Daniels C, Bayer T, Banguera-Hinestroza E, Barbrook A, Howe CJ, LaJeunesse TC, Voolstra CR (2014) Assessing *Symbiodinium* diversity in scleractinian corals via next-generation sequencing-based genotyping of the ITS2 rDNA region. Molecular Ecology 23:4418–4433

Arthur R, Done TJ, Marsh H, Harriott V (2006) Local processes strongly influence post-bleaching benthic recovery in the Lakshadweep Islands. Coral Reefs 25:427–440

Ateweberhan M, Feary DA, Keshavmurthy S, Chen A, Schleyer MH, Sheppard C (2013) Climate change impacts on coral reefs: Synergies with local effects, possibilities for acclimation, and management implications. Marine Pollution Bulletin 74:526–539

Baird AH, Bhagooli R, Ralph PJ, Takahashi S (2009) Coral bleaching: the role of the host. Trends in Ecology and Evolution 24:16–20

Baird AH, Cumbo VR, Leggat W, Rodriguez-Lanetty M (2007) Fidelity and flexibility in coral symbioses. Marine Ecology Progress Series 347:307–309

Baird AH, Guest JR, Willis BL (2009) Systematic and biogeographical patterns in the reproductive biology of scleractinian sorals. Annual Review of Ecology, Evolution, and Systematics 40:551–571

Baird AH, Marshall PA (2002) Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Marine Ecology Progress Series 237:133–141

Baker AC (2001) Ecosystems: Reef corals bleach to survive change. Nature 411:765–766

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. Annual Review of Ecology, Evolution, and Systematics 34:661–689

Baker DM, Andras JP, Jordán-Garza AG, Fogel ML (2013) Nitrate competition in a coral symbiosis varies with temperature among *Symbiodinium* clades. ISME Journal 7:1248–1251

Baker AC, Cunning R (2016) Bulk gDNA extraction from coral samples. protocols.io

Baker DM, Freeman CJ, Wong JCY, Fogel ML, Knowlton N (2018) Climate change promotes parasitism in a coral symbiosis. ISME J 12:921–930

Baker AC, Glynn PW, Riegl BM (2008) Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. Estuarine, Coastal and Shelf Science 80:435–471

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Coral reefs: corals' adaptive response to climate change. Nature 430:741

Bamston AG, Chelliah M, Goldenberg SB (1997) Documentation of a highly ENSO-related SST region in the equatorial pacific: Research note. Atmosphere-Ocean 35:367–383

Ban SS, Graham NAJ, Connolly SR (2014) Evidence for multiple stressor interactions and effects on coral reefs. Global Change Biology 20:681–697

Banaszak AT, LaJeunesse TC, Trench RK (2000) The synthesis of mycosporine-like amino acids (MAAs) by cultured, symbiotic dinoflagellates. Journal of Experimental Marine Biology and Ecology 249:219–233

Barnett J, Adger WN (2003) Climate dangers and atoll countries. Climatic Change 61:321–337

Barott K, Smith J, Dinsdale E, Hatay M, Sandin S, Rohwer F (2009) Hyperspectral and physiological analyses of coral-algal interactions. PLoS One 4:e8043

Baskett ML, Gaines SD, Nisbet RM (2009) Symbiont diversity may help coral reefs survive moderate climate change. Ecological Applications 19:3–17

Baskett ML, Nisbet RM, Kappel C V, Mumby PJ, Gaines SD (2010) Conservation management approaches to protecting the capacity for corals to respond to climate change: a theoretical comparison. Global Change Biology 16:1229–1246

Baum JK, Worm B (2009) Cascading top-down effects of changing oceanic predator abundances. Journal of Animal Ecology 78:699–714

Baums IB, Devlin-Durante MK, LaJeunesse TC (2014) New insights into the dynamics between reef corals and their associated dinoflagellate endosymbionts from population genetic studies. Molecular Ecology 23:4203–4204

Beijbom O, Edmunds PJ, Roelfsema C, Smith J, Kline DI, Neal BP, Dunlap MJ, Moriarty V, Fan T-Y, Tan C-J, Chan S, Treibitz T, Gamst A, Mitchell BG, Kriegman D (2015) Towards automated annotation of benthic survey images: Variability of human experts and operational modes of automation. PLoS ONE 10:e0130312

Bellwood DR, Hughes TP, Folke C, Nyström M (2004) Confronting the coral reef crisis. Nature 429:827–833

Belyea LR, Lancaster J (1999) Assembly rules within a contingent ecology. Oikos 86:402–416

Ben-Haim Y, Banim E, Kushmaro A, Loya Y, Rosenberg E (1999) Inhibition of photosynthesis and bleaching of zooxanthellae by the coral pathogen *Vibrio shiloi*. Environmental Microbiology 1:223–229

Bender EA, Case TJ, Gilpin ME (1984) Perturbation experiments in community ecology: Theory and practice. Ecology 65:1–13

Bensadia F, Boudreault S, Guay J-F, Michaud D, Cloutier C (2006) Aphid clonal resistance to a parasitoid fails under heat stress. Journal of Insect Physiology 52:146–157

Béraud E, Gevaert F, Rottier C, Ferrier-Pagès C (2013) The response of the scleractinian coral *Turbinaria reniformis* to thermal stress depends on the nitrogen status of the coral holobiont. The Journal of Experimental Biology 216:2665–2674

Berkelmans R, De'ath G, Kininmonth S, Skirving WJ (2004) A comparison of the 1998 and 2002 coral bleaching events on the Great Barrier Reef: spatial correlation, patterns, and predictions. Coral Reefs 23:74–83

Berkelmans R, Oppen MJH van (2006) The role of zooxanthellae in the thermal tolerance of corals: a "nugget of hope" for coral reefs in an era of climate change. Proceedings of the Royal Society B: Biological Sciences 273:2305–2312

Black CC, Burris JE (1983) Diurnal carbon-14 partitioning between zooxanthellae and the coral animal tissue of intact *Seriatopora hystrix* colonies. Marine Biology 75:117–120

Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG (2013) Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nature Methods 10:57–59

Bongaerts P, Frade PR, Ogier JJ, Hay KB, Bleijswijk J van, Englebert N, Vermeij MJA, Bak RP, Visser PM, Hoegh-Guldberg O (2013) Sharing the slope: depth partitioning of agariciid corals and associated *Symbiodinium* across shallow and mesophotic habitats (2-60 m) on a Caribbean reef. BMC Evolutionary Biology 13:205

Boschma H (1925a) The nature of the association between anthozoa and zooxanthellae. Proceedings of the National Academy of Sciences 11:65–67

Boschma H (1925b) On the feeding reactions and digestion in the coral polyp *Astrangia danae*, with notes on its symbiosis with zooxanthellae. The Biological Bulletin 49:407–439

Boulotte NM, Dalton SJ, Carroll AG, Harrison PL, Putnam HM, Peplow LM, Oppen MJ van (2016) Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals. The ISME Journal 10:2693–2701

Bourne D, Iida Y, Uthicke S, Smith-Keune C (2008) Changes in coral-associated microbial communities during a bleaching event. The ISME Journal 2112:350–363

Bourne DG, Morrow KM, Webster NS (2016) Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. Annual Review of Microbiology 70:317–340

Boylan P, Kleypas JA (2008) New insights into the exposure and sensitivity of coral reefs to ocean warming. In: Proceedings of the 11th International Coral Reef Symposium. Ft. Lauderdale, FL, p 854–858

Boyle S, Anda V De, Koenig K, O’Reilly E, Schafer M, Acoba T, Dillon A, Heenan A, Oliver TTA, Swanson DW, Others (2017) Coral reef ecosystems of the Pacific Remote Islands Marine National Monument: a 2006-2016 overview.

Boyle MA, Samaha AL, Rodewald AM, Hoffmann AN (2013) Evaluation of the reliability and validity of GraphClick as a data extraction program. Computers in Human Behavior 29:1023–1027

Brainard RE, Oliver T, McPhaden MJ, Cohen A, Venegas R, Heenan A, Vargas-Ángel B, Rotjan R, Mangubhai S, Flint E, Hunter SA (2018) Ecological Impacts of the 2015/16 El Niño in the Central Equatorial Pacific. Bulletin of the American Meteorological Society 99:S21–S26

Brandt KA (1881) Uber das Zusammenleben von Thieren und Algen. Verhandlungen der Physiologischen Gesellschaft zu Berlin

Brook BW, Sodhi NS, Bradshaw CJA (2008) Synergies among extinction drivers under global change. Trends in Ecology and Evolution 23:453–460

Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs 16:S129–S138

Brown BE, Suharsono (1990) Damage and recovery of coral reefs affected by El Niño related seawater warming in the Thousand Islands, Indonesia. Coral Reefs 8:163–170

Browne NK, Tay JKL, Low J, Larson O, Todd PA (2015) Fluctuations in coral health of four common inshore reef corals in response to seasonal and anthropogenic changes in water quality. Marine Environmental Research 105:39–52

Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. Trends in Ecology and Evolution 18:119–125

Buddemeier RW, Baker AC, Fautin DG, Jacobs JR (2004) The adaptive hypothesis of bleaching. In: Coral Health and Disease.p 427–444

Buddemeier RW, Fautin DG (1993) Coral bleaching as an adaptive mechanism. Bioscience 43:320–326

Burke LM, Reytar K, Spalding M, Perry A (2011) Reefs at Risk Revisited. World Resources Institute

Burriesci MS, Raab TK, Pringle JR (2012) Evidence that glucose is the major transferred metabolite in dinoflagellate-cnidarian symbiosis. The Journal of Experimental Biology 215:3467–3477

Byler KA, Carmi-Veal M, Fine M, Goulet TL (2013) Multiple symbiont acquisition strategies as an adaptive mechanism in the coral Stylophora pistillata. PLoS ONE 8:e59596

Cáceres M De (2013) How to use the indicspecies package (ver. 1.7. 1). Centre Tecnològic Forestal de Catalunya, Catalonia

Cai W, Borlace S, Lengaigne M, Rensch P van, Collins M, Vecchi G, Timmermann A, Santoso A, McPhaden MJ, Wu L, England MH, Wang G, Guilyardi E, Jin F-F (2014) Increasing frequency of extreme El Niño events due to greenhouse warming. Nature Climate Change 4:111

Calcagno V, Mazancourt C de (2010) glmulti: an R package for easy automated model selection with (generalized) linear models. Journal of Statistical Software 34:1–28

Callahan BJ, McMurdie PJ, Holmes SP (2017) Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME Journal 11:2639–2643

Cantin NE, Oppen MJH van, Willis BL, Mieog JC (2009) Juvenile corals can acquire more carbon from high-performance algal symbionts. Coral Reefs 28:405

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal 6:1621–1624

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, Fierer N, Knight R (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108:4516–4522

Carilli J, Walsh S (2012) Benthic foraminiferal assemblages from Kiritimati (Christmas) Island indicate human-mediated nutrification has occurred over the scale of decades. Marine Ecology Progress Series 456:87–99

Carvalho TS de, Jesus E da C, Barlow J, Gardner TA, Soares IC, Tiedje JM, Moreira FM de S (2016) Land use intensification in the humid tropics increased both alpha and beta diversity of soil bacteria. Ecology 97:2760–2771

Carvalho FM, Souza RC, Barcellos FG, Hungria M, Vasconcelos ATR (2010) Genomic and evolutionary comparisons of diazotrophic and pathogenic bacteria of the order Rhizobiales. BMC Microbiology 10:37

Cayuela L, Golicher DJ, Benayas JMR, González-Espinosa M, Ramírez-Marcial N (2006) Fragmentation, disturbance and tree diversity conservation in tropical montane forests. Journal of Applied Ecology 43:1172–1181

Ceh J, Kilburn MR, Cliff JB, Raina J-B, Keulen M van, Bourne DG (2013) Nutrient cycling in early coral life stages: *Pocillopora damicornis* larvae provide their algal symbiont (*Symbiodinium*) with nitrogen acquired from bacterial associates. Ecology and Evolution 3:2393–2400

Chan YL, Pochon X, Fisher MA, Wagner D, Concepcion GT, Kahng SE, Toonen RJ, Gates RD (2009) Generalist dinoflagellate endosymbionts and host genotype diversity detected from mesophotic (67-100 m depths) coral *Leptoseris*. BMC Ecology 9:21

Chao A, Gotelli NJ, Hsieh TC, Sander EL, Ma KH, Colwell RK, Ellison AM (2014) Rarefaction and extrapolation with Hill numbers: a framework for sampling and estimation in species diversity studies. Ecological Monographs 84:45–67

Chen C-P, Tseng C-H, Chen CA, Tang S-L (2011) The dynamics of microbial partnerships in the coral *Isopora palifera*. ISME J 5:728–740

Chou LM, Toh TC, Toh K Ben, Ng CSL, Cabaitan P, Tun K, Goh E, Afiq-Rosli L, Taira D, Du RCP, Loke HX, Khalis A, Li J, Song T (2016) Differential response of coral assemblages to thermal stress underscores the complexity in predicting bleaching susceptibility. PLoS One 11:e0159755

Cinner JE, Huchery C, MacNeil MA, Graham NAJ, McClanahan TR, Maina J, Maire E, Kittinger JN, Hicks CC, Mora C, Allison EH, D'Agata S, Hoey AS, Feary DA, Crowder L, Williams ID, Kulbicki M, Vigliola L, Wantiez L, Edgar GJ, Stuart-Smith RD, Sandin SA, Green AL, Hardt MJ, Beger M, Friedlander AM, Campbell

SJ, Holmes KE, Wilson SK, Brokovich E, Brooks AJ, Cruz-Motta JJ, Booth DJ, Chabanet P, Gough C, Tupper M, Ferse SCA, Sumaila UR, Mouillot D (2016) Bright spots among the world's coral reefs. Nature 535:416–419

Claar DC, Baum JK (2018) Timing matters: survey timing during extended heat stress can influence perceptions of coral susceptibility to bleaching. Coral Reefs

Claar DC, Fabina NS, Putnam HM, Cunning R, Sogin E, Baum JK, Gates RD (2017) Embracing complexity in coral-algal symbioses. In: Algal and Cyanobacteria Symbioses. World Scientific, p 467–492

Claar DC, Szostek L, McDevitt-Irwin JM, Schanze JJ, Baum JK (2018) Global patterns and impacts of El Niño events on coral reefs: A meta-analysis. PLoS One 13:e0190957

Clavel J, Julliard R, Devictor V (2011) Worldwide decline of specialist species: toward a global functional homogenization? Frontiers in Ecology and the Environment 9:222–228

Coffroth MA, Lewis CF, Santos SR, Weaver JL (2006) Environmental populations of symbiotic dinoflagellates in the genus *Symbiodinium* can initiate symbioses with reef cnidarians. Current Biology 16:R985-7

Coffroth MA, Poland DM, Petrou EL, Brazeau DA, Holmberg JC (2010) Environmental symbiont acquisition may not be the solution to warming seas for reef-building corals. PLoS ONE 5:e13258

Condamine FL, Rolland J, Morlon H (2013) Macroevolutionary perspectives to environmental change. Ecology Letters 16:72–85

Connolly SR, Baird AH (2010) Estimating dispersal potential for marine larvae: dynamic models applied to scleractinian corals. Ecology 91:3572–3583

Cooper TF, Berkelmans R, Ulstrup KE, Weeks S, Radford B, Jones AM, Doyle J, Canto M, O'Leary RA, Oppen MJH van (2011) Environmental factors controlling the distribution of *Symbiodinium* harboured by the coral *Acropora millepora* on the Great Barrier Reef. PLoS ONE 6:e25536

Corbin C, Heyworth ER, Ferrari J, Hurst GDD (2017) Heritable symbionts in a world of varying temperature. Heredity 118:10–20

Correa AMS, McDonald MD, Baker AC (2009) Development of clade-specific *Symbiodinium* primers for quantitative PCR (qPCR) and their application to detecting clade D symbionts in Caribbean corals. Marine Biology 156:2403–2411

Costanza R, D’Arge R, Groot R De, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O’Neill R V, Paruelo J, Raskin RG, Sutton P, Belt M Van Den (1997) The value of the world’s ecosystem services and natural capital. Nature 387:253–260

Côté IM, Darling ES (2010) Rethinking ecosystem resilience in the face of climate change. PLoS Biology 8:e1000438

Côté IM, Darling ES, Brown CJ (2016) Interactions among ecosystem stressors and their importance in conservation. Proceedings of the Royal Society B: Biological Sciences 283:20152592

Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters 11:1304–1315

Cumbo VR, Oppen M van, Baird AH (2018) Temperature and *Symbiodinium* physiology affect the establishment and development of symbiosis in corals. Marine Ecology Progress Series 587:117–127

Cunning R, Baker AC (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nature Climate Change 3:259–262

Cunning R, Gates RD, Edmunds PJ (2017) Using high-throughput sequencing of ITS2 to describe *Symbiodinium* metacommunities in St. John, US Virgin Islands. PeerJ 5:e3472

Cunning JR, Ritson-Williams R GR (2016) Patterns of bleaching and recovery of *Montipora capitata* in Kāneʻohe Bay, Hawaiʻi, USA. Marine Ecology Progress Series 551:131–139

Cunning R, Silverstein RN, Baker AC (2015) Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. Proceedings of the Royal Society B: Biological Sciences 282:20141725

Cunning R, Yost DM, Guarinello ML, Putnam HM, Gates RD (2015) Variability of *Symbiodinium* communities in waters, sediments, and corals of thermally distinct reef pools in American Samoa. PLoS One 10:e0145099

D'Croz L, Maté JL (2004) Experimental responses to elevated water temperature in genotypes of the reef coral *Pocillopora damicornis* from upwelling and non-upwelling environments in Panama. Coral Reefs 23:473–483

Darling ES, Alvarez-Filip L, Oliver TA, McClanahan TR, Côté IM, Bellwood DR (2012) Evaluating life-history strategies of reef corals from species traits. Ecology Letters 15:1378–1386

Darling ES, McClanahan TR, Côté IM (2010) Combined effects of two stressors on Kenyan coral reefs are additive or antagonistic, not synergistic. Conservation Letters 3:122–130

Davey MK, Anderson DLT (1998) A comparison of the 1997/98 El Niño with other such events. Weather 53:295–302

Diamond J (1997) Guns, germs, and steel: the fates of human societies. [Chicago] University of Chicago Press.

Dinsdale EA, Pantos O, Smriga S, Edwards RA, Angly F, Wegley L, Hatay M, Hall D, Brown E, Haynes M, Krause L, Sala E, Sandin SA, Thurber RV, Willis BL, Azam F, Knowlton NN, Rohwer F (2008) Microbial ecology of four coral atolls in the Northern Line Islands. PLoS ONE 3:e1584

Donner SD (2011) An evaluation of the effect of recent temperature variability on the prediction of coral bleaching events. Ecological Applications 21:1718–1730

Dornelas M, Gotelli NJ, McGill B, Shimadzu H, Moyes F, Sievers C, Magurran AE (2014) Assemblage time series reveal biodiversity change but not systematic loss. Science 344:296–299

Douglas AE (2003) Coral bleaching - how and why? Marine Pollution Bulletin 46:385–392

Drew EA (1972) The biology and physiology of alga-invertebrates symbioses. II. The density of symbiotic algal cells in a number of hermatypic hard corals and alcyonarians from various depths. Journal of Experimental Marine Biology and Ecology 9:71–75

Dunn SR, Thomas MC, Nette GW, Dove SG (2012) A lipidomic approach to understanding free fatty acid lipogenesis derived from dissolved inorganic carbon within Cnidarian-dinoflagellate symbiosis. PLoS ONE 7:e46801–e46801

Dustan P (1982) Depth-dependent photoadaption by zooxanthellae of the reef coral *Montastrea annularis*. Marine Biology 68:253–264

Eakin CM (2001) A tale of two ENSO events: carbonate budgets and the influence of two warming disturbances and intervening variability, Uva Island, Panama. Bulletin of Marine Science 69:171–186

Eakin CM, Liu G, Gomez AM, JL DLC, Heron SF, Skirving WJ, Geiger EF, Tirak K V, Strong AE (2016) Global coral bleaching 2014-2017: status and an appeal for observations. Reef Encounter 31:20–26

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461

Edge SE, Shearer TL, Morgan MB, Snell TW (2013) Sub-lethal coral stress: detecting molecular responses of coral populations to environmental conditions over space and time. Aquatic Toxicology 128–129:135–146

Edmunds PJ (2007) Evidence for a decadal-scale decline in the growth rates of juvenile scleractinian corals. Marine Ecology Progress Series 341:1–13

Edmunds PJ (2017) Intraspecific variation in growth rate is a poor predictor of fitness for reef corals. Ecology 98:2191–2200

Edmunds PJ, Pochon X, Levitan DR, Yost DM, Belcaid M, Putnam HM, Gates RD (2014) Long-term changes in *Symbiodinium* communities in *Orbicella annularis* in St. John, US Virgin Islands. Marine Ecology Progress Series 506:129–144

Edwards AJ, Clark S, Zahir H, Rajasuriya A, Naseer A, Rubens J (2001) Coral bleaching and mortality on artificial and natural reefs in Maldives in 1998, sea surface temperature anomalies and initial recovery. Marine Pollution Bulletin 42:7–15

Emerich DW, Krishnan HB (2014) Symbiosomes: temporary moonlighting organelles. Biochemical Journal 460:1–11

Enríquez S, Pantoja-Reyes NI (2005) Form-function analysis of the effect of canopy morphology on leaf self-shading in the seagrass *Thalassia testudinum*. Oecologia 145:234–242

Eren AM, Maignien L, Sul WJ, Murphy LG, Grim SL, Morrison HG, Sogin ML (2013) Oligotyping: differentiating between closely related microbial taxa using 16S rRNA gene data. Methods in Ecology and Evolution 4:1111–1119

Eren AM, Morrison HG, Lescault PJ, Reveillaud J, Vineis JH, Sogin ML (2015) Minimum entropy decomposition: unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. The ISME Journal 9:968–979

Eren AM, Vineis JH, Morrison HG, Sogin ML (2013) A filtering method to generate high quality short reads using Illumina paired-end technology. PLoS ONE 8

Everett KDE, Bush RM, Andersen AA (1999) Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards. International Journal of Systematic and Evolutionary Microbiology 49:415–440

Ezard THG, Aze T, Pearson PN, Purvis A (2011) Interplay between changing climate and species' ecology drives macroevolutionary dynamics. Science 332:349–351

Fabina NS, Putnam HM, Franklin EC, Stat M, Gates RD (2012) Transmission mode predicts specificity and interaction patterns in coral-Symbiodinium networks. PLoS ONE 7:e44970

Fabina NS, Putnam HM, Franklin EC, Stat M, Gates RD (2013) Symbiotic specificity, association patterns, and function determine community responses to global changes: Defining critical research areas for coral-*Symbiodinium* symbioses. Global Change Biology 19:3306–3316

Fagoonee I, Wilson HB, Hassell MP, Turner JR (1999) The dynamics of zooxanthellae populations: a long-term study in the field. Science 283:843–845

Finney JC, Pettay DT, Sampayo EM, Warner ME, Oxenford HA, LaJeunesse TC (2010) The relative significance of host-habitat, depth, and geography on the ecology, endemism, and speciation of coral endosymbionts in the genus *Symbiodinium*. Microbial Ecology 60:250–263

Fitt WK, McFarland FK, Warner ME, Chilcoat GC (2000) Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. Limnology and Oceanography 45:677–685

Fitt WK, Trench RK (1983) The relation of diel patterns of cell division to diel patterns of motility in the symbiotic dinoflagellate *Symbiodinium microadriaticum* Freudenthal in culture. New Phytologist 94:421–432

Fouw J de, Govers LL, Koppel J van de, Belzen J van, Dorigo W, Sidi Cheikh MA, Christianen MJA, Reijden KJ van der, Geest M van der, Piersma T, Smolders AJP, Olff H, Lamers LPM, Gils JA van, Heide T van der (2016) Drought, mutualism breakdown, and landscape-scale degradation of seagrass beds. Current Biology 26:1051–1056

Frade PR, Englebert N, Faria J, Visser PM, Bak RPM (2008) Distribution and photobiology of *Symbiodinium* types in different light environments for three colour morphs of the coral *Madracis pharensis*: Is there more to it than total irradiance? Coral Reefs 27:913–925

Franklin EC, Stat M, Pochon X, Putnam HM, Gates RD (2012) GeoSymbio: a hybrid, cloud-based web application of global geospatial bioinformatics and ecoinformatics for *Symbiodinium*-host symbioses. Molecular Ecology Resources 12:369–373

Freilich MA, Wieters E, Broitman BR, Marquet PA, Navarrete SA (2018) Species co-occurrence networks: Can they reveal trophic and non-trophic interactions in ecological communities? Ecology 99:690–699

Freudenthal HD (1962) *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a Zooxanthella: taxonomy, life cycle, and morphology. The Journal of Protozoology 9:45–52

Friedlander AM, Parrish JD (1998) Habitat characteristics affecting fish assemblages on a Hawaiian coral reef. Journal of Experimental Marine Biology and Ecology:1–30

Furby KA, Bouwmeester J, Berumen ML (2013) Susceptibility of central Red Sea corals during a major bleaching event. Coral Reefs 32:505–513

Garcia GD, Santos E de O, Sousa G V, Zingali RB, Thompson CC, Thompson FL (2016) Metaproteomics reveals metabolic transitions between healthy and diseased stony coral *Mussismilia braziliensis*. Molecular Ecology 25:4632–4644

Garde LA, Spillman CM, Heron SF, Beeden RJ (2014) Reef Temp Next Generation: A new operational system for monitoring reef thermal stress. Journal of Operational Oceanography 7:21–33

Garren M, Son K, Raina J-B, Rusconi R, Menolascina F, Shapiro OH, Tout J, Bourne DG, Seymour JR, Stocker R (2014) A bacterial pathogen uses dimethylsulfoniopropionate as a cue to target heat-stressed corals. ISME J 8:999–1007

Gates RD (1990) Seawater temperature and sublethal coral bleaching in Jamaica. Coral Reefs 8:193–197

Gates RD, Ainsworth TD (2011) The nature and taxonomic composition of coral symbiomes as drivers of performance limits in scleractinian corals. Journal of Experimental Marine Biology and Ecology 408:94–101

Gates RD, Baghdasarian G, Muscatine L (1992) Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. The Biological Bulletin 182:324–332

Gates RD, Edmunds PJ (1999) The physiological mechanisms of acclimatization in tropical reef corals. Integrative and Comparative Biology 39:30–43

Gates RD, Hoegh-Guldberg O, McFall-Ngai MJ, Bil KY, Muscatine L (1995) Free amino acids exhibit anthozoan “host factor” activity: they induce the release of photosynthate from symbiotic dinoflagellates in vitro. Proceedings of the National Academy of Sciences 92:7430–7434

Gilleland E, Katz RW, Others (2016) extRemes 2.0: an extreme value analysis package in R. Journal of Statistical Software 72:1–39

Gilman SE, Urban MC, Tewksbury J, Gilchrist GW, Holt RD (2010) A framework for community interactions under climate change. Trends in Ecology and Evolution 25:325–331

Gleason MG (1993) Effects of disturbance on coral communities - bleaching in Moorea, French Polynesia. Coral Reefs 12:193–201

Gleeson MW, Strong AE (1995) Applying MCSST to coral reef bleaching. Advances in Space Research 16:151–154

Glenn TC (2011) Field guide to next-generation DNA sequencers. Molecular Ecology Resources 11:759–769

Glynn PW (1984) Widespread coral mortality and the 1982-83 El Niño warming event. Environmental Conservation 11:133–146

Glynn PW (1988) El Niño Southern Oscillation 1982-1983: nearshore population, community, and ecosystem responses. Annual Review of Ecology and Systematics 19:309–345

Glynn PW (1990) Coral mortality and disturbances to coral reefs in the tropical Eastern Pacific. Elsevier Oceanography Series 52:55–126

Glynn PW (1993) Coral reef bleaching: ecological perspectives. Coral Reefs 12:1–17

Glynn PW (1996) Coral reef bleaching: facts, hypotheses and implications. Global Change Biology 2:495–509

Glynn PW, D'Croz L (1990) Experimental evidence for high temperature stress as the cause of El Niño-coincident coral mortality. Coral Reefs 8:181–191

Glynn PW, Maté JL, Baker AC, Calderón MO (2001) Coral bleaching and mortality in Panama and Ecuador during the 1997-1998 El Niño-Southern Oscillation Event: spatial/temporal patterns and comparisons with the 1982-1983 event. Bulletin of Marine Science 69:79–109

Glynn PW, Weerdt W de (1991) Elimination of two reef-building hydrocorals following the 1982-83 El Niño warming event. Science 253:69–71

Golbuu Y, Victor S, Penland L, Idip D, Emaurois C, Okaji K, Yukihira H, Iwase A, Woesik R van (2007) Palau's coral reefs show differential habitat recovery following the 1998-bleaching event. Coral Reefs 26:319–332

Goldsmith DB, Kellogg CA, Morrison CL, Gray MA, Stone RP, Waller RG, Brooke SD, Ross SW (2018) Comparison of microbiomes of cold-water corals *Primnoa pacifica* and *Primnoa resedaeformis,* with possible link between microbiome composition and host genotype. Scientific Reports 8:12383

Gordon BR, Leggat W (2010) *Symbiodinium*-invertebrate symbioses and the role of metabolomics. Marine Drugs 8:2546–2568

Goreau TF (1963) Calcium carbonate deposition by coralline algae and corals in relation to their roles as reef-builders. Annals of the New York Academy of Sciences 109:127–167

Goreau TF, Hayes RL (1994) Coral bleaching and ocean "hot spots." Ambio 23:176–180

Goreau TF, McClanahan TR, Hayes RL, Strong AE (2000) Conservation of coral reefs after the 1998 global bleaching event. Conservation Biology 14:5–15

Gove JM, Williams GJ, McManus MA, Heron SF, Sandin SA, Vetter OJ, Foley DG (2013) Quantifying climatological ranges and anomalies for Pacific coral reef ecosystems. PLoS One 8:e61974

Gower JC (1975) Generalized procrustes analysis. Psychometrika 40:33–51

Graham NAJ, Bellwood DR, Cinner JE, Hughes TP, Norström A V, Nyström M (2013) Managing resilience to reverse phase shifts in coral reefs. Frontiers in Ecology and the Environment 11:541–548

Graham NAJ, Jennings S, Aaron MacNeil M, Mouillot D, Wilson SK (2015) Predicting climate-driven regime shifts versus rebound potential in coral reefs. Nature 518:94–97

Granados-Cifuentes C, Neigel J, Leberg P, Rodriguez-Lanetty M (2015) Genetic diversity of free-living *Symbiodinium* in the Caribbean: the importance of habitats and seasons. Coral Reefs 34:927–939

Green EA, Davies SW, Matz M V, Medina M (2014) Quantifying cryptic *Symbiodinium* diversity within *Orbicella faveolata* and *Orbicella franksi* at the Flower Garden Banks, Gulf of Mexico. PeerJ 2:e386

Grottoli AG, Warner ME, Levas SJ (2014) The cumulative impact of annual coral bleaching can turn some coral species winners into losers. Global Change Biology 20:3823–3833

Guest JR, Baird AH, Maynard JA, Muttaqin E, Edwards AJ, Campbell SJ, Yewdall K, Affendi YA, Chou LM (2012) Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. PLoS ONE 7:e33353–e33353

Guzman HM, Cortes J (2006) Reef recovery 20 years after the 1982-1983 El Niño massive mortality. Marine Biology 151:401–411

Halpern BS, Frazier M, Potapenko J, Casey KS, Koenig K, Longo C, Lowndes JS, Rockwood RC, Selig ER, Selkoe KA, Walbridge S (2015) Spatial and temporal changes in cumulative human impacts on the world's ocean. Nature Communications 6:7615

Harii S, Yamamoto M, Hoegh-Guldberg O (2010) The relative contribution of dinoflagellate photosynthesis and stored lipids to the survivorship of symbiotic larvae of the reef-building corals. Marine Biology 157:1215–1224

Harley CDG, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL (2006) The impacts of climate change in coastal marine systems. Ecology Letters 9:228–241

Harvey C (2016) Why dead coral reefs could mark the beginning of `dangerous' climate change. The Washington Post

Hedges L V, Olkin I (1985) Statistical methods for meta-analysis. Academic Press

Hedges L V, Pigott TD (2004) The power of statistical tests for moderators in meta-analysis. Psychological Methods 9:426–445

Helmuth BST, Timmerman BEH, Sebens KP (1997) Interplay of host morphology and symbiont microhabitat in coral aggregations. Marine Biology 130:1–10

Heron SF, Liu G, Rauenzahn JL, Christensen TRL, Skirving WJ, Burgess TFR, Eakin CM, Morgan JA (2014) Improvements to and continuity of operational global thermal stress monitoring for coral bleaching. Journal of Operational Oceanography 7:3–11

Heron SF, Maynard JA, Hooidonk R van, Eakin CM (2016) Warming trends and bleaching stress of the world's coral reefs 1985-2012. Scientific Reports 6:38402

Hirose M, Reimer JD, Hidaka M, Suda S (2008) Phylogenetic analyses of potentially free-living *Symbiodinium* spp. isolated from coral reef sand in Okinawa, Japan. Marine Biology 155:105–112

Hobbs RJ, Huenneke LF (1992) Disturbance, diversity, and invasion: implications for conservation. Conservation Biology 6:324–337

Hodgson D, McDonald JL, Hosken DJ (2015) What do you mean, “resilient”? Trends in Ecology and Evolution 30:503–506

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world’s coral reefs. Marine and Freshwater Research 50:839–866

Hoegh-Guldberg O (2004) Coral reefs in a century of rapid environmental change. Symbiosis 37:1–31

Hoegh-Guldberg O (2011) Coral reef ecosystems and anthropogenic climate change. Regional Environmental Change Change 11:215–227

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world’s marine ecosystems. Science 328:1523–1528

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell D, Sale PF, Edwards AJ, Caldeira K, Knowlton NN, Eakin CM, Iglesias-Prieto R, Muthiga NA, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742

Holbrook SJ, Adam TC, Edmunds PJ, Schmitt RJ, Carpenter RC, Brooks AJ, Lenihan HS, Briggs CJ (2018) Recruitment drives spatial variation in recovery rates of resilient coral reefs. Scientific Reports 8:7338

Holling CS (1973) Resilience and stability of ecological systems. Annual Review of Ecology and Systematics 4:1–23

Hongo C, Yamano H (2013) Species-specific responses of corals to bleaching events on anthropogenically turbid reefs on Okinawa Island, Japan, over a 15-year period (1995-2009). PLoS One 8:e60952

Hoogenboom M, Rodolfo-Metalpa R, Ferrier-Pagès C (2010) Co-variation between autotrophy and heterotrophy in the Mediterranean coral *Cladocora caespitosa*. The Journal of Experimental Biology 213:2399–2409

Hooidonk R van, Maynard JA, Planes S (2013) Temporary refugia for coral reefs in a warming world. Nature Climate Change 3:508

Hooidonk R van, Maynard J, Tamelander J, Gove J, Ahmadia G, Raymundo L, Williams G, Heron SF, Planes S (2016) Local-scale projections of coral reef futures and implications of the Paris Agreement. Scientific Reports 6:39666

Howells EJ, Oppen MJH van, Willis BL (2009) High genetic differentiation and cross-shelf patterns of genetic diversity among Great Barrier Reef populations of *Symbiodinium*. Coral Reefs 28:215–225

Huang H, Zhou G, Yang J, Liu S, You F, Lei X (2013) Diversity of free-living and symbiotic *Symbiodinium* in the coral reefs of Sanya, South China Sea. Marine Biology Research 9:117–128

Hughes TP, Anderson KD, Connolly SR, Heron SF, Kerry JT, Lough JM, Baird AH, Baum JK, Berumen ML, Bridge TC, Claar DC, Eakin CM, Gilmour JP, Graham NAJ, Harrison H, Hobbs J-PA, Hoey AS, Hoogenboom M, Lowe RJ, McCulloch MT, Pandolfi JM, Pratchett M, Schoepf V, Torda G, Wilson SK (2018) Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. Science 359:80–83

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas JA, Lough JM, Marshall PA, Nyström M, Palumbi SR, Pandolfi JM, Rosen B, Roughgarden J (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301:929–933

Hughes TP, Bellwood DR, Folke C, Steneck RS, Wilson J (2005) New paradigms for supporting the resilience of marine ecosystems. Trends in Ecology and Evolution 20:380–386

Hughes TP, Connell JH (1999) Multiple stressors on coral reefs: a long-term perspective. Limnology and Oceanography 44:932–940

Hughes TP, Graham NAJ, Jackson JBC, Mumby PJ, Steneck RS (2010) Rising to the challenge of sustaining coral reef resilience. Trends in Ecology and Evolution 25:633–642

Hughes TP, Kerry J, Álvarez-Noriega M, Álvarez-Romero J, Anderson K, Baird A, Babcock R, Beger M, Bellwood D, Berkelmans R, Bridge T, Butler I, Byrne M, Cantin N, Comeau S, Connolly S, Cumming G, Dalton S, Diaz-Pulido G, Eakin CM, Figueira W, Gilmour J, Harrison H, Heron S, Hoey AS, Hobbs J-P, Hoogenboom M, Kennedy E, Kuo C, Lough J, Lowe R, Liu G, Malcolm McCulloch HM, McWilliam M, Pandolfi J, Pears R, Pratchett M, Schoepf V, Simpson T, Skirving W, Sommer B, Torda G, Wachenfeld D, Willis B, Wilson S (2017) Global warming and recurrent mass bleaching of corals. Nature 543:373–378

Hughes TP, Kerry JT, Baird AH, Connolly SR, Dietzel A, Eakin CM, Heron SF, Hoey AS, Hoogenboom MO, Liu G, McWilliam MJ, Pears RJ, Pratchett MS, Skirving WJ, Stella JS, Torda G (2018) Global warming transforms coral reef assemblages. Nature 556:492–496

Iacarella JC, Adamczyk E, Bowen D, Chalifour L, Eger A, Heath W, Helms S, Hessing-Lewis M, Hunt BP V, MacInnis A, O'Connor MI, Robinson CLK, Yakimishyn J, Baum JK (2018) Anthropogenic disturbance homogenizes seagrass fish communities. Global Change Biology 24:1904–1918

Iglesias-Prieto R, Beltran VH, LaJeunesse TC, Reyes-Bonilla H, Thome PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. Proceedings of the Royal Society B: Biological Sciences 271:1757–1763

Igulu MM, Nagelkerken I, Dorenbosch M, Grol MGG, Harborne AR, Kimirei IA, Mumby PJ, Olds AD, Mgaya YD (2014) Mangrove habitat use by juvenile reef fish: meta-analysis reveals that tidal regime matters more than biogeographic region. PLoS ONE 9:e114715

Imhoff JF, Wiese J (2014) The Order Kiloniellales. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds) The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Springer Berlin Heidelberg, Berlin, Heidelberg, p 301–306

Jokiel PL, York J, Richard H (1982) Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae. Bulletin of Marine Science 32:301–315

Jones RJ (2008) Coral bleaching, bleaching-induced mortality, and the adaptive significance of the bleaching response. Marine Biology 154:65–80

Jones AM, Berkelmans R, Oppen MJH van, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proceedings of the Royal Society B: Biological Sciences 275:1359–1365

Jones RJ, Yellowlees D (1997) Regulation and control of intracellular algae (= zooxanthellae) in hard corals. Philosophical Transactions of the Royal Society B: Biological Sciences 352:457–468

Karp DS, Rominger AJ, Zook J, Ranganathan J, others (2012) Intensive agriculture erodes β-diversity at large scales. Ecology 15:963–970

Kellogg CA, Piceno YM, Tom LM, DeSantis TZ, Gray MA, Andersen GL (2014) Comparing bacterial community composition of healthy and dark spot-affected *Siderastrea siderea* in Florida and the Caribbean. PLoS ONE

Kelmo F, Attrill MJ (2013) Severe impact and subsequent recovery of a coral assemblage following the 1997-8 El Niño event: a 17-year study from Bahia, Brazil. PLoS ONE 8:e65073

Kenkel CD, Goodbody-Gringley G, Caillaud D, Davies SW, Bartels E, Matz M V (2013) Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments. Molecular Ecology 22:4335–4348

Kennedy E V, Tonk L, Foster NL, Chollett I, Ortiz J-C, Dove S, Hoegh-Guldberg O, Mumby PJ, Stevens JR (2016) *Symbiodinium* biogeography tracks environmental patterns rather than host genetics in a key Caribbean reef-builder, *Orbicella annularis*. Proceedings of the Royal Society B: Biological Sciences 283

Kenyon JC, Brainard RE, Hoeke RK, Parrish FA, Wilkinson CB (2006) Towed-diver surveys, a method for mesoscale spatial assessment of Benthic Reef habitat: A case study at Midway Atoll in the Hawaiian Archipelago. Coastal Management 34:339–349

Kinzie RA, Chee GS (1979) The effect of different zooxanthellae on the growth of experimentally reinfected hosts. Biological Bulletin 156:315–327

Kirk NL, Andras JP, Harvell CD, Santos SR, Coffroth MA (2009) Population structure of *Symbiodinium* sp. associated with the common sea fan, *Gorgonia ventalina*, in the Florida Keys across distance, depth, and time. Marine Biology 156:1609–1623

Knowlton NN, Rohwer F (2003) Multispecies microbial mutualisms on coral reefs: the host as a habitat. The American Naturalist 162:S51–S62

Kojis BL, Quinn NJ (1981) Reproductive strategies in four species of *Porites* (Scleractinia). In: Proceedings of the 4th International Coral Reef Symposium.p 145–151

Koricheva J, Gurevitch J (2014) Uses and misuses of meta-analysis in plant ecology. Journal of Ecology 102:828–844

Krediet CJ, Ritchie KB, Paul VJ, Teplitski M (2013) Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. Proceedings of the Royal Society B: Biological Sciences 280:20122328

Kühl M, Cohen Y, Dalsgaard T, Jorgensen BB, Revsbech NP (1995) Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O2, pH and light. Marine Ecology Progress Series 117:159–172

LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a “species” level marker. Journal of Phycology 37:866–880

LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Marine Biology 141:387–400

LaJeunesse TC (2005) Species radiations of symbiotic dinoflagellates in the Atlantic and Indo-Pacific since the Miocene-Pliocene transition. Molecular Biology and Evolution 22:570–581

LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Current Biology 28:2570–2580

LaJeunesse TC, Smith R, Walther M, Pinzon J, Pettay DT, McGinley MP, Aschaffenburg MD, Medina-Rosas P, Cupul-Magana AL, Perez AL, Reyes-Bonilla H, Warner ME (2010) Host-symbiont recombination versus natural selection in the response of coral-dinoflagellate symbioses to environmental disturbance. Proceedings of the Royal Society B: Biological Sciences 277:2925–2934

Land LS, Lang JC, Smith BN (1975) Preliminary observations on the carbon isotopic composition of some reef coral tissues and symbiotic zooxanthellae. Limnology and Oceanography 20:283–287

Larsson J (2018) eulerr: Area-proportional Euler and Venn diagrams with circles or ellipses.

Lebrija-Trejos E, Pérez-García EA, Meave JA, Bongers F, Poorter L (2010) Functional traits and environmental filtering drive community assembly in a species-rich tropical system. Ecology 91:386–398

Lee-Cruz L, Edwards DP, Tripathi BM, Adams JM (2013) Impact of logging and forest conversion to oil palm plantations on soil bacterial communities in Borneo. Applied and Environmental Microbiology 79:7290–7297

Lee MJ, Jeong HJ, Jang SH, Lee SY, Kang NSS (2016) Most low-abundance “background” *Symbiodinium* spp. are transitory and have minimal functional significance for symbiotic corals. Microbial Ecology 71:771–83

Lema KA, Willis BL, Bourne DG (2012) Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. Applied and Environmental Microbiology 78:3136–3144

Lenth R V (2016) Least-squares means: the R package lsmeans. Journal of Statistical Software 69:1–33

Lesser MP (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. Physiology 68:253–278

Lesser MP (2013) Using energetic budgets to assess the effects of environmental stress on corals: are we measuring the right things? Coral Reefs 32:25–33

Lesser MP, Mazel CH, Gorbunov MY, Falkowski PG (2004) Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. Science 305:997–1000

Lesser MP, Stat M, Gates RD (2013) The endosymbiotic dinoflagellates (*Symbiodinium* sp.) of corals are parasites and mutualists. Coral Reefs 32:603–611

Levas SJ, Grottoli AG, Hughes A, Osburn CL, Matsui Y (2013) Physiological and biogeochemical traits of bleaching and recovery in the mounding species of coral *Porites lobata*: implications for resilience in mounding corals. PLoS One 8:e63267

Levin SA (1992) The problem of pattern and scale in ecology: The Robert H. MacArthur award lecture. Ecology 73:1943–1967

Lewis DH, Smith DC (1971) The autotrophic nutrition of symbiotic marine coelenterates with special reference to hermatypic corals. i. Movement of photosynthetic products between the symbionts. Proceedings of the Royal Society B: Biological Sciences 178:111–129

Lisboa FJG, Peres-Neto PR, Chaer GM, Jesus E da C, Mitchell RJ, Chapman SJ, Berbara RLL (2014) Much beyond Mantel: bringing Procrustes association metric to the plant and soil ecologist's toolbox. PLoS One 9:e101238

Little AF, Oppen MJH van, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. Science 304:1492–1494

Littman RA, Bourne DG, Willis BL (2010) Responses of coral-associated bacterial communities to heat stress differ with Symbiodinium type on the same coral host. Molecular Ecology 19:1978–1990

Littman RA, Oppen MJH van, Willis BL (2008) Methods for sampling free-living *Symbiodinium* (zooxanthellae) and their distribution and abundance at Lizard Island (Great Barrier Reef). Journal of Experimental Marine Biology and Ecology:48–53

Littman R, Willis BL, Bourne DG (2011) Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. Environmental Microbiology Reports 3:651–660

Liu G, Matrosova LE, Penland C (2008) NOAA Coral Reef Watch coral bleaching outlook system. In: Proceedings of the 11th International Coral Reef Symposium. Ft. Lauderdale, Florida, p 951–955

Liu G, Rauenzahn JL, Heron SF, Eakin CM, Skirving WJ, Christensen TRL, Strong AE, Li J (2013) NOAA coral reef watch 50 km satellite sea surface temperature-based decision support system for coral bleaching management. NOAA, NESDIS

Liu H, Stephens TG, González-Pech RA, Beltran VH, Lapeyre B, Bongaerts P, Cooke I, Aranda M, Bourne DG, Forêt S, Miller DJ, Oppen MJH van, Voolstra CR, Ragan MA, Chan CX (2018) *Symbiodinium* genomes reveal adaptive evolution of functions related to coral-dinoflagellate symbiosis. Communications Biology 1:95

Liu G, Strong AE, Skirving WJ (2003) Remote sensing of sea surface temperatures during 2002 Barrier Reef coral bleaching. Eos, Transactions American Geophysical Union 84:137–141

Liu G, Strong AE, Skirving WJ, Arzayus LF (2006) Overview of NOAA coral reef watch program’s near-real time satellite global coral bleaching monitoring activities. In: Proceedings of 10th International Coral Reef Symposium.p 1783–1793

Lix JK, Venkatesan R, Grinson G, Rao RR, Jineesh VK, Arul MM, Vengatesan G, Ramasundaram S, Sundar R, Atmanand MA (2016) Differential bleaching of corals based on El Niño type and intensity in the Andaman Sea, southeast Bay of Bengal. Environmental Monitoring and Assessment 188:175

Lough JM, Anderson KD, Hughes TP (2018) Increasing thermal stress for tropical coral reefs: 1871-2017. Scientific Reports 8:6079

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15:550

Loya Y, Sakai K, Yamazato K, Nakano Y (2001) Coral bleaching: the winners and the losers. Ecology Letters 4:122–131

Lozupone CA, Hamady M, Kelley ST, Knight R (2007) Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. Applied and Environmental Microbiology 73:1576–1585

Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R (2011) UniFrac: an effective distance metric for microbial community comparison. The ISME Journal 5:169–172

Luo YJ, Wang L-H, Chen WNU, Peng SE, Tzen JTC, Hsiao YY, Huang HJ, Fang L-S, Chen CS (2009) Ratiometric imaging of gastrodermal lipid bodies in coral-dinoflagellate endosymbiosis. Coral Reefs 28:289–301

Madin JS, Hoogenboom MO, Connolly SR, Darling ES, Falster DS, Huang D, Keith SA, Mizerek T, Pandolfi JM, Putnam HM, Baird AH (2016) A trait-based approach to advance coral reef science. Trends in Ecology and Evolution 31:419–428

Magurran AE, Dornelas M, Moyes F, Gotelli NJ, McGill B (2015) Rapid biotic homogenization of marine fish assemblages. Nature Communications 6:8405

Maina J, McClanahan TR, Venus V, Ateweberhan M, Madin J (2011) Global gradients of coral exposure to environmental stresses and implications for local management. PLoS One 6:e23064

Maina J, Venus, McClanahan TR, Ateweberhan M (2008) Modelling susceptibility of coral reefs to environmental stress using remote sensing data and GIS models. Ecological Modelling 212:20

Manning MM, Gates RD (2008) Diversity in populations of free-living Symbiodinium from a Caribbean and Pacific reef. Limnology and Oceanography 53:1853

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs 19:155–163

McClanahan TR (2004) The relationship between bleaching and mortality of common corals. Marine Biology 144:1239–1245

McClanahan TR, Donner SD, Maynard JA, MacNeil MA, Graham NAJ, Maina J, Baker AC, Alemu I JB, Beger M, Campbell SJ, Darling ES, Eakin CM, Heron SF, Jupiter SD, Lundquist CJ, McLeod E, Mumby PJ, Paddack MJ, Selig ER, Woesik R van (2012) Prioritizing key resilience indicators to support coral reef management in a changing climate. PLoS ONE 7:e42884

McClanahan TR, Maina J, Moothien-Pillay R, Baker AC (2005) Effects of geography, taxa, water flow, and temperature variation on coral bleaching intensity in Mauritius. Marine Ecology Progress Series 298:131–142

McClanahan TR, Starger CJ, Baker AC (2014) Decadal changes in common reef coral populations and their associations with algal symbionts (*Symbiodinium* spp.). Marine Ecology 36:1215–1229

McDevitt-Irwin JM, Baum JK, Garren M, Vega Thurber RL (2017) Responses of coral-associated bacterial communities to local and global stressors. Frontiers in Marine Science 4:262

McDevitt-Irwin JM, Iacarella JC, Baum JK (2016) Reassessing the nursery role of seagrass habitats from temperate to tropical regions: a meta-analysis. Marine Ecology Progress Series 557:133–143

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME Journal 6:610–618

McGill BJ, Dornelas M, Gotelli NJ, Magurran AE (2015) Fifteen forms of biodiversity trend in the Anthropocene. Trends in Ecology and Evolution 30:104–113

McKinney ML, Lockwood JL (1999) Biotic homogenization: a few winners replacing many losers in the next mass extinction. Trends in Ecology and Evolution 14:450–453

McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8:e61217

McPhaden MJ (1999) Genesis and evolution of the 1997-98 El Niño. Science 283:950–954

Menge BA, Olson AM (1990) Role of scale and environmental factors in regulation of community structure. Trends in Ecology and Evolution 5:52–57

Menge BA, Sutherland JP (1987) Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. American Naturalist 130:730–757

Mieog JC, Olsen JL, Berkelmans R, Bleuler-Martinez SA, Willis BL, Oppen MJH van (2009) The roles and interactions of symbiont, host and environment in defining coral fitness. PLoS ONE 4:e6364–e6364

Mieog JC, Oppen MJH van, Berkelmans R, Stam WT, Olsen JL (2009) Quantification of algal endosymbionts (*Symbiodinium*) in coral tissue using real-time PCR. Molecular Ecology Resources 9:74–82

Millard SP (2013) EnvStats: An R package for environmental statistics, 2nd ed. 20. Springer

Moberg F, Folke C (1999) Ecological goods and services of coral reef ecosystems. Ecological Economics 29:215–233

Montgomery RS, Strong AE (1994) Coral bleaching threatens oceans, life. Eos, Transactions American Geophysical Union 75:145–147

Moothien-Pillay R, Willis BL, Terashima H (2005) Trends in the density of zooxanthellae in *Acropora millepora* (Ehrenberg, 1834) at the Palm Island Group, Great Barrier Reef, Australia. Symbiosis 38:209–226

Morate O (2016) 2015 Population and Housing Census. Volume 1: Management Report and Basic Tables.

Morris EK, Caruso T, Buscot F, Fischer M, Hancock C, Maier TS, Meiners T, Müller C, Obermaier E, Prati D, Socher SA, Sonnemann I, Wäschke N, Wubet T, Wurst S, Rillig MC (2014) Choosing and using diversity indices: insights for ecological applications from the German Biodiversity Exploratories. Ecology and Evolution 4:3514–3524

Morrow KM, Paul VJ, Liles MR, Chadwick NE (2011) Allelochemicals produced by Caribbean macroalgae and cyanobacteria have species-specific effects on reef coral microorganisms. Coral Reefs 30:309

Mumby PJ, Chisholm JRM, Edwards AJ, Andrefouet S, Jaubert J (2001) Cloudy weather may have saved Society Island reef corals during the 1998 ENSO event. Marine Ecology Progress Series 222:209–216

Mumby PJ, Harborne AR (2010) Marine reserves enhance the recovery of corals on Caribbean reefs. PLoS ONE 5:1–7

Muscatine L (1967) Glycerol excretion by symbiotic algae from corals and tridacna and its control by the host. Science 156:516–519

Muscatine L, Cernichiari E (1969) Assimilation of photosynthetic products of zooxanthellae by a reef coral. The Biological Bulletin 137:506–523

Muscatine L, Porter JW (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. Bioscience 27:454–460

Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4:133–142

National Climatic Data Center (2007) GHRSST Level 4 AVHRR_OI Global Blended Sea Surface Temperature Analysis. Ver. 1.0.

Neal BP, Khen A, Treibitz T, Beijbom O, O'Connor G, Coffroth MA, Knowlton N, Kriegman D, Mitchell BG, Kline DI (2017) Caribbean massive corals not recovering from repeated thermal stress events during 2005–2013. Ecology and Evolution 7:1339–1353

Nelson MC, Morrison HG, Benjamino J, Grim SL, Graf J (2014) Analysis, optimization and verification of Illumina-generated 16s rRNA gene amplicon surveys. PLoS ONE 9:e94249

Nitschke MR (2015) The free-living *Symbiodinium* reservoir and scleractinian coral symbiont acquisition. espace.library.uq.edu.au

Nitschke MR, Davy SK, Ward S (2016) Horizontal transmission of *Symbiodinium* cells between adult and juvenile corals is aided by benthic sediment. Coral Reefs 35:335–344

NOAA Coral Reef Watch (2000) NOAA Coral Reef Watch Operational 50-km Satellite Coral Bleaching Degree Heating Weeks Product.

NOAA CRW (2013) NOAA Coral Reef Watch Daily Global 5-km Satellite Coral Bleaching Degree Heating Week Product.

Norström A V, Nyström M, Jouffray J-B, Folke C, Graham NAJ, Moberg F, Olsson P, Williams GJ (2016) Guiding coral reef futures in the Anthropocene. Frontiers in Ecology and the Environment 14:490–498

Novak M, Yeakel JD, Noble AE, Doak DF, Emmerson M, Estes JA, Jacob U, Tinker MT, Wootton JT (2016) Characterizing species interactions to understand press perturbations: What is the community matrix? Annual Review of Ecology, Evolution, and Systematics 47:409–432

Nugues MM, Smith GW, Hooidonk RJ Van, Seabra MI, Bak RPM (2004) Algal contact as a trigger for coral disease. Ecology Letters 7:919–923

Nyström M, Folke C, Moberg F (2000) Coral reef disturbance and resilience in a human-dominated environment. Trends in Ecology and Evolution 15:413–417

Oksanen J (2017) Vegan: an introduction to ordination.

Olden JD, Poff NL (2004) Ecological processes driving biotic homogenization: testing a mechanistic model using fish faunas. Ecology 85:1867–1875

Olson ND, Ainsworth TD, Gates RD, Takabayashi M (2009) Diazotrophic bacteria associated with Hawaiian *Montipora* corals: Diversity and abundance in correlation with symbiotic dinoflagellates. Journal of Experimental Marine Biology and Ecology 371:140–146

Oppen MJH van, Bongaerts P, Frade P, Peplow LM, Boyd SE, Nim HT, Bay LK (2018) Adaptation to reef habitats through selection on the coral animal and its associated microbiome. Molecular Ecology 27:2956–2971

Oppen MJH van, Gates RD (2006) Conservation genetics and the resilience of reef-building corals. Molecular Ecology 15:3863–3883

Oppen MJH van, Mieog JC, Sánchez CA, Fabricius KE (2005) Diversity of algal endosymbionts (zooxanthellae) in octocorals: the roles of geography and host relationships. Molecular Ecology 14:2403–2417

Oppen MJ van, Willis BL, Vugt HW, Miller DJ (2000) Examination of species boundaries in the *Acropora cervicornis* group (Scleractinia, cnidaria) using nuclear DNA sequence analyses. Molecular Ecology 9:1363–1373

Pagès H, Aboyoun P, Gentleman R, DebRoy S (2017) Biostrings: String objects representing biological sequences, and matching algorithms.

Paine RT, Tegner MJ, Johnson EA (1998) Compounded perturbations yield ecological surprises. Ecosystems 1:535–545

Pandolfi JM, Connolly SR, Marshall DJ, Cohen AL (2011) Projecting coral reef futures under global warming and ocean acidification. Science 333:418–422

Pantos O, Bongaerts P, Dennis PG, Tyson GW, Hoegh-Guldberg O (2015) Habitat-specific environmental conditions primarily control the microbiomes of the coral *Seriatopora hystrix*. The ISME Journal 9:1916–1927

Pearse VB, Muscatine L (1971) Role of symbiotic algae (Zooxanthellae) in coral calcification. The Biological Bulletin 141:350–363

Peay KG, Garbelotto M, Bruns TD (2009) Spore heat resistance plays an important role in disturbance-mediated assemblage shift of ectomycorrhizal fungi colonizing *Pinus muricata* seedlings. Journal of Ecology 97:537–547

Peixoto RS, Rosado PM, Leite DC de A, Rosado AS, Bourne DG (2017) Beneficial microorganisms for corals (BMC): Proposed mechanisms for coral health and resilience. Frontiers in Microbiology 8:341

Peres-Neto PR, Jackson DA (2001) How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. Oecologia 129:169–178

Pernice M, Meibom A, Heuvel A Van Den, Kopp C, Domart-Coulon I, Hoegh-Guldberg O, Dove S (2012) A single-cell view of ammonium assimilation in coral-dinoflagellate symbiosis. The ISME Journal 6:1314–1324

Philander SGH (1983) Anomalous El Niño of 1982-83. Nature 305:16

Pochon X, Gates RD (2010) A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawaii. Molecular Phylogenetics and Evolution 56:6

Pochon X, Pawlowski J, Others (2006) Evolution of the soritids-*Symbiodinium* symbiosis. Symbiosis 42:77–88

Pochon X, Stat M, Takabayashi M, Chasqui L, Chauka LJ, Logan D, Gates RD (2010) Comparison of endosymbiotic and free-living *Symbiodinium* (Dinophyceae) diversity in a Hawaiian reef environment. Journal of Phycology 46:53–65

Post E, Forchhammer MC, Bret-Harte MS, Callaghan T V, Christensen TR, Elberling B, Fox AD, Gilg O, Hik DS, Høye TT, Ims RA, Jeppesen E, Klein DR, Madsen J, McGuire AD, Rysgaard S, Schindler DE, Stirling I, Tamstorf MP, Tyler NJC, Wal R van der, Welker J, Wookey PA, Schmidt NM, Aastrup P (2009) Ecological dynamics across the Arctic associated with recent climate change. Science 325:1355–1358

Przeslawski R, Byrne M, Mellin C (2015) A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. Global Change Biology 21:2122–2140

Putnam HM, Barott KL, Ainsworth TD, Gates RD (2017) The vulnerability and resilience of reef-building corals. Current Biology 27:R528–R540

Putnam HM, Stat M, Pochon X, Gates RD (2012) Endosymbiotic flexibility associates with environmental sensitivity in scleractinian corals. Proceedings of the Royal Society B: Biological Sciences 279:4352–4361

Quigley KM, Bay LK, Willis BL (2017) Temperature and water quality-related patterns in sediment-associated *Symbiodinium* communities impact symbiont uptake and fitness of juveniles in the genus *Acropora*. Frontiers in Marine Science 4:401

Quigley KM, Davies SW, Kenkel CD, Willis BL, Matz M V, Bay LK (2014) Deep-sequencing method for quantifying background abundances of *Symbiodinium* types: exploring the rare *Symbiodinium* biosphere in reef-building corals. PLoS ONE 9:e94297

Quigley KM, Warner PA, Bay LK, Willis BL (2018) Unexpected mixed-mode transmission and moderate genetic regulation of Symbiodinium communities in a brooding coral. Heredity 121:524–536

R Development Core Team (2008) R: A language and environment for statistical computing.

Rädecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C (2015) Nitrogen cycling in corals: the key to understanding holobiont functioning? Trends in Microbiology 23:490–497

Raina J-B, Tapiolas DM, Forêt S, Lutz A, Abrego DD, Ceh J, Seneca FO, Clode PL, Bourne DG, Willis BL, Motti CA (2013) DMSP biosynthesis by an animal and its role in coral thermal stress response. Nature 502:677–680

Republic of Kiribati Island Report Series: Kiritimati (2012)

Reynolds RW, Smith TM, Liu C, Chelton DB, Casey KS, Schlax MG (2007) Daily high-resolution-blended analyses for sea surface temperature. Journal of Climate 20:5473–5496

Richmond RH, Hunter CL (1990) Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. Marine Ecology Progress Series 60:185–203

Roder C, Arif C, Bayer T, Aranda M, Daniels C, Shibl A, Chavanich S, Voolstra CR (2014) Bacterial profiling of White Plague Disease in a comparative coral species framework. ISME Journal

Rodrigues-Lanetty M, Loh WK, Carter D, Hoegh-Guldberg O (2001) Latitudinal variability in symbiont specificity within the widespread scleractinian coral *Plesiastrea versipora*. Marine Biology 138:1175–1181

Rodrigues LJ, Grottoli AG (2007) Energy reserves and metabolism as indicators of coral recovery from bleaching. Limnology and Oceanography 52:1874–1882

Rohwer F, Youle M, Vosten D (2010) Coral reefs in the microbial seas. Plaid Press Granada Hills

Rosenberg MS (2005) The file-drawer problem revisited: a general weighted method for calculating fail-safe numbers in meta-analysis. Evolution 59:464–468

Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nature Reviews Microbiology 5:355–362

Röthig T, Costa RM, Simona F, Baumgarten S, Torres AF, Radhakrishnan A, Aranda M, Voolstra CR (2016) Distinct bacterial communities associated with the coral model Aiptasia in aposymbiotic and symbiotic states with Symbiodinium. Frontiers in Marine Science 3:403

Rowan R (1995) Intraspecific diversity and ecological zonation in coral-algal symbiosis. Proceedings of the National Academy of Sciences 92:2850–2853

Rowan R, Powers DA (1991) A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbioses. Science 251:1348–1351

Rowan R, Powers DA (1992) Ribosomal RNA sequences and the diversity of symbiotic dinoflagellates (zooxanthellae). Proceedings of the National Academy of Sciences 89:3639–3643

Sachs JL, Wilcox TP (2006) A shift to parasitism in the jellyfish symbiont Symbiodinium microadriaticum. Proceedings of the Royal Society B: Biological Sciences 273:425–429

Salih A, Larkum A, Cox G, Kühl M, Hoegh-Guldberg O (2000) Fluorescent pigments in corals are photoprotective. Nature 408:850–853

Salvat B (1992) Coral reefs - a challenging ecosystem for human societies. Global Environmental Change 2:12–18

Sandin SA, Smith JE, Demartini EE, Dinsdale EA, Donner SD, Friedlander AM, Konotchick T, Malay M, Maragos JE, Obura DO, Pantos O, Paulay G, Richie M, Rohwer F, Schroeder RE, Walsh SM, Jackson JBC, Knowlton NN, Sala E (2008) Baselines and degradation of coral reefs in the Northern Line Islands. PLoS ONE 3:e1548--e1548

Santos SR, Coffroth MA (2003) Molecular genetic evidence that dinoflagellates belonging to the genus Symbiodinium freudenthal are haploid. The Biological Bulletin 204:10–20

Schliep KP (2011) phangorn: phylogenetic analysis in R. Bioinformatics 27:592–593

Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. Global Change Biology 21:236–249

Silverstein RN, Cunning R, Baker AC (2017) Tenacious D: Symbiodinium in clade D remain in reef corals at both high and low temperature extremes despite impairment. Journal of Experimental Biology 220:1192–1196

Smith EG, Hume BCC, Delaney P, Wiedenmann J, Burt JA (2017) Genetic structure of coral-*Symbiodinium* symbioses on the world's warmest reefs. PLoS One 12:e0180169

Socolar JB, Gilroy JJ, Kunin WE, Edwards DP (2016) How should beta-diversity inform biodiversity conservation? Trends in Ecology and Evolution 31:67–80

Soffer N, Zaneveld J, Vega Thurber R (2015) Phage-bacteria network analysis and its implication for the understanding of coral disease. Environmental Microbiology 17:1203–1218

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere." Proceedings of the National Academy of Sciences 103:12115–12120

Souter DW, Lindén O (2000) The health and future of coral reef systems. Ocean and Coastal Management 43:657–688

Spurgeon JPG (1992) The economic valuation of coral reefs. Marine Pollution Bulletin 24:529–536

Starzomski BM, Srivastava DS (2007) Landscape geometry determines community response to disturbance. Oikos 116:690–699

Stat M, Baker AC, Bourne DG, Correa AMS, Forsman ZH, Huggett MJ, Pochon X, Skillings D, Toonen RJ, Oppen MJH van, Gates RD (2012) Molecular delineation of species in the coral holobiont. Advances in Marine Biology 63:1–65

Stat M, Carter D, Hoegh-Guldberg O (2006) The evolutionary history of *Symbiodinium* and scleractinian hosts - symbiosis, diversity, and the effect of climate change. Perspectives in Plant Ecology 8:23–43

Stat M, Gates RD (2011) Clade D Symbiodinium in scleractinian corals: a “nugget” of hope, a selfish opportunist, an ominous sign, or all of the above? Journal of Marine Biology 730715:1–9

Stat M, Loh WKW, LaJeunesse TC, Hoegh-Guldberg O, Carter DA (2009) Stability of coral-endosymbiont associations during and after a thermal stress event in the southern Great Barrier Reef. Coral Reefs 28:709–713

Stat M, Morris E, Gates RD (2008) Functional diversity in coral-dinoflagellate symbiosis. Proceedings of the National Academy of Sciences 105:9256–9261

Stat M, Pochon X, Franklin EC, Bruno JF, Casey KS, Selig ER, Gates RD (2013) The distribution of the thermally tolerant symbiont lineage (*Symbiodinium* clade D) in corals from Hawaii: correlations with host and the history of ocean thermal stress. Ecology and Evolution 3:1317–1329

Stone L, Rajagopalan B, Bhasin H, Loya Y (1999) Mass coral reef bleaching: A recent outcome of increased El Niño activity? Ecology Letters 2:325–330

Strong AE, Barrientos CS, Duda C (1997) Improved satellite techniques for monitoring coral reef bleaching. In: Proceedings of the 8th International Coral Reef Symposium. Panama City, Panama, p 1495–1498

Sweatman H, Abdo D, Burgess S, Coleman G, Delean S, Emslie M, Miller I, Osborne K, Oxley W, Page C, others (2004) Long-term monitoring of the Great Barrier Reef. Status Report Number 6.

Sweet MJ (2014) *Symbiodinium* diversity within *Acropora muricata* and the surrounding environment. Marine Ecology 35:343–353

Takabayashi M, Adams LM, Pochon X, Gates RD (2012) Genetic diversity of free-living *Symbiodinium* in surface water and sediment of Hawai`i and Florida. Coral Reefs 31:157–167

Thøgersen MS, Delpin MW, Melchiorsen J, Kilstrup M, Månsson M, Bunk B, Spröer C, Overmann J, Nielsen KF, Gram L (2016) Production of the bioactive compounds violacein and indolmycin is conditional in a maeA mutant of Pseudoalteromonas luteoviolacea S4054 lacking the malic enzyme. Frontiers in Microbiology

Thomas L, Kendrick GA, Kennington WJ, Richards ZT, Stat M (2014) Exploring *Symbiodinium* diversity and host specificity in *Acropora* corals from geographical extremes of Western Australia with 454 amplicon pyrosequencing. Molecular Ecology 23:3113–3126

Thornhill DJ, Howells EJ, Wham DC, Steury TD, Santos SR (2017) Population genetics of reef coral endosymbionts (*Symbiodinium*, Dinophyceae). Molecular Ecology 26:2640–2659

Thornhill DJ, LaJeunesse TC, Kemp DW, Fitt WK, Schmidt GW (2006) Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. Marine Biology 148:711–722

Thurber RV, Burkepile DE, Correa AMS, Thurber AR, Shantz AA, Welsh R, Pritchard C, Rosales S (2012) Macroalgae decrease growth and alter microbial community structure of the reef-building coral, *Porites astreoides*. PLoS One 7:e44246

Tolman H, Chalikov D (1994) Development of a third-generation ocean wave model at NOAA/NCEP. In: M. Isaacson MCQ (ed) Int. Sym. Waves: Physical & Numerical Modelling. University of British Columbia Press, p 724–733

Tolman HL, Others (2009) User manual and system documentation of WAVEWATCH III version 3.14. Technical note, MMAB Contribution 276:220

Tonk L, Bongaerts P, Sampayo EM, Hoegh-Guldberg O (2013) SymbioGBR: a web-based database of Symbiodinium associated with cnidarian hosts on the Great Barrier Reef. BMC Ecology 13:7

Toscano MA, Casey KS, Shannon J (2002) Improved prediction of coral bleaching using high-resolution HotSpot anomaly mapping. In: Seventh International Conference on Remote Sensing for Marine and Coastal Environments. Miami, FL

Toscano MA, Liu G, Guch IC, Casey KS (2002) Improved prediction of coral bleaching using high-resolution HotSpot anomaly mapping. In: Proceedings of the 9th International Coral Reef Symposium. Bali, Indonesia

Treml EA, Halpin PN, Urban DL, Pratson LF (2008) Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. Landscape Ecology 23:19–36

Trenberth KE, Hoar TJ (1997) El Niño and climate change. Geophysical Research Letters 24:3057–3060

Trench RK (1971a) The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. III. The effect of homogenates of host tissues on the excretion of photosynthetic products in vitro by zooxanthellae from two marine coelenterates. Proceedings of the Royal Society B: Biological Sciences 177:251–264

Trench RK (1971b) The physiology and biochemistry of zooxanthellae symbiotic with marine coelenterates. I. The assimilation of photosynthetic products of zooxanthellae by two marine coelenterates. Proceedings of the Royal Society B: Biological Sciences 177:225–235

Trench RK (1993) Microalgal-invertebrate symbioses - A review. Endocytobiosis and Cell Research 9:135–175

Tylianakis JM, Didham RK, Bascompte J, Wardle DA (2008) Global change and species interactions in terrestrial ecosystems. Ecology Letters 11:1351–1363

Ulstrup KE, Berkelmans R, Ralph PJ, Oppen MJH van (2006) Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae. Marine Ecology Progress Series 314:135–148

Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, Mcminds R, Zaneveld JR (2014) Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Global Change Biology 20:544–554

Vega Thurber R, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F, Dinsdale E, Kelly L, Rohwer F (2009) Metagenomic analysis of stressed coral holobionts. Environmental Microbiology 11:2148–2163

Venn A, Tambutté É, Lotto S, Zoccola D, Allemand D, Tambutté S (2009a) pH regulation in symbiotic anemones and corals: A delicate balancing act. Proceedings of the National Academy of Sciences 106:16541–16542

Venn AA, Tambutté E, Lotto S, Zoccola D, Allemand D, Tambutté S (2009b) Imaging intracellular pH in a reef coral and symbiotic anemone. Proceedings of the National Academy of Sciences 106:16574–16579

Viechtbauer W (2010) Conducting meta-analyses in R with the metafor package. Journal of Statistical Software 36:1–48

Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104:451–457

Vorburger C, Perlman SJ (2018) The role of defensive symbionts in host-parasite coevolution: Symbiont-mediated coevolution. Biological Reviews 4:79

Wagner M, Horn M (2006) The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. Current Opinion in Biotechnology 17:241–249

Wagner D, Pochon X, Irwin L, Toonen RJ, Gates RD (2011) Azooxanthellate? Most Hawaiian black corals contain *Symbiodinium*. Proceedings of the Royal Society B: Biological Sciences 278:1323–1328

Walker B, Holling CS, Carpenter S, Kinzig A (2004) Resilience, adaptability and transformability in social-ecological systems. Ecology and Society 9:5

Walsh SM (2011) Ecosystem-scale effects of nutrients and fishing on coral reefs. Journal of Marine Biology 2011:1–13

Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J-M, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416:389–395

Wangpraseurt D, Larkum A, Ralph PJ, Kühl M (2012) Light gradients and optical microniches in coral tissues. Frontiers in Microbiology 3:316

Ware JR, Fautin DG, Buddemeier RW (1996) Patterns of coral bleaching: modeling the adaptive bleaching hypothesis. Ecological Modelling 84:199–214

Watson MS, Claar DC, Baum JK (2016) Subsistence in isolation: Fishing dependence and perceptions of change on Kiritimati, the world's largest atoll. Ocean and Coastal Management 123:1–8

Wellington GM, Glynn PW, Strong AE, Navarrete SA, Wieters E, Hubbard D (2001) Crisis on coral reefs linked to climate change. Eos, Transactions American Geophysical Union 82:1–5

West JM, Salm R V (2003) Resistance and resilience to coral bleaching: Implications for coral reef conservation and management. Conservation biology : the journal of the Society for Conservation Biology 17:956–967

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer

Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret F-E, Postle AD, Achterberg EP (2012) Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nature Climate Change 3:160

Wilkinson CR (1996) Global change and coral reefs: impacts on reefs, economies and human cultures. Global Change Biology 2:547–558

Wilkinson CR, Hodgson G (1999) Coral reefs and the 1997-1998 mass bleaching and mortality. Nature and Resources 35:16–25

Wilkinson CR, Lindén O, Cesar H, Hodgson G, Rubens J, Strong AE (1999) Ecological and socioeconomic impacts of 1998 coral mortality in the Indian Ocean. An ENSO impact and a warning of future change? Ambio 28:188–196

Williams EH, Bunkley-Williams L (1990) The world-wide coral reef bleaching cycle and related sources of coral mortality. Atoll Research Bulletin 35:67

Williams JW, Jackson ST (2007) Novel climates, no-analog communities, and ecological surprises. Frontiers in Ecology and the Environment 5:475–482

Willis BL, Babcock RC, Harrison PL, Oliver JK (1985) Patterns in the mass spawning of corals on the Great Barrier Reef from 1981 to 1984. In: Proceedings of the Fifth International Coral Reef Congress, Tahiti, 1985, Vol. 4.p 343–348

Woesik R van, Shiroma K, Koksal S (2010) Phenotypic variance predicts symbiont population densities in corals: a modeling approach. PLoS ONE 5:e9185–e9185

Wooldridge SA (2009) Water quality and coral bleaching thresholds: Formalising the linkage for the inshore reefs of the Great Barrier Reef, Australia. Marine Pollution Bulletin 58:745–751

Wooldridge SA, Done TJ (2009) Improved water quality can ameliorate effects of climate change on corals. Ecological Applications 19:1492–9

Work TM, Aeby GS (2014) Microbial aggregates within tissues infect a diversity of corals throughout the Indo-Pacific. Marine Ecology Progress Series 500:1–9

Yamashita H, Koike K (2013) Genetic identity of free-living Symbiodinium obtained over a broad latitudinal range in the Japanese coast. Phycological Research 61:68–80

Yellowlees D, Rees TA V, Leggat W (2008) Metabolic interactions between algal symbionts and invertebrate hosts. Plant, Cell and Environment 31:679–694

Yost DM, Wang L-H, Fan T-Y, Chen C-S, Lee RW, Sogin E, Gates RD (2013) Diversity in skeletal architecture influences biological heterogeneity and *Symbiodinium* habitat in corals. Zoology (Jena, Germany) 116:262–269

Zaneveld JR, Burkepile DE, Shantz AA, Pritchard CE, McMinds R, Payet JP, Welsh R, Correa AMS, Lemoine NP, Rosales S, Fuchs C, Maynard JA, Thurber RV (2016) Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. Nature Communications 7:11833

Zaneveld JR, McMinds R, Vega Thurber R (2017) Stress and stability: applying the Anna Karenina principle to animal microbiomes. Nature Microbiology 2:17121

Zapata FA, Rodríguez-Ramírez A, Caro-Zambrano C, Garzón-Ferreira J (2010) Mid-term coral-algal dynamics and conservation status of a Gorgona Island (Tropical Eastern Pacific) coral reef. Revista de Biologia Tropical 58 Suppl 1:81–94

Ziegler M, Stone E, Colman D, Takacs-Vesbach C, Shepherd U (2018) Patterns of *Symbiodinium* (Dinophyceae) diversity and assemblages among diverse hosts and the coral reef environment of Lizard Island, Australia. Journal of Phycology 54:447–460

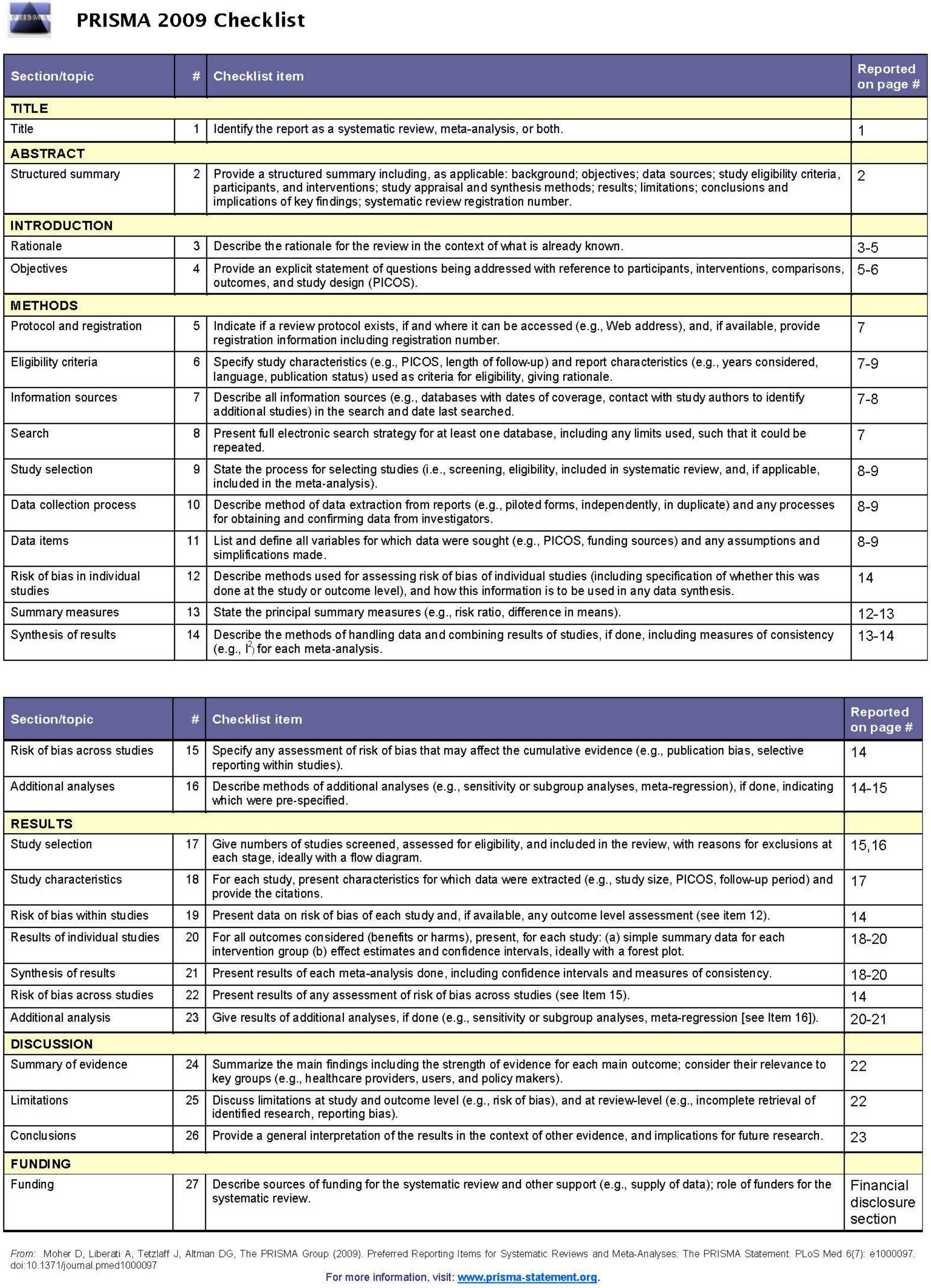
Appendix A
Chapter 3 Supplementary Material

Appendix A. Table S1. Overview of all papers reviewed for the meta-analysis.
Table S1 is available online as a csv in the supporting information section of this manuscript at PLOS ONE https://doi.org/10.1371/journal.pone.0190957

Appendix A. Table S2. All coral cover data included in the meta-analysis.
Table S2 is available online as a csv in the supporting information section of this manuscript at PLOS ONE https://doi.org/10.1371/journal.pone.0190957

Appendix A. Table S3. Coral bleaching after-only data included in the meta-analysis.
Table S3 is available online as a csv in the supporting information section of this manuscript at PLOS ONE https://doi.org/10.1371/journal.pone.0190957

Appendix A. Figure S1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2009 Checklist Official PRISMA checklist, with criteria and corresponding manuscript pages.



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	7
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7-9
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7-8
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	8-9
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8-9
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8-9
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	14
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	12-13
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	13-14
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	14
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	14-15
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	15,16
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	17
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	14
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	18-20
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	18-20
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	14
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	20-21
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	22
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	22
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	23
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Financial disclosure section

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

Appendix A. Figure S2. *Animation of progression of maximum El Niño impact by event, from 1982-2016.*

Figure S2 is available online in the supporting information section of this manuscript at PLOS ONE https://doi.org/10.1371/journal.pone.0190957

Appendix A. Figure S3. All model results. a. Moderators included in top models. Output from the R package 'glmulti', as well as reverse stepwise ANOVAs are included, and two data sets were included for each method (all coral cover data, and only data collected within 1 year of the peak El Niño/La Niña event. Moderators are denoted as either positive (+) or negative (-). A dark grey background indicates that the moderator was not included in the final model and white background indicates that the moderator was included in the final model and was significant at $p < 0.05$. b. Top ten best models for coral cover, showing included model terms (moderators), AICc values and weights. c. Top ten best models for coral bleaching, showing included model terms (moderators), AICc values and weights.

a. Moderators included in top models

Model Method	Included Data Type	Mean Temp	Max DHW	Max DHW: TimeLag
glmulti	Cover, All Data	-	x	-
	Cover, 1 Year Only	-	x	x
Reverse Stepwise ANOVAs	Cover, All Data	-	-	x
	Cover, 1 Year Only	-	-	x
glmulti	Bleaching, collected + simulated “before” data	+	x	-
	Bleaching, only simulated “before” data	x	x	-
Reverse Stepwise ANOVAs	Bleaching, collected + simulated “before” data	+	x	x
	Bleaching, only simulated “before” data			

b. Coral Cover (up to 1 year after maximum stress).

Included Model Terms	**AICc**	**Weight**
Max DHW + Mean Temp (Top Model)	552.70	0.0358
Mean Temp + Mean Temp:Max DHW	552.87	0.0330
Max DHW + Temp Var	553.48	0.0242
Temp Var + Mean Temp:Max DHW	553.56	0.0232
Max DHW + Temp Var:Mean Temp	553.69	0.0218
Mean Temp:Max DHW + Temp Var:Mean Temp	553.70	0.0217
Max DHW + Mean Temp + Time Lag:Max DHW	554.02	0.0185
Max DHW + Mean Temp + Temp Var:Mean Temp	554.04	0.0184
Max DHW + Mean Temp + Mean Temp:Time Lag	554.05	0.0183
Max DHW + Time Lag + Mean Temp	554.07	0.0181

c. Bleaching (including measured and simulated before-bleaching values)

Included Model Terms	**AICc**	**Weight**
Mean Temp + Time Lag:Max DHW	433.64	0.0377
Time Lag + Mean Temp	434.33	0.0267
Mean Temp + Mean Temp:Time Lag	434.39	0.0260
Mean Temp + Temp Var + Time Lag:Max DHW	434.59	0.0235
Mean Temp + Time Lag:Max DHW + Temp Var:Mean Temp	434.63	0.0230
Mean Temp + Temp Var + Temp Var:Time Lag	434.83	0.0208
Mean Temp + Temp Var: Time Lag + Temp Var:Mean Temp	434.84	0.0207
Mean Temp + Temp Var:Time Lag	434.91	0.0200
Time Lag + Mean Temp + Mean Temp:Time Lag	435.42	0.0155
Time Lag + Mean Temp + Temp Var	435.43	0.0154

Appendix B
Chapter 5 Supplementary Material

I. Sample Processing

DNA Extraction and Sequencing

DNA was extracted from all samples using a guanidinium-based extraction protocol (Stat et al. 2009, Cunning, Silverstein, et al. 2015), modified to include three 70% ethanol washes (rather than one). After extraction, DNA was cleaned using Zymo Genomic DNA Clean and Concentrator$^{\text{TM}}$ -25 (Catalog Nos. D4064 & D4065) to improve downstream processing (http://www.zymoresearch.com/downloads/dl/file/id/638/d4064i.pdf).

Library preparation and Illumina MiSeq ITS-2 amplicon sequencing was performed by the Hawaii Institute of Marine Biology (HIMB) Genetics Core Lab following the Illumina 16S Metagenomic Sequencing Library Preparation (Illumina protocol, Part # 15044223 Rev. B) with modifications to generalize this protocol for ITS sequences. ITS primers (ITS-forward: 5’-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGAATTGCAGAACTCCGTC-3’ and ITS-reverse: 5’-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTCCGCTTACTTATATGCTT-3’ (Stat et al. 2009)) were used instead of the 16S primers. Prepared libraries were sequenced on the Illumina MiSeq platform with 2x300 paired-end read chemistry.

Appendix B. Supplementary Table 1. Sample size of water, sediment, and corals by site and time point (August 2014, Before; January 2015, Pre-Storm; January 2015, Post-Storm; May 2015, After). Number of samples reported in "All Coral" is the sum of all samples in *Pocillopora grandis, Montipora aequituberculata*, and *Porites lobata*.

Site Name	Site ID	Field Season	Water	Sediment	All Coral	*Pocillopora grandis*	*Montipora aequituberculata*	*Porites lobata*	TOTAL
M1	8	Before	4	6	6	2	3	1	16
		Pre-Storm	-	-	-	-	-	-	0
		Post-Storm	2	2	6	2	3	1	10
		After	2	5	12	3	6	3	19
		Total this Site	*8*	*13*	*24*	*7*	*12*	*5*	
VH2	27	Before	-	5	13	3	5	5	18
		Pre-Storm	-	-	-	-	-	-	0
		Post-Storm	1	4	14	4	5	5	19
		After	2	6	16	4	7	5	24
		Total this Site	*3*	*15*	*43*	*11*	*17*	*15*	
VH1	30	Before	5	6	18	5	6	7	29
		Pre-Storm	-	-	-	-	-	-	0
		Post-Storm	4	4	18	5	6	7	26
		After	4	4	29	10	9	10	37
		Total this Site	*13*	*14*	*65*	*20*	*21*	*24*	
M2	35	Before	7	5	22	7	7	8	34
		Pre-Storm	-	-	16	6	6	4	16
		Post-Storm	4	5	21	8	6	7	30
		After	4	6	30	11	9	10	40
		Total this Site	*15*	*16*	*89*	*32*	*28*	*29*	
TOTAL			39	58	221	70	73	78	318

Appendix B. Supplementary Table 2. Volumes of water filtered for each sample.

Sample ID	**Field Season**	**SiteID**	**Site Name**	**Volume (L)**
KI14WSYM004	2014	8	M1	6.0
KI14WSYM003	2014	8	M1	8.0
KI14WSYM031	2014	8	M1	8.0
KI14WSYM021	2014	8	M1	7.2
KI14WSYM015	2014	30	VH1	5.5
KI14WSYM016	2014	30	VH1	6.5
KI14WSYM032	2014	30	VH1	7.6
KI14WSYM008	2014	30	VH1	7.7
KI14WSYM017	2014	30	VH1	5.7
KI14WSYM001	2014	35	M2	7.0
KI14WSYM005	2014	35	M2	6.5
KI14WSYM028	2014	35	M2	8.6
KI14WSYM029	2014	35	M2	8.4
KI14WSYM030	2014	35	M2	4.7
KI14WSYM006	2014	35	M2	6.2
KI14WSYM007	2014	35	M2	7.4
KI15aWSYM005	2015a	8	M1	8.0
KI15aWSYM007	2015a	8	M1	9.0
KI15aWSYM013	2015a	27	VH2	9.0
KI15aWSYM017	2015a	30	VH1	6.0
KI15aWSYM018	2015a	30	VH1	7.0
KI15aWSYM019	2015a	30	VH1	7.6
KI15aWSYM020	2015a	30	VH1	7.8
KI15aWSYM009	2015a	35	M2	8.4
KI15aWSYM010	2015a	35	M2	8.0
KI15aWSYM011	2015a	35	M2	9.0
KI15aWSYM012	2015a	35	M2	7.0
KI15bWSYM023	2015b	8	M1	6.5
KI15bWSYM024	2015b	8	M1	7.5
KI15bWSYM018	2015b	27	VH2	6.2
KI15bWSYM019	2015b	27	VH2	7.1
KI15bWSYM005	2015b	30	VH1	3.9
KI15bWSYM006	2015b	30	VH1	4.4
KI15bWSYM007	2015b	30	VH1	4.8
KI15bWSYM008	2015b	30	VH1	10.5
KI15bWSYM001	2015b	35	M2	3.0
KI15bWSYM002	2015b	35	M2	6.7
KI15bWSYM003	2015b	35	M2	8.1
KI15bWSYM004	2015b	35	M2	6.2

Appendix B. Supplementary Table 3. Volume of sediment filtered for each sample.

Sample ID	Field Season	SiteID	Site Name	Sediment (mL)
KI14SSYM003	2014	8	M1	7
KI14SSYM004	2014	8	M1	6.2
KI14SSYM005	2014	8	M1	3
KI14SSYM006	2014	8	M1	3.5
KI14SSYM007	2014	8	M1	3
KI14SSYM008	2014	8	M1	2
KI14SSYM058	2014	27	VH2	3
KI14SSYM059	2014	27	VH2	3
KI14SSYM060	2014	27	VH2	3.5
KI14SSYM061	2014	27	VH2	3
KI14SSYM063	2014	27	VH2	3
KI14SSYM015	2014	30	VH1	3.8
KI14SSYM016	2014	30	VH1	3
KI14SSYM017	2014	30	VH1	3
KI14SSYM018	2014	30	VH1	3
KI14SSYM019	2014	30	VH1	3
KI14SSYM020	2014	30	VH1	3
KI14SSYM010	2014	35	M2	3
KI14SSYM011	2014	35	M2	3
KI14SSYM012	2014	35	M2	3
KI14SSYM013	2014	35	M2	3
KI14SSYM050	2014	35	M2	3
KI15aSSYM026	2015a	8	M1	3
KI15aSSYM027	2015a	8	M1	3
KI15aSSYM019	2015a	27	VH2	3
KI15aSSYM020	2015a	27	VH2	3
KI15aSSYM022	2015a	27	VH2	3
KI15aSSYM024	2015a	27	VH2	3
KI15aSSYM013	2015a	30	VH1	3
KI15aSSYM014	2015a	30	VH1	3
KI15aSSYM015	2015a	30	VH1	3
KI15aSSYM016	2015a	30	VH1	3
KI15aSSYM009	2015a	35	M2	3
KI15aSSYM010	2015a	35	M2	3
KI15aSSYM011	2015a	35	M2	3
KI15aSSYM012	2015a	35	M2	3
KI15bSSYM019	2015b	8	M1	3
KI15bSSYM024	2015b	8	M1	3
KI15bSSYM021	2015b	8	M1	3
KI15bSSYM023	2015b	8	M1	3
KI15bSSYM022	2015b	8	M1	3
KI15bSSYM014	2015b	27	VH2	3

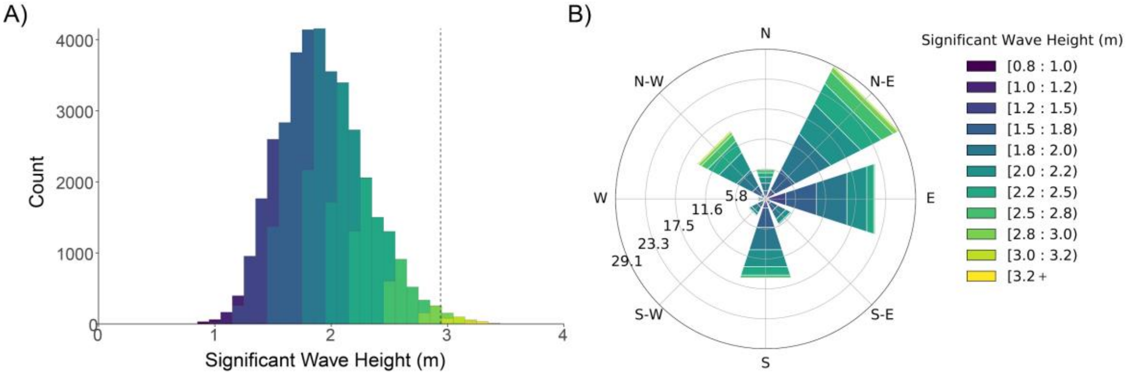
KI15bSSYM013	2015b	27	VH2	3
KI15bSSYM017	2015b	27	VH2	3
KI15bSSYM016	2015b	27	VH2	3
KI15bSSYM018	2015b	27	VH2	3
KI15bSSYM015	2015b	27	VH2	3
KI15bSSYM007	2015b	30	VH1	3
KI15bSSYM009	2015b	30	VH1	3
KI15bSSYM010	2015b	30	VH1	3
KI15bSSYM012	2015b	30	VH1	3
KI15bSSYM004	2015b	35	M2	3
KI15bSSYM001	2015b	35	M2	3
KI15bSSYM006	2015b	35	M2	3
KI15bSSYM002	2015b	35	M2	3
KI15bSSYM005	2015b	35	M2	3
KI15bSSYM003	2015b	35	M2	3

II. Environmental data

Significant Wave Height

We quantified the intensity of storm waves using NOAA's Multi-grid Wavewatch III Hindcast (Tolman & Chalikov 1994, Tolman & Others 2009). First, significant wave height and wave direction were extracted for Kiritimati from February 2005 to December 2017. To visualize the wave climate on Kiritimati, a histogram of significant wave heights for the available time period of wave data (~12 years; Appendix B Fig. S1a) and a wave rose (Appendix B Fig. S1b) were plotted. This wave rose shows the primary wave direction (polar coordinates), as well as the distribution of significant wave heights coming from each direction. Waves on Kiritimati primarily come from the E-NE, consistent with the forcing of the trade winds. Waves also occasionally come from the N-NW or the S-SW; these generally represent storm waves and swell coming from the Western Pacific.

To determine the severity of the January 2015 storm waves in the context of the broader wave climate on Kiritimati, we extracted the maximum wave height during this event (significant wave height = 2.94 m). Next, a three-parameter generalized extreme value (GEV) distribution was fit to the significant wave height data using the R package (R Development Core Team 2008) extRemes (Gilleland et al. 2016). GEV parameters were fit as follows: location = 1.784 ± 0.002 s.e.; scale = 0.345 ± 0.001 s.e.; and shape = -0.151 ± 0.003 s.e. Next, the R package EnvStats (Millard 2013) was used to model the fitted GEV distribution and calculate the percentile of the GEV at the maximum wave height during this event (wave height = 2.94 m, 94th percentile of significant wave height at this location). This allows us to define this storm wave event as a sizeable, but not unprecedented, storm wave event on Kiritimati.



Appendix B. Supplementary Figure 1. Wave climate on Kiritimati (2005-2017) extracted from NOAA's Wavewatch III hindcast product. Plots are based on 3-hourly wave models. A) Histogram of significant wave height. Colors indicate significant wave height, and dashed line on panel a indicates maximum wave height on Kiritimati in January 2015 (2.94 m). B) Polar rose showing average wave conditions. Each bin represents 45° increments. Colors indicate significant wave height, angle of each bin indicates direction of incoming waves, and distance from center represents the probability (percentage) of waves coming from each direction.

III. ITS2 sequences from Symbiodinium

To visualize overlap in ITS2 subclades, taxonomic information is shown in a table by each compartment (Appendix B Table S4), and by each coral species (Appendix B Table S5). Next, bar plots of *Symbiodinium* clade are plotted for water, sediment, and each coral species (Appendix B Fig. S2), followed by bar plots by *Symbiodinium* subclade (Appendix B Fig. S3).

Symbiodinium taxa found exclusively in symbiosis with corals were dominated by clade C OTUs but included one clade D OTU (D2; Appendix B Table S4, Appendix B Figs. S2, S3). *Symbiodinium* taxa detected only in the free-living state (i.e. in water and/or sediment, but not coral) included OTUs from clades A, C, D, F, and I (Appendix B Table S4, Appendix B Figs. S2, S3); these taxa may represent symbiotic *Symbiodinium* that associate with other coral species not sampled in this study, or may be exclusively free-living *Symbiodinium*. OTUs from clade I were primarily found in sediment samples, supporting previous research documenting this clade as endosymbiotic within benthic forams (although members of this clade may also be free-living) (Pochon & Gates 2010). Generalists *Symbiodinium* C1 and C3 were found in all three compartments. Multiple C1-like types were found consistently associated with coral (e.g., C1b/C1e, C1j), coral and sediment (e.g., C1c/C45, C1f, C1a, C1198), and all three ecological compartments (e.g., C1, C1d). Additionally, C15-like types (C15, C1223) were found across all three ecological compartments.

We also evaluated compartment-specific indicator taxa. These indicator taxa are *Symbiodinium* subclades that are over-represented in one compartment compared to the others. Sediment-specific indicator *Symbiodinium* subclades included A101/A2_multiple, A113, A114/A134, A121, A133, F3.2, F5 (F5.1a, F5_multiple, F5.2b, F5.2c), and I4. Water-specific indicator *Symbiodinium* subclades included A4 and one OTU of C15_AY239369. Coral-specific indicator *Symbiodinium* subclades included C21/C32_multiple, C3 (C3_multiple, C3.1), C31, C42, and C15. Free-living indicator *Symbiodinium* subclades (i.e. indicators for both water and sediment) included C3 (C3_multiple, C3_multiple/C65a/C1p/C1.v1a) and C1.

Appendix B. Supplementary Table 4. *Symbiodinium* subclades found in each compartment (i.e. coral, water, sediment). Superscripts identify named *Symbiodinium* species: ^{1}*S. kawagutii,* ^{2}*S. tridacnidorum,* ^{3}*S. pilosum,* ^{4}*S. trenchii*). Matrix shows which compartment each *Symbiodinium* subclade was detected within. For example, subclades in row "Coral" and column "Coral" were only detected in coral (but not in the water or sediment); subclades in row "Sediment" and column "Water" were detected only in water and sediment, but not in coral. The clade is denoted by the first letter in each subclade name.

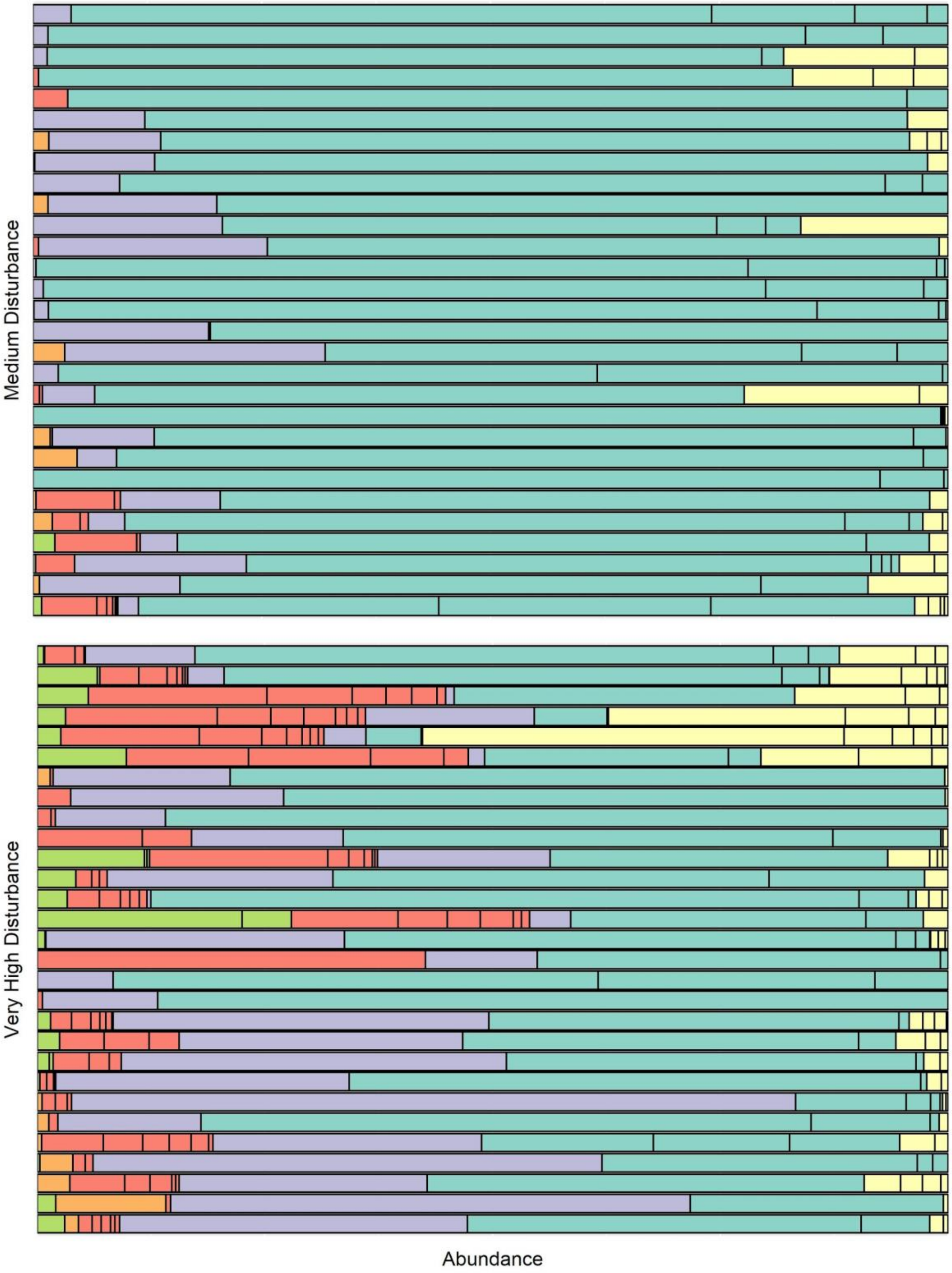
	Coral	Sediment	Water	Coral, Water, & Sediment
Coral	C1b/C1e C1j C4 C21 C21/3d/C3k C1103 C1197 C1226 D2	A113 C1a C1c/C45 C1f C3k C15a C1198	C21/C32 C1094	A121 C1 C1d C3 C15 C31 C31a C32 C42 C1233 D1a ^{4} D1 F5 F3 F114 G100 G3
Sediment		A114 A133 A112 A141 C1p C66 C91 C92 C1005 C1051 C1139 D120 F2b F104/F5 F1 ^{1} I2	A4 A5 A3/A6 ^{2} A101/A2 ^{3} I4	
Water			A7 C1232	

Appendix B. Supplementary Table 5. *Symbiodinium* subclades found in each coral species (i.e. *Pocillopora grandis, Montipora aequituberculata, Porites lobata*). Superscripts identify named *Symbiodinium* species: [1] *S. trenchii*). Matrix shows which coral species each *Symbiodinium* subclade was detected within. For example, subclades in row "*P. grandis*" and column "*P. grandis*" were only detected in *P. grandis* (but not in the other two species); subclades in row "*P. grandis*" and column "*M. aequituberculata*" were detected only in those two species, but not in *P. lobata*. The clade is denoted by the first letter in each subclade name.

	P. grandis	*M. aequituberculata*	*P. lobata*	All 3 species
P. grandis	A113 C1a C15.4 C1226 C4 C1103 C1197 C3.2 C1c/C45 C1j C1198 C3.22 C1b/C1e C1094 C1.6 C15a C15.22 D2	C15.6 C21/3d/3k C15.2 C1f C32.1 C3.1 C21.2 C21.10 D1.3 F114	C31a F5.1a F3.2a F3.2 G3.1	A121 C15 C42 C3k C3 C31 C1 C1233 C21/32 C1d D1a[1] D1 D1a.1 G100 G3.3
M. aequituberculata		NONE	NONE	
P. lobata			NONE	

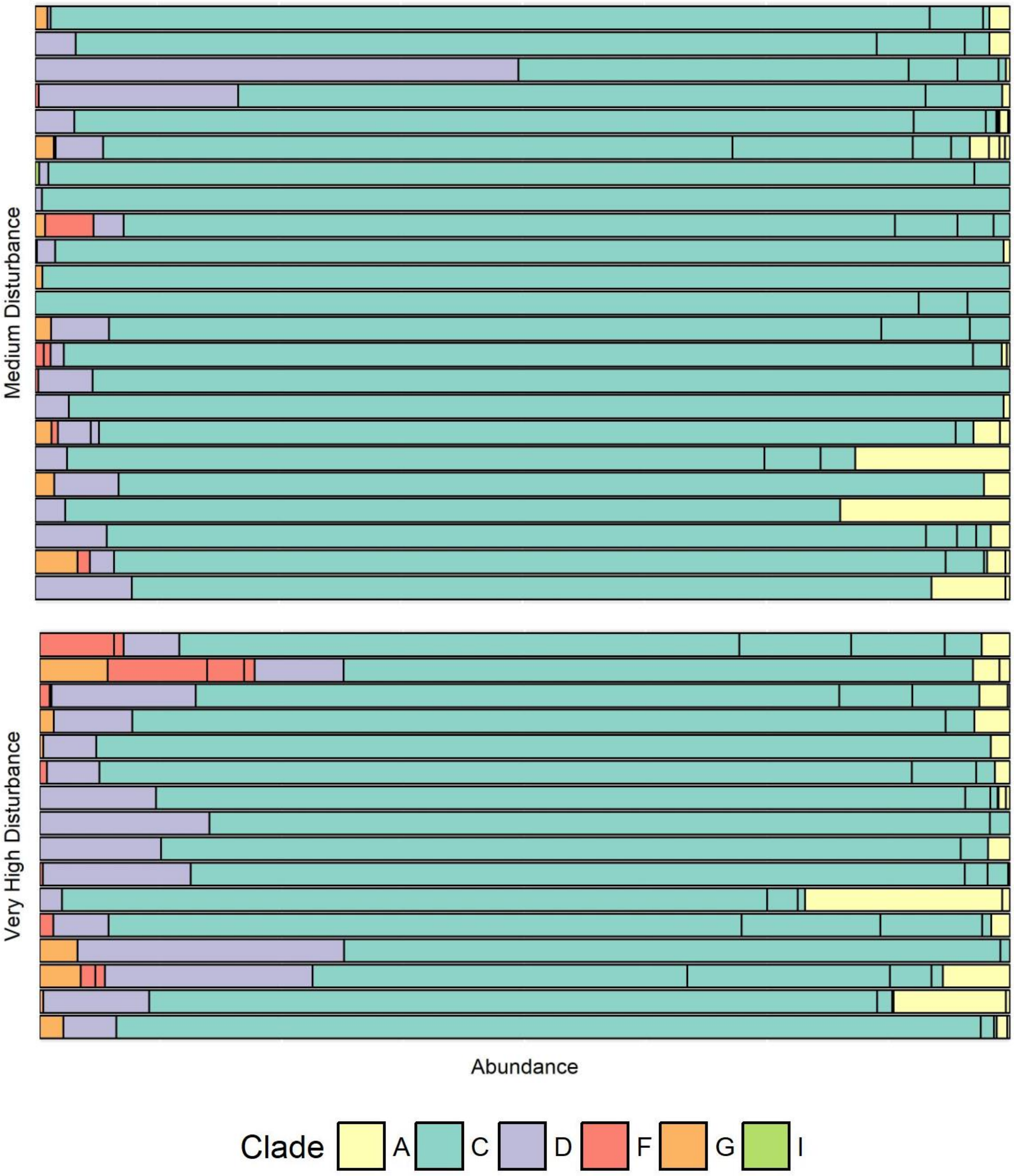
Appendix B. Supplementary Figure 2. Bar plot of *Symbiodinium* taxa, colored by clade.

A) Sediment

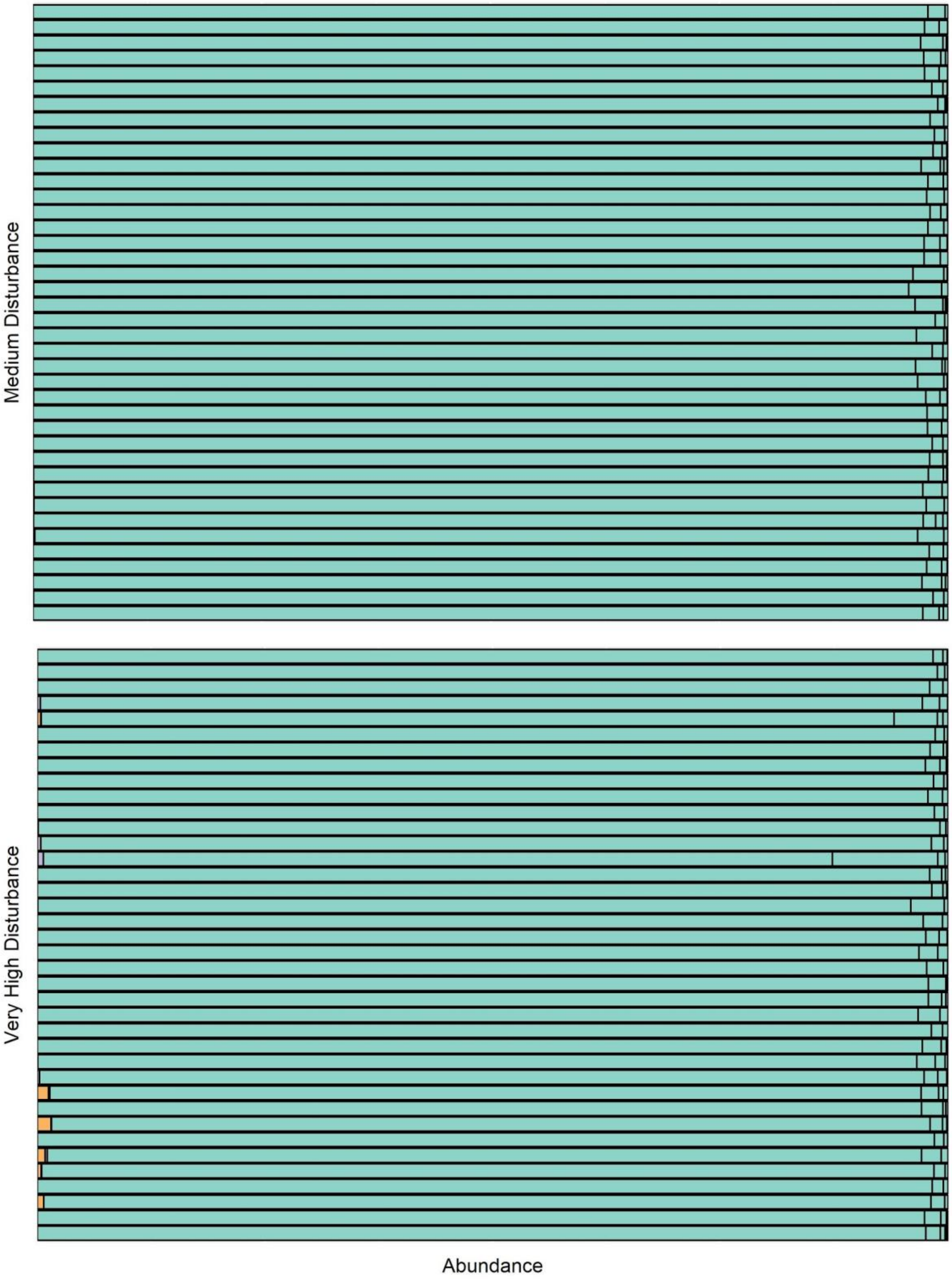


Appendix B. Supplementary Figure 2. Bar plot of *Symbiodinium* taxa, colored by clade.

B) Water

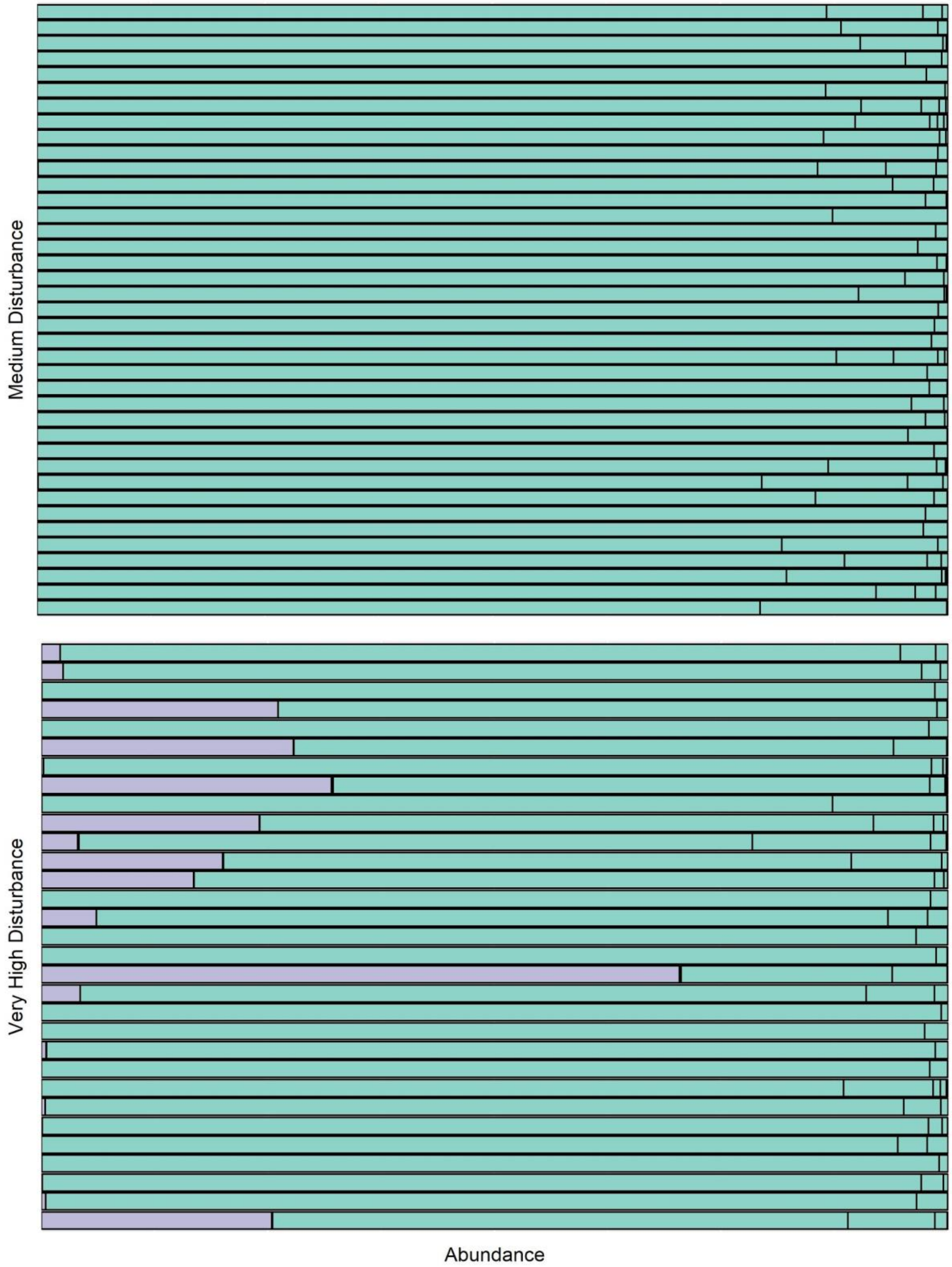


Appendix B. Supplementary Figure 2. Bar plot of *Symbiodinium* taxa, colored by clade.
C) *Montipora aequituberculata*



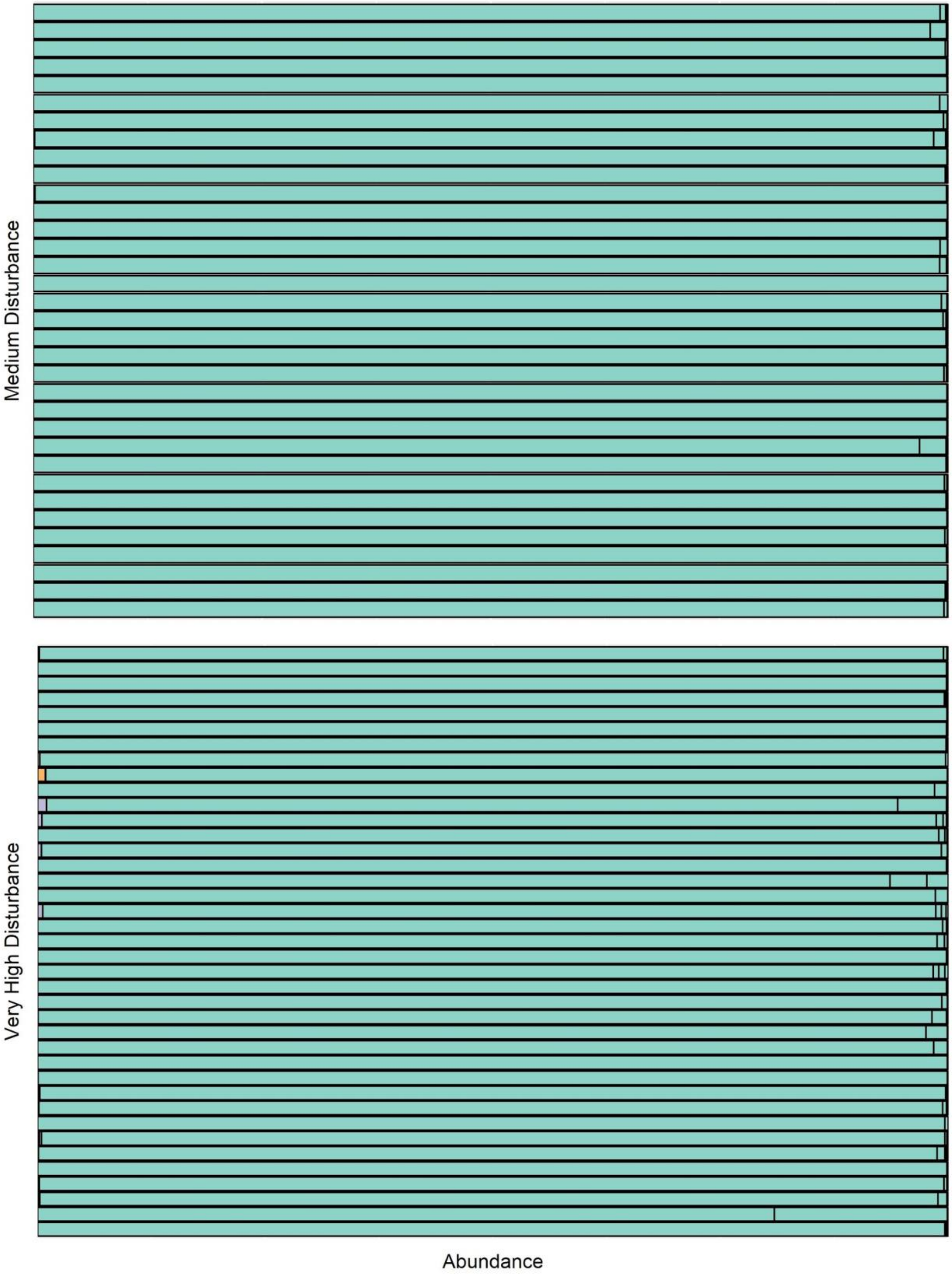
Appendix B. Supplementary Figure 2. Bar plot of *Symbiodinium* taxa, colored by clade.

D) *Pocillopora grandis*

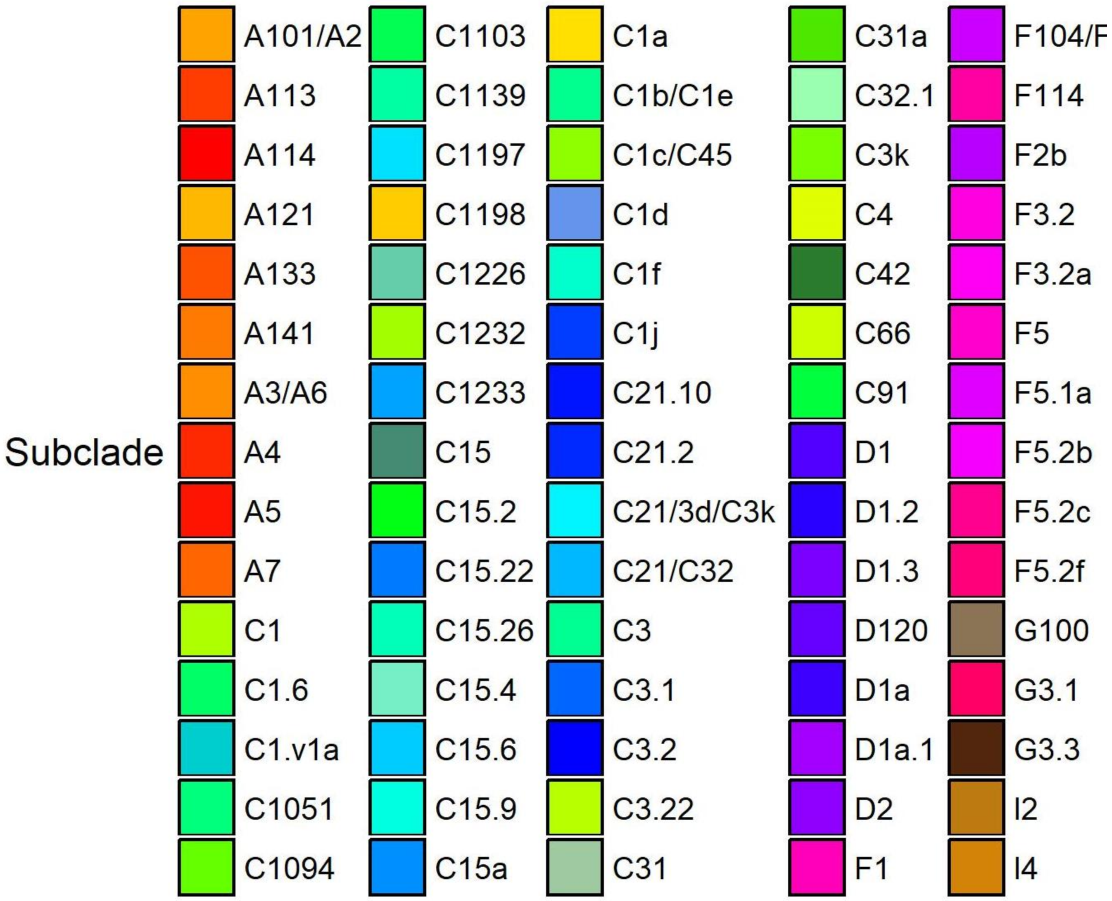


Appendix B. Supplementary Figure 2. Bar plot of *Symbiodinium* taxa, colored by clade.

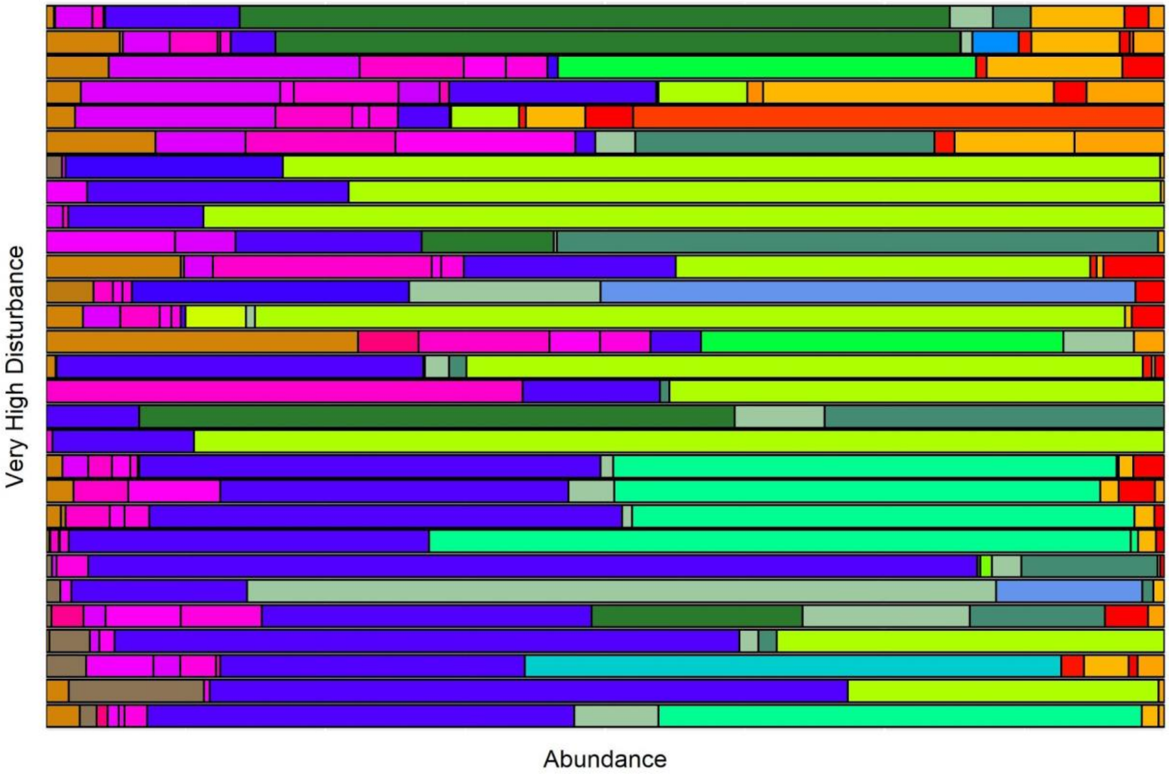
E) *Porites lobata*

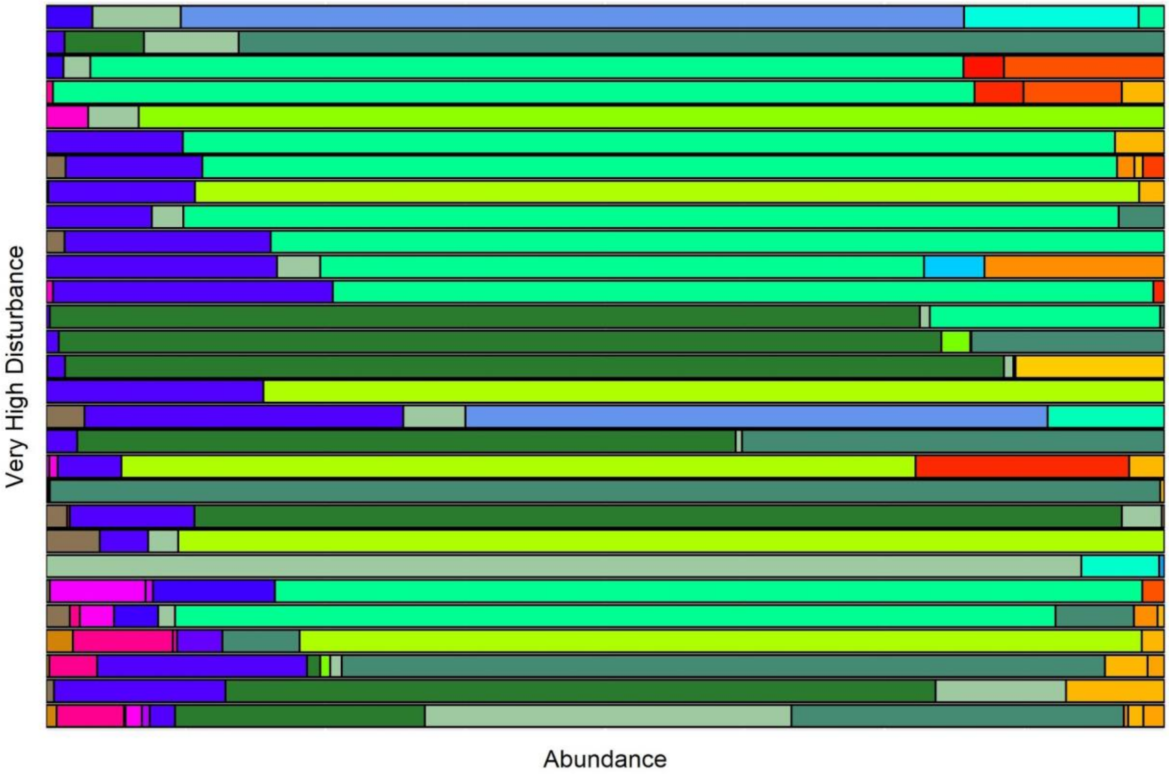


Appendix B. Supplementary Figure 3. Bar plot of *Symbiodinium* subclades. A) Color legend

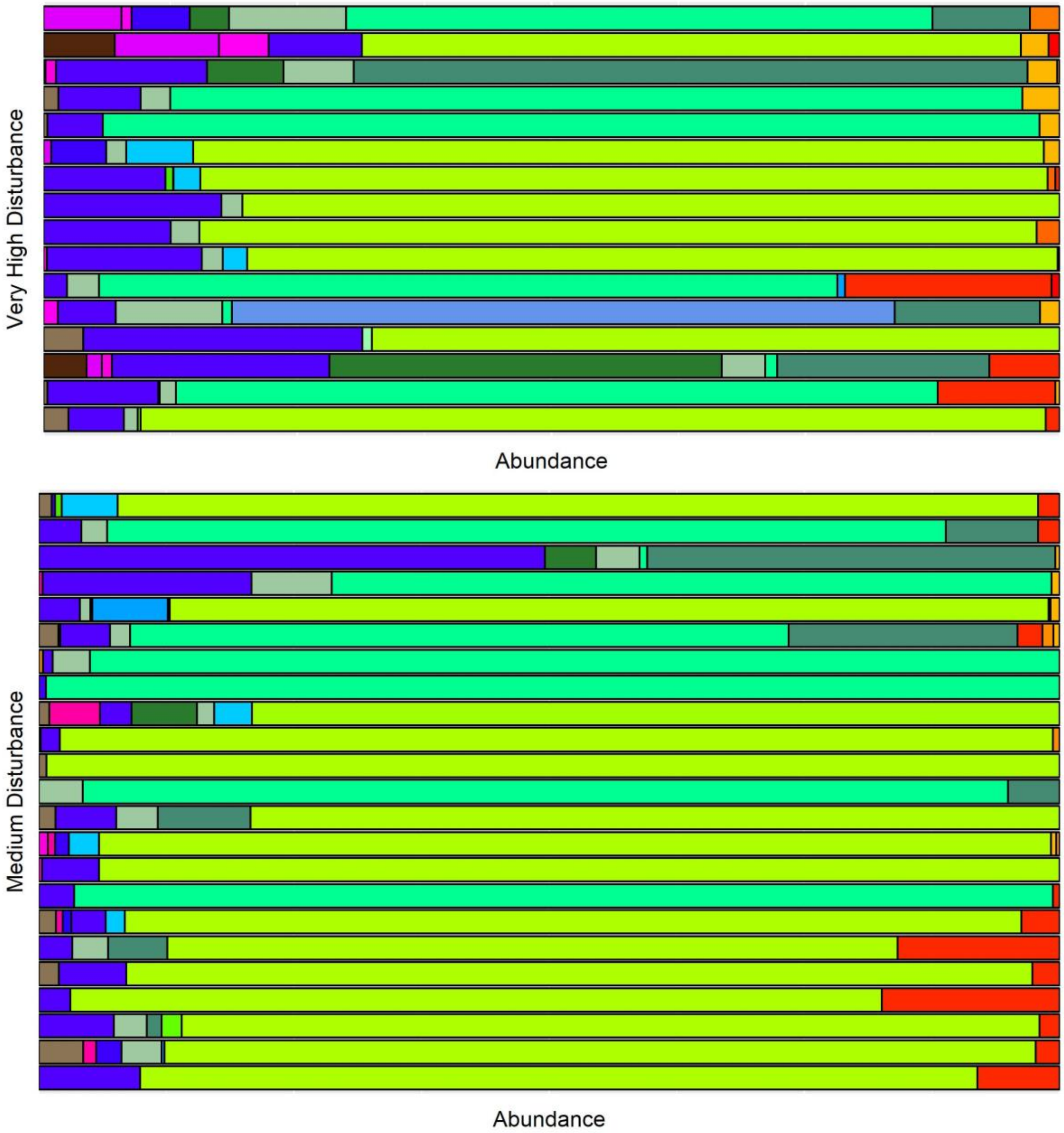


Appendix B. Supplementary Figure 3. B) Sediment

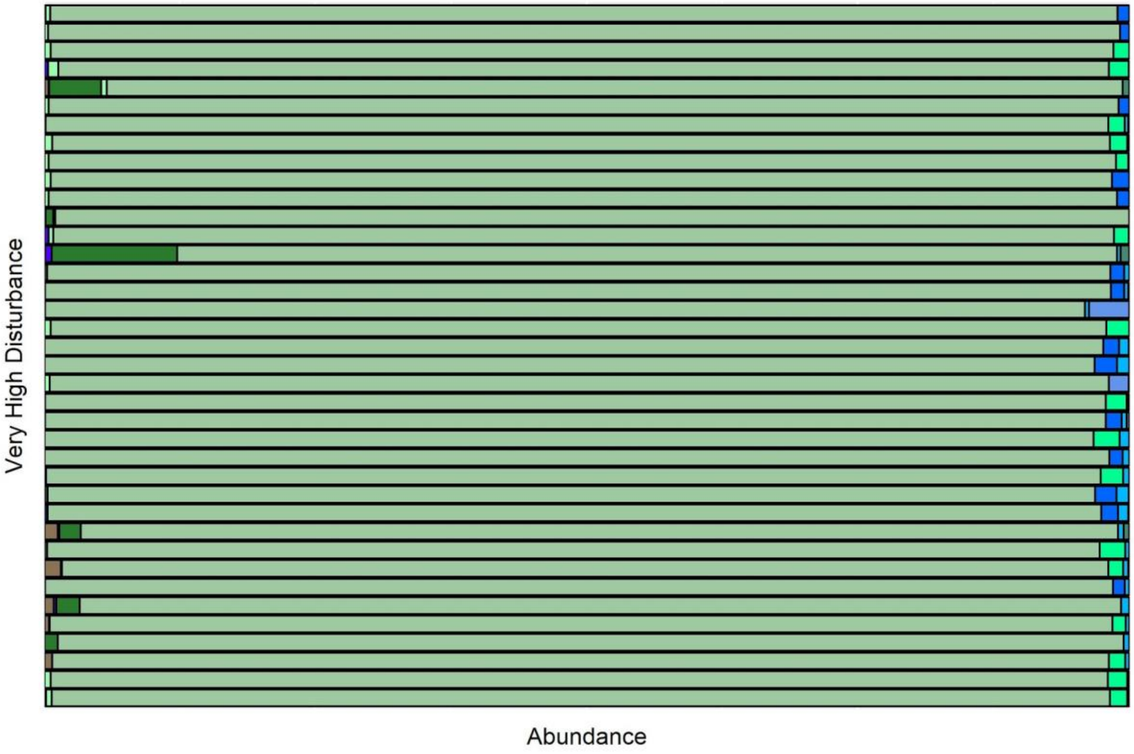




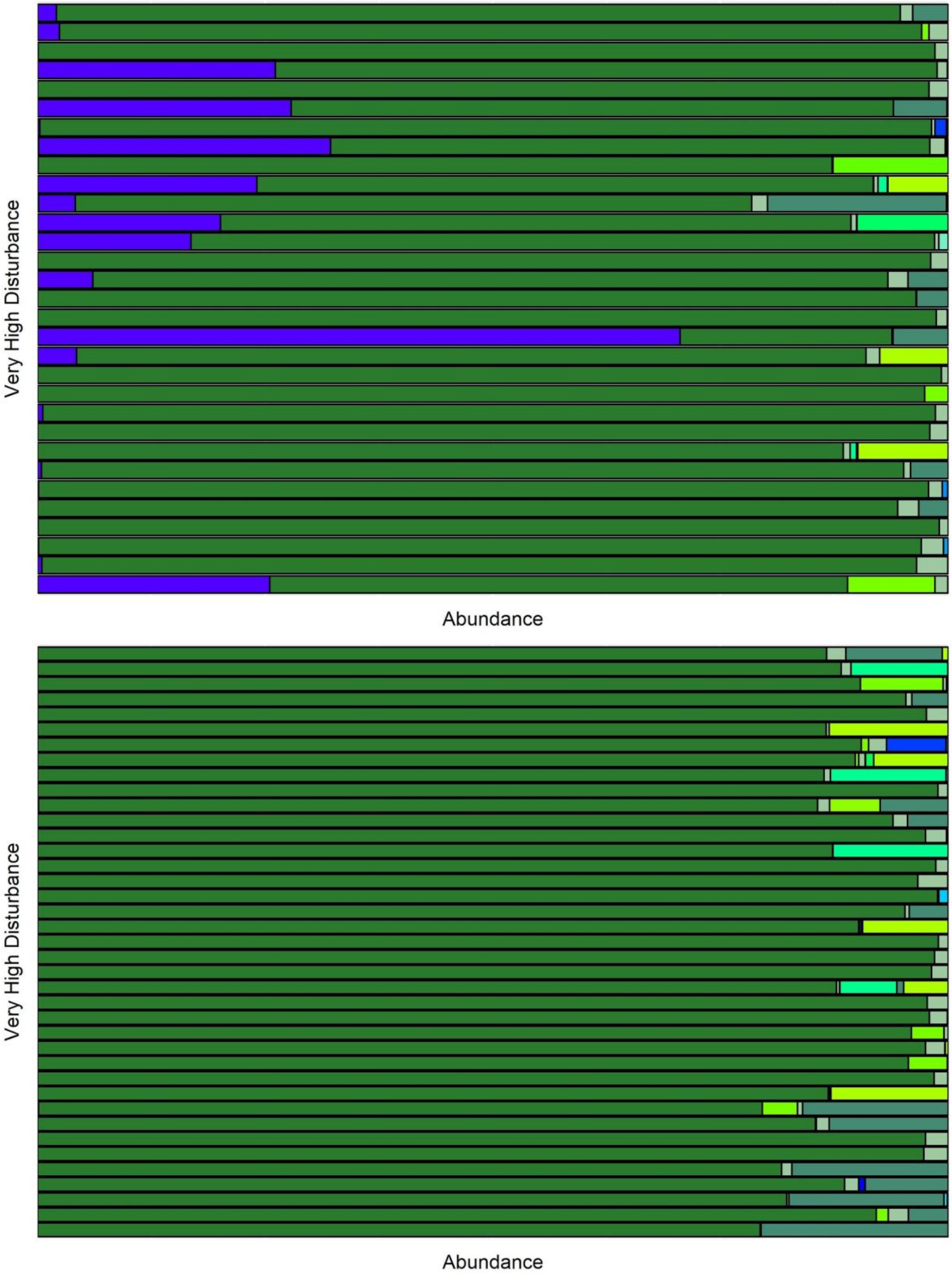
Appendix B. Supplementary Figure 3. C) Water



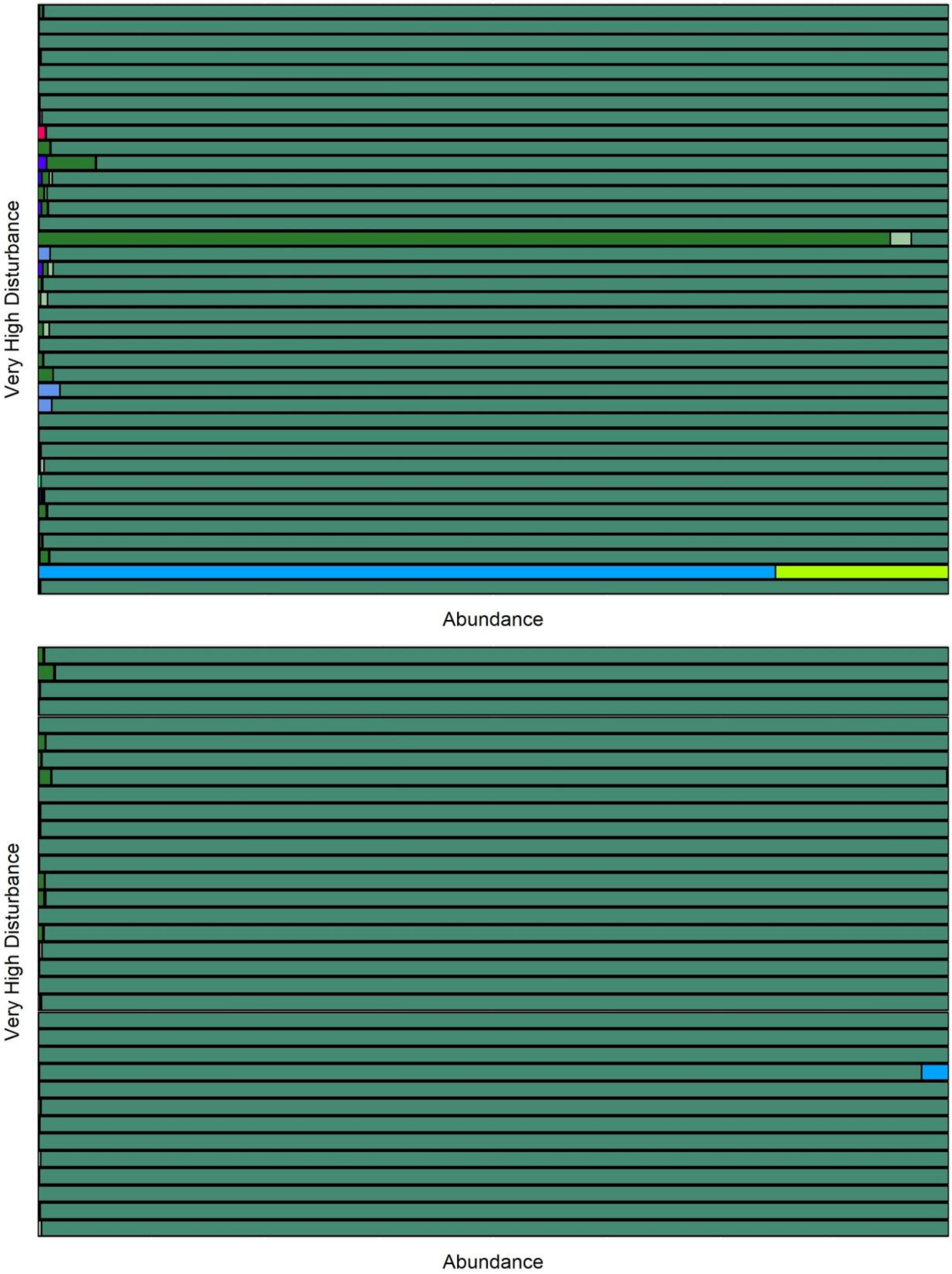
Appendix B. Supplementary Figure 3. D) *Montipora aequituberculata*



Appendix B. Supplementary Figure 3. E) *Pocillopora grandis*



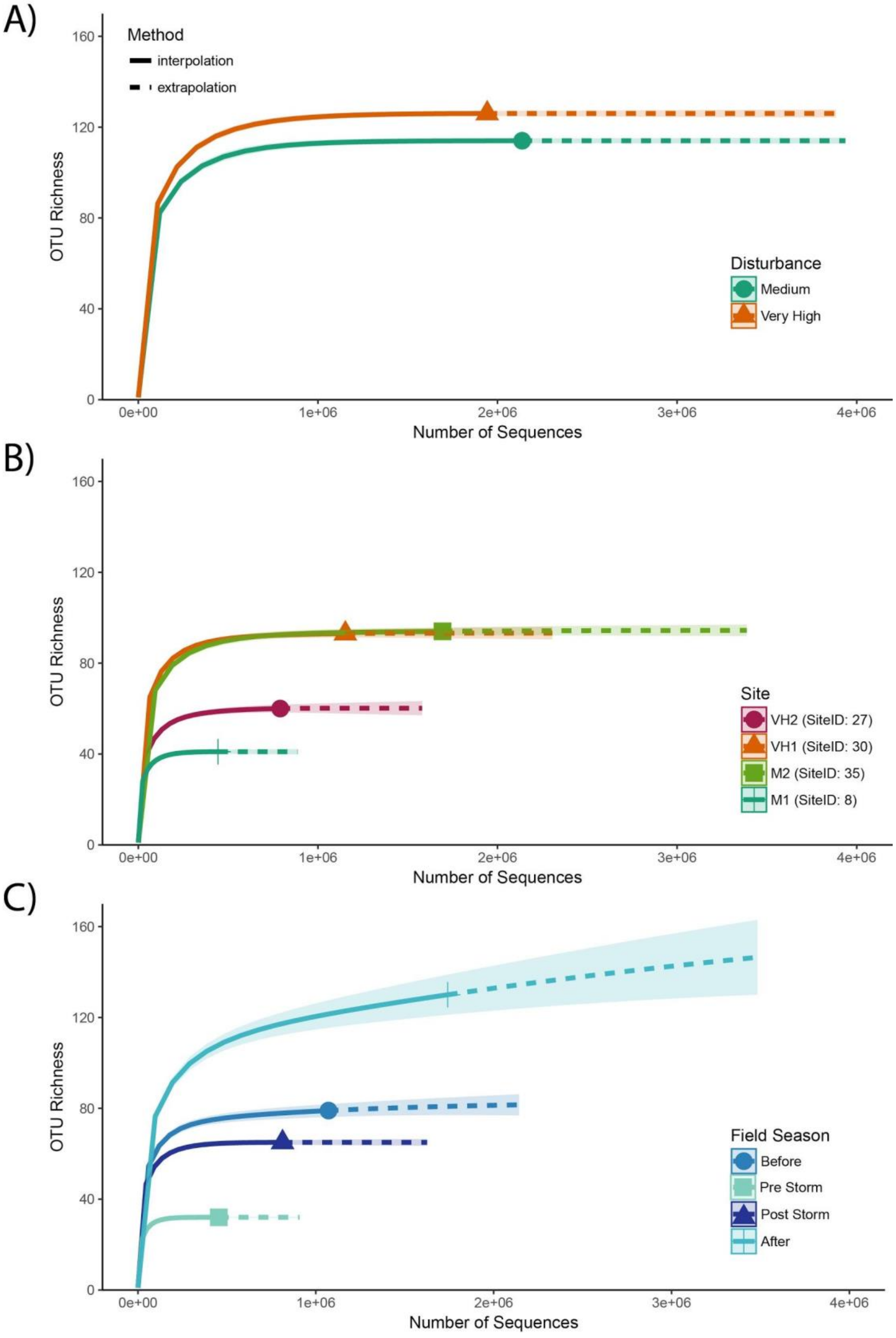
Appendix B. Supplementary Figure 3. F) *Porites lobata*



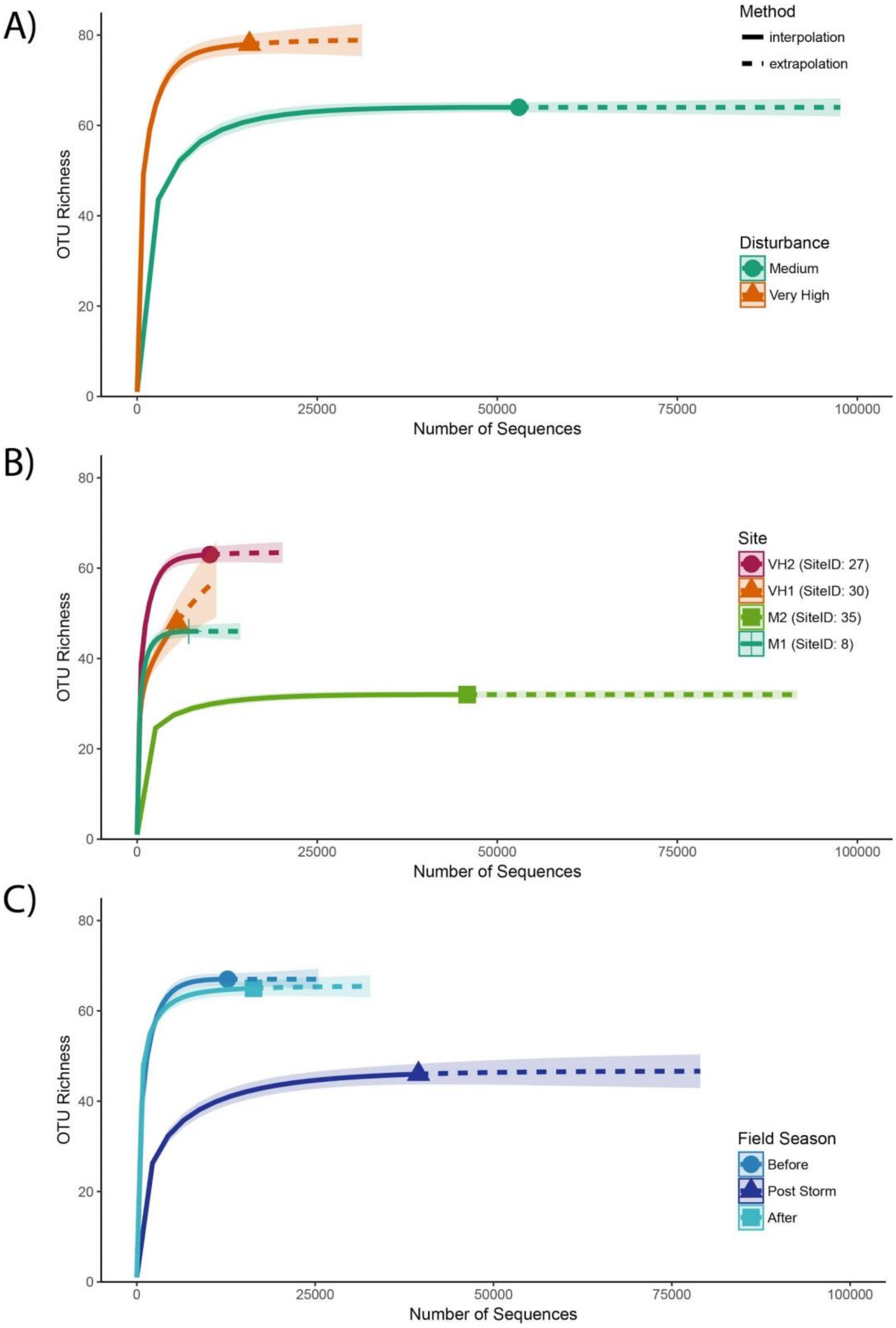
IV. Species (OTU) Accumulation Curves

Species (OTU) accumulation curves were generated using the package iNEXT (Chao et al. 2014). Three curves were generated for each compartment (coral, sediment, water): by disturbance level, by site, and by field season (Appendix B Figs. S4, S5, S6). Curves generally reached an asymptote at the sampling depth (with the exception of sediment at site 30), indicating that sequencing depth was sufficient to capture the full *Symbiodinium* community at these aggregated levels.

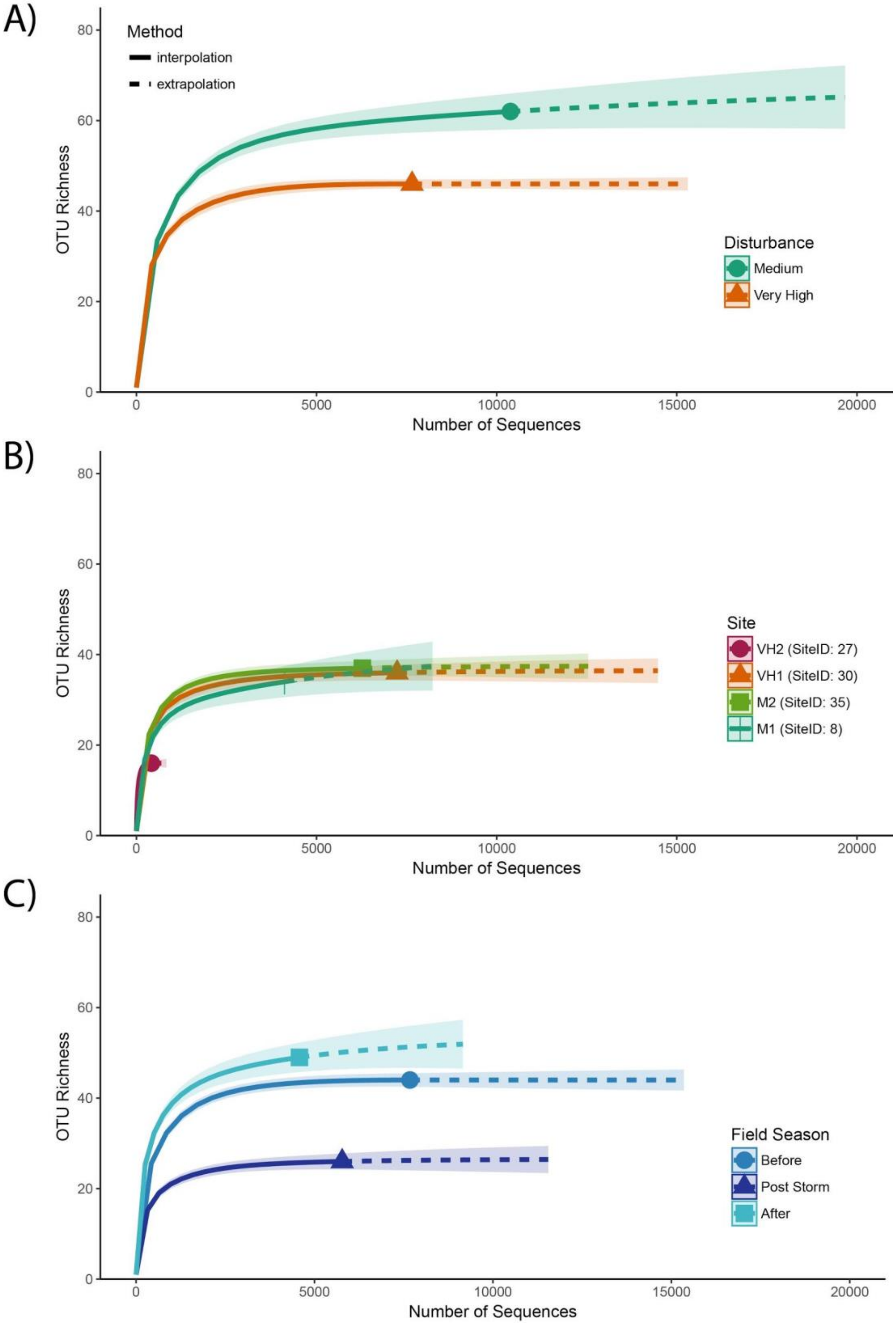
Appendix B. Supplementary Figure 4. Interpolation-extrapolation plots for coral A) by disturbance level; B) by site; C) by field season



Appendix B. Supplementary Figure 5. Sediment A) by disturbance level; B) by site; C) by field season

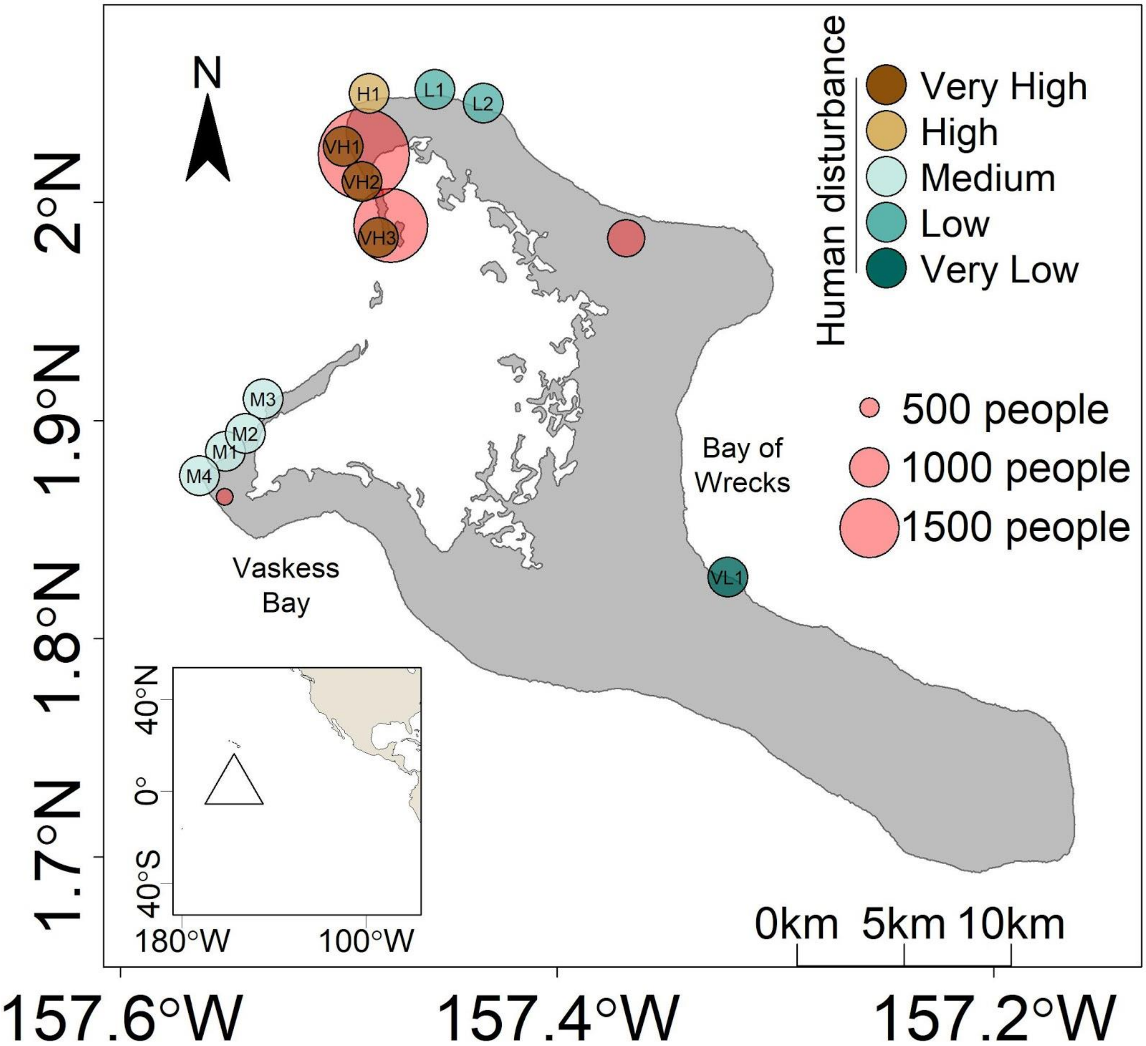


Appendix B. Supplementary Figure 6. Water A) by disturbance level; B) by site; C) by field season

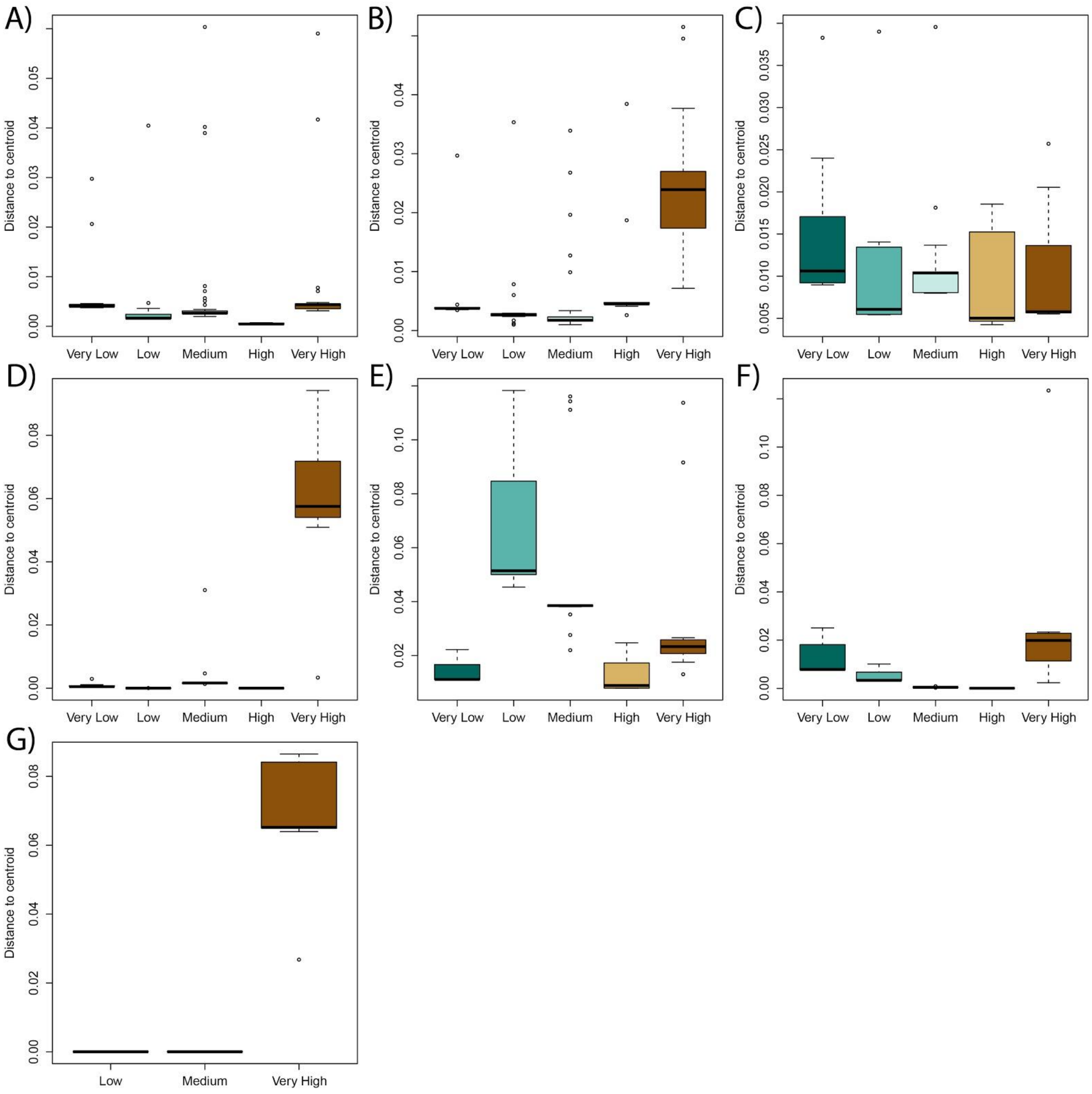


Appendix C
Chapter 6 Supplementary Material

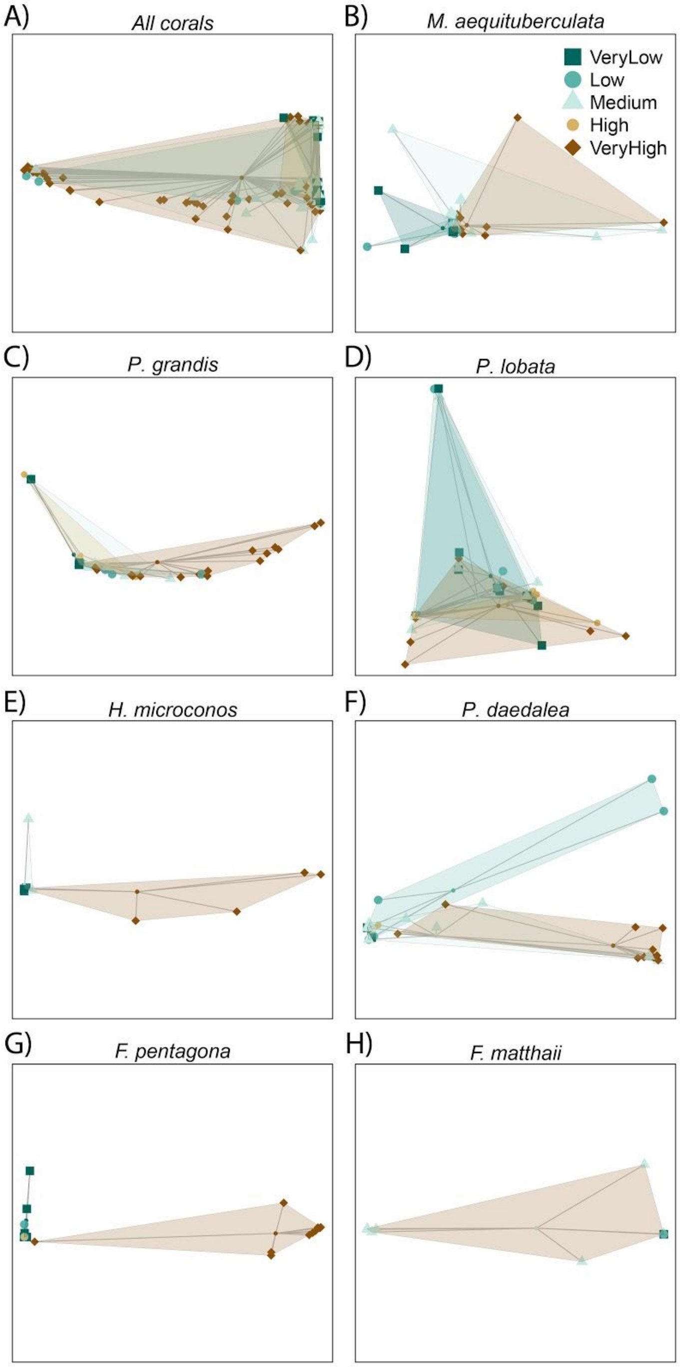
Appendix C. Figure S1. Study sites (n = 11) across five levels of human disturbance on Kiritimati. Villages are denoted with red circles, scaled by population size.



Appendix C. Figure S2. Beta diversity variation (i.e. distance to centroid) of Symbiodiniaceae communities in individual coral species across the disturbance gradient. A) *M. aequituberculata*, B) *P. grandis,* C) *P. lobata*, D) *H. microconos,* E) *P. daedalea*, F) *F. pentagona*, and G) *F. matthaii*.

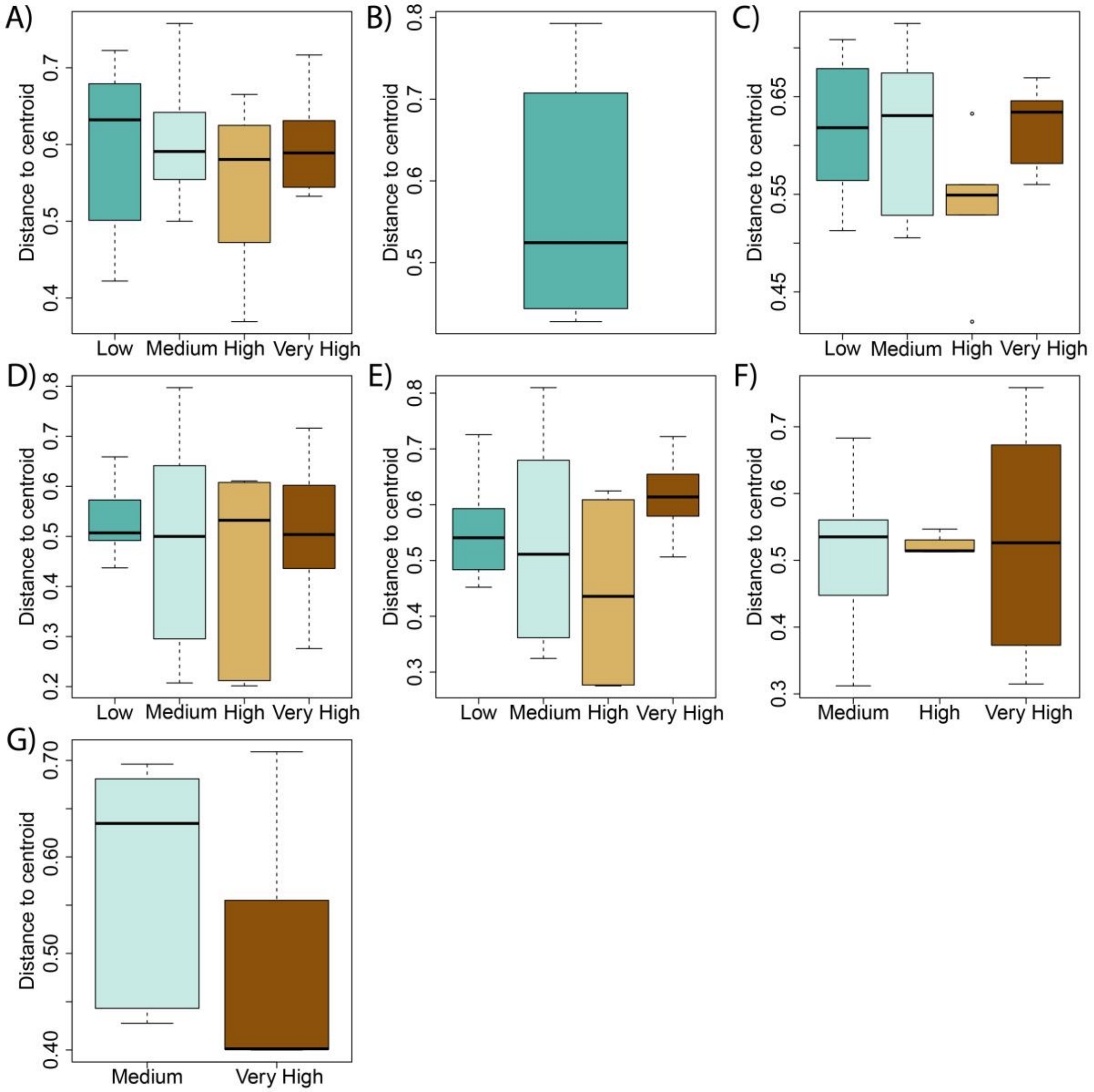


Appendix C. Figure S3. Multivariate ordination (PCoA) of Symbiodiniaceae communities associated with each coral species, showing turnover (differences in multivariate location) and variation (multivariate dispersion). Points indicate individual samples (connected to the centroid point in the center), color indicates sampling time point, and shaded areas indicate boundaries of observed community structure

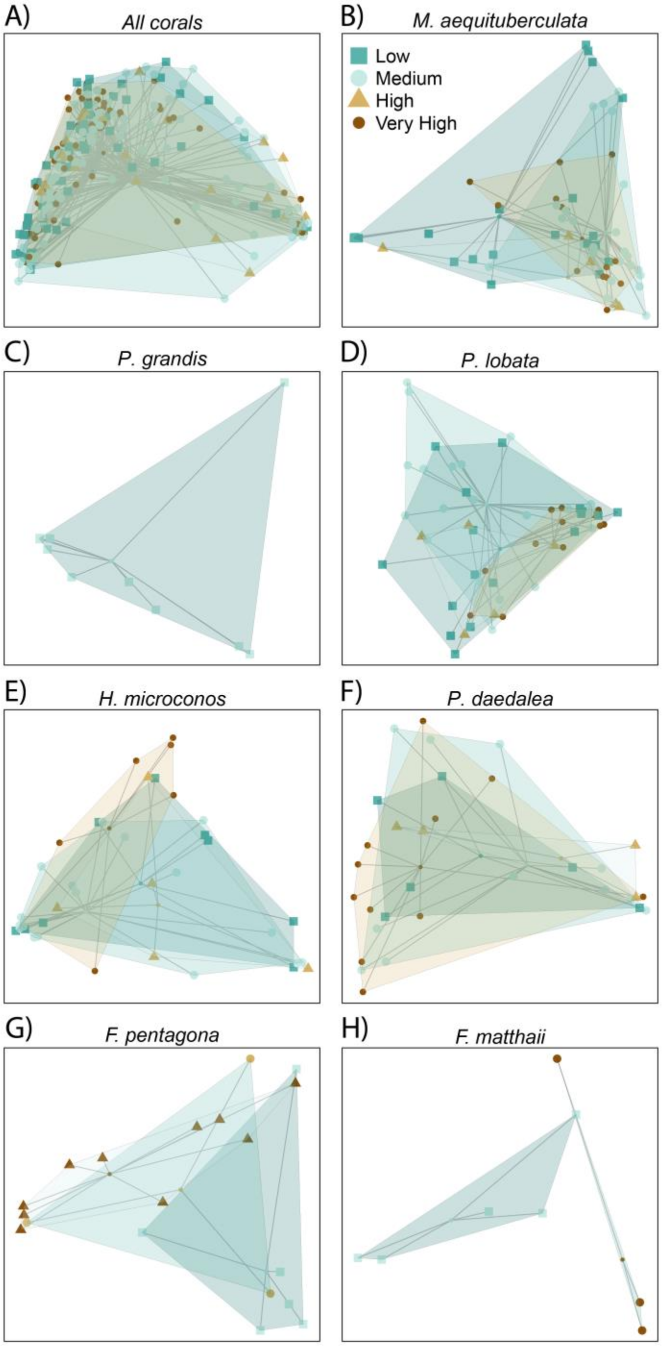


.

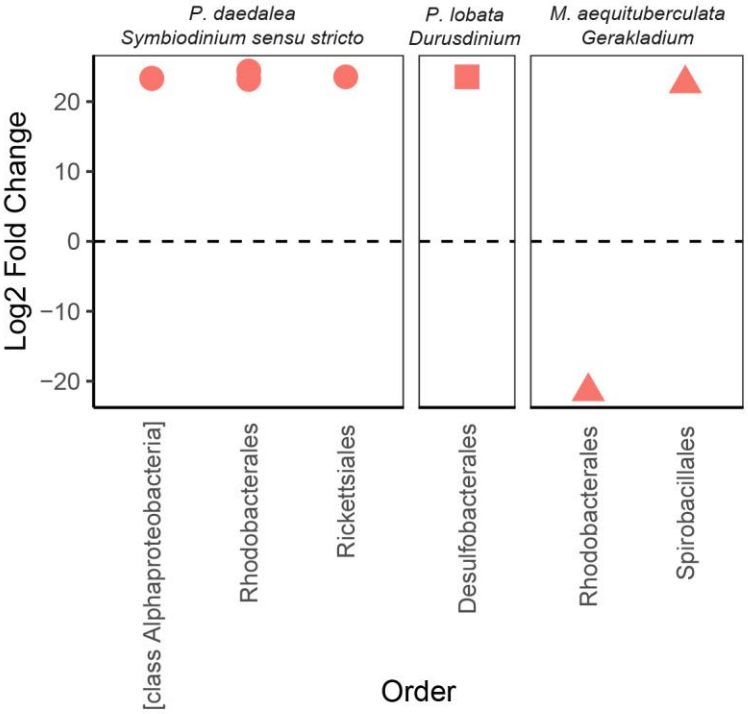
Appendix C. Figure S4. Beta diversity variation (i.e. distance to centroid) of microbe communities. A) *M. aequituberculata*, B) *P. grandis*, C) *P. lobata*, D) *H. microconos*, E) *P. daedalea*, F) *F. pentagona*, and G) *F. matthaii*.



Appendix C. Figure S5. Multivariate ordination (PCoA) of microbe communities associated with each coral species, showing community structure changes (differences in multivariate location) and variation (multivariate dispersion). Points indicate individual samples (connected to the centroid point in the center), color indicates sampling time point, and shaded areas indicate boundaries of observed community structure.



Appendix C. Figure S6. DESeq coral species-specific differential abundance. Each column represents a different coral species/Symbiodiniaceae combination. For example, the first column is differential abundance of bacterial taxa associated with the presence of *Symbiodinium sensu stricto* (previously *Symbiodinium* clade A) within *Platygyra daedalea* colonies.



Appendix C. Table S1. Symbiodiniaceae sample size by coral species (ordered from most to least available samples). Coral life history strategies (Stress tolerant - *Porites lobata*, *Hydnophora microconos*, *Platygyra daedalea*, *Favites pentagona*; Competitive - *Pocillopora eydouxi*; Generalist - *Montipora aequituberculata*) (Darling et al. 2012).

	SITE											
Coral Species	**VH1**	**VH2**	**VH3**	**H1**	**M1**	**M2**	**M3**	**M4**	**L1**	**L2**	**VL1**	**All**
M. aequituberculata	10	13	5	12	11	12	12	13	12	12	12	124
P. grandis	12	10	5	9	12	11	12	12	12	12	9	116
P. lobata	10	11	4	7	8	11	9	10	9	11	11	101
H. microconos	4	3	1	5	5	4	7	4	5	4	7	49
P. daedalea	3	7	2	4	5	1	5	5	6	1	3	42
F. pentagona	3	4	3	2	2	1	2	2	1	3	6	29
F. matthaii	5	3	2	0	2	2	2	0	1	1	0	18
Total	47	51	22	39	45	42	49	46	46	44	48	479

Appendix C. Table S2. Microbiome (bacteria) sample size by coral species (ordered from most to least available samples).

	SITE									
Coral Species	**VH1**	**VH2**	**H1**	**M1**	**M2**	**M3**	**M4**	**L1**	**L2**	**All**
M. aequituberculata	5	10	5	7	6	5	8	12	9	67
P. lobata	4	8	5	5	6	5	6	10	7	56
H. microconos	4	3	5	4	4	5	4	5	4	38
P. daedalea	3	8	4	3	1	3	4	5	1	32
F. pentagona	3	7	3	1	1	2	2	1	3	23
P. grandis	0	0	0	7	3	0	0	1	4	15
F. matthaii	1	2	1	2	1	1	1	1	0	10
Total	20	38	23	29	22	21	25	35	28	241

Appendix C. Table S3. Sample size by coral species for coral colonies where sequence data is available for both the microbiome and Symbiodiniaceae (ordered from most to least available samples). This is a subset of samples from Tables S1 and S2.

	SITE									
Coral Species	**VH1**	**VH2**	**H1**	**M1**	**M2**	**M3**	**M4**	**L1**	**L2**	**All**
M. aequituberculata	5	10	5	6	6	5	8	12	9	66
P lobata	4	7	2	2	6	3	4	7	7	42
H. microconos	4	3	5	4	4	5	4	5	4	38
P. daedalea	3	8	4	3	1	3	4	5	1	32
F. pentagona	3	6	2	1	1	2	1	1	3	20
P. grandis	0	0	0	7	3	0	0	1	4	15
F. matthaii	1	2	0	1	1	1	0	1	0	7
Total	20	36	18	24	22	19	21	32	28	220

Appendix C. Table S4. Symbiodiniaceae beta diversity variation (i.e. distance to centroid). Individual coral species by disturbance level that are not included in this table were not significant.

Model	**Model Test**	**Significant Post-hoc Groups**
Disturbance (pooled across coral species)	F = 63, df = 4, p < 0.001	Very High - High; p < 0.001 Very High - Medium; p < 0.001 Very High - Low; p < 0.001 Very High - Very Low; p < 0.001
Species (pooled across disturbance levels)	F = 122, df = 6, p < 0.001	*M. aequituberculata - P. grandis;* p = 0.038 *M. aequituberculata - H. microconos;* p < 0.001 *M. aequituberculata - P. daedalea;* p < 0.001 *M. aequituberculata - F. pentagona;* p < 0.001 *M. aequituberculata - F. matthaii;* p < 0.001 *P. grandis - P. daedalea*; p < 0.001 *P. grandis - F. pentagona;* p < 0.001 *P. grandis - F. matthaii;* p < 0.001 *P. lobata - P. daedalea;* p < 0.001 *P. lobata - F. pentagona;* p <0.001 *P. lobata - F. matthaii;* p < 0.001 *H. microconos - P. daedalea;* p < 0.001 *H. microconos - F. pentagona;* p < 0.001 *H. microconos - F. matthaii*; p <0.001 *P. daedalea - F. matthaii;* p = 0.012
Individual coral species, by disturbance level (only significant models shown)		
P. grandis	F = 27, df = 4, p < 0.001	Very High - High; p = 0.003 Very High - Medium; p < 0.001 Very High - Low; p < 0.001 Very High - Very Low; p < 0.001
F. matthaii	F = 55, df = 3, p < 0.001	Very High - Medium; p < 0.001 Very High - Low; p < 0.001
H. microconos	F = 39, df = 4, p < 0.001	Very High - High; p < 0.001 Very High - Medium; p < 0.001 Very High - Low; p < 0.001 Very High - Very Low; p < 0.001
P. daedalea	F = 3.5, df = 4, p = 0.016	Low - High; p = 0.035

Appendix C. Table S5. Microbe beta diversity variation (i.e. distance to centroid). When coral species were evaluated individually, no species showed a significant difference in variation by disturbance category.

Model	**Model Test**	**Significant Post-hoc Groups**
Pooled models		
Disturbance (pooled across coral species)	F = 5.5, df = 3, p = 0.001	Very High - High; p < 0.001 High – Low; p = 0.007 High – Medium; p = 0.01
Species (pooled across disturbance levels)	F = 7.9, df = 6, p < 0.001	*M. aequituberculata - H. microconos;* p < 0.001 *P. lobata - H. microconos;* p < 0.001 *H. microconos - P. daedalea;* p = 0.011 *H. microconos - F. pentagona;* p = 0.015 *H. microconos - F. matthaii*; p = 0.031

Appendix D
Chapter 7 Supplementary Material

Materials and Methods

Study location

Kiritimati (Christmas Island), Republic of Kiribati, is located in the central equatorial Pacific Ocean (01°52′N 157°24′W), at the center of the Niño 3.4 region (a region which is used to quantify El Niño presence and strength; Bamston et al. 1997). Kiritimati is the world's largest atoll by landmass (388 km^2; 150 km in perimeter), and all fourteen surveyed reefs surrounding the atoll are sloping, fringing reefs with no back reef or significant reef crest formations. During the 2015-2016 El Niño event, Kiritimati experienced ten months of sustained temperature stress (detailed below), causing a mass bleaching and mortality event (Figure 7.1; Harvey 2016).

Kiritimati has a strong gradient of human disturbance around the island, with the majority of the human population residing in two villages on the west side of the atoll (Walsh 2011, Watson et al. 2016) (Figure 7.2). Human use, including subsistence fishing and waste-water runoff, are densely concentrated in this area, while the north, east, and south regions of the atoll are minimally impacted (Walsh 2011, Watson et al. 2016). We quantified local human disturbance at each of our sites by combining (with equal weight) two sources of information: 1) the number of people residing within 2 km of each site, as a measure of localized impacts, based upon the Government of Kiribati's population census data for each village on Kiritimati ("Republic of Kiribati Island Report Series: Kiritimati" 2012, Morate 2016)*;* 2) spatial fishing pressure, quantified through detailed semi-structured interviews conducted with heads of households in each of the atoll's villages in 2013, and represented using a kernel density function (Walsh 2011, Watson et al. 2016). This resulted in five distinct levels of local human disturbance across our study sites (very low, low, medium, high, very high) (Figure 7.2; S3).

Temperature quantification

Corals are sensitive to temperatures warmer than 1°C above their normal summertime maximum mean sea surface temperature (SST), defined as the 'bleaching threshold'. Temperatures above the long-term maximum monthly mean SST + 1°C are considered 'Coral Bleaching HotSpots' (Strong et al. 1997). Degree Heating Week (DHW) is a measure of accumulated thermal stress, defined as the rolling sum of Coral Bleaching HotSpots during the preceding twelve weeks (Wellington et al. 2001). DHW is commonly used as a metric to quantify coral bleaching risk, with bleaching typically occurring at >4°C-weeks, and mass mortality usually occurring at >8°C-weeks (Liu et al. 2006).

In situ loggers

We deployed temperature loggers (SBE 56, Sea-Bird Scientific; ±0.001°C precision) around the atoll at twelve of our fourteen study sites (all between 10-12 m depth; one to three loggers deployed in each disturbance treatment) from 2011-2016, to measure *in situ* thermal stress. We sub-sampled temperature measurements to a consistent 1-hour sampling grid, and then averaged these measurements to determine 'half-weekly' temperatures (similar to NOAA's DHW product, https://coralreefwatch.noaa.gov/satellite/dhw.php, NOAA CRW 2013). Next, we calculated hotspot values, where a hotspot is defined as when (half-weekly temperature) – (baseline temperature) is a positive number; baseline temperature is the long-term maximum monthly mean, 28.14°C. Following NOAA, for degree heating week calculations (DHW; Gleeson & Strong 1995, Liu et al. 2003), the value of a hotspot was included if it was >1°C (values of 0-1°C are discarded), with each of the 24 half-week cumulative hotspot measurements divided by two.

In situ temperature regimes were quite consistent around the atoll (with the exception of two sites subject to very low disturbance, within the Bay of Wrecks, during non-warming conditions) (Fig. S2A). Although these two sites have slightly higher temperatures during non-warming conditions, the corals and *Symbiodinium* at these sites responded similarly to all other sites, with the exception of those in the very high disturbance treatment. Moreover, temperatures were the same for the very high disturbance sites and the remainder of the sites (except for the Bay of Wrecks), but these areas had extremely different coral survivorship and symbionts, confirming that temperature variance is unlikely explain the observed differences among sites.

Given that temperature profiles were fairly similar amongst sites (Fig. S2A), with the above-noted exceptions, we averaged DHW values across sites to create a single island-wide *in situ* DHW metric. Using a threshold of DHW > 0, heat stress would be considered to have extended for one year, from late May 2015 to early May 2016 (Figure 7.1). More conservatively, using the bleaching threshold (SST > (mean monthly maximum + 1°C)), heat stress on Kiritimati extended for approximately ten months, from early May 2015 to mid-February (Figure 7.1).

NOAA 5-km satellite thermal stress

NOAA's 5-km Degree Heating Week satellite product (v3 released in 2017) shows very similar amounts of heat stress as our *in situ* measurements, demonstrating that each disturbance treatment experienced a similar temperature trajectory (Fig S2B). The maximum heat stress (extracted from NOAA's 5-km Coral Reef Watch product; measured as degree heat weeks, DHW) for each disturbance treatment varied by less than one DHW (<4% overall; Table S1). Furthermore, the extremely small thermal differences between areas are such that the sites with marginally lower DHW are the ones with lower coral survival (i.e. the opposite of what one would expect if differences in DHW had influenced coral survivorship).

Coral tagging and sampling

We tagged colonies of *Platygyra daedalea* along a 60 m transect at 10-12 m depth at 14 different fore reef sites spanning 5 local disturbance levels around Kiritimati (Figure 7.2). We tagged and sampled a total of 80 coral colonies. We sampled the tagged coral colonies three times before (August 2014, January/February 2015, April/May 2015), once two months into (July 2015), once near the end of (March 2016), and twice after (November 2016, July 2017) the El Niño warming. Not all sites could be visited during all field seasons, and some site surveys were only partially completed during some field seasons due to inclement weather conditions. We photographed each coral colony at its initial tagging and at each revisit to record colony measurements and bleaching. We sampled corals using a small chisel and stored the small tissue samples extracted in seawater on ice until preservation. After collection, we preserved one portion of each coral tissue sample in guanidinium buffer (50% w/v guanidinium isothiocyanate; 50 mM Tris pH 7.6; 10 µM EDTA; 4.2% w/v sarkosyl; 2.1% v/v-mercaptoethanol) which we stored at 4°C until extraction for sequencing. We froze a second portion of each sample at -20°C in the field, and subsequently stored these samples at -80°C until DNA extraction.

DNA Extraction

For the samples prepared for Illumina MiSeq amplicon sequencing, we performed DNA extraction using a guanidinium-based extraction protocol (Stat et al. 2009, Cunning, Silverstein, et al. 2015) with the modification that the DNA pellet was washed with 70% ethanol three times rather than once. After extraction, we cleaned the DNA using Zymo Genomic DNA Clean and Concentrator$^{\text{TM}}$-25 (Catalog Nos. D4064 & D4065) following the standard protocol (http://www.zymoresearch.com/downloads/dl/file/id/638/d4064i.pdf). We measured DNA using the dsDNA Qubit BR assay. Any samples that had concentrations below detection levels of this assay were quantified using the dsDNA Qubit HS assay. For the samples prepared for qPCR, we placed a portion of the frozen sample in 1% SDS in DNAB and extracted these sub-samples using an organic extraction protocol for qPCR assays (Baker & Cunning 2016).

Quantitative-PCR assays

Measurement of symbiont to host cell ratios

We used quantitative PCR assays targeting specific loci in *P. daedalea*, *Symbiodinium* clade C, and *Symbiodinium* clade D to measure symbiont to host cell ratios (Mieog, van Oppen, et al. 2009). Assays targeting actin loci specific to *Symbiodinium* clades C and D were multiplexed following the methods of (Cunning & Baker 2013) in 10 µL reactions. We designed a new assay targeting the PaxC intron of *P. daedalea* (which is single copy in corals; van Oppen et al. 2000) based on 14 sequences obtained from NCBI Nucleotide database (accession numbers KX026897 - KX026910; (Smith et al. 2017)). Forward and reverse primers (PaxC-F: 5'-GGATACCCGCGTCGACTCT-3'; PaxC-R: 5'-CCCTAAGTTTGCTTTTTATTGTTCCT-3') were designed using Primer Express v3.0 (Applied Biosystems) to amplify a 72 bp region of the PaxC intron. We performed amplification of the coral host target locus in 12.5 µL qPCR reactions containing 900 nM of each primer using SYBR Green Chemistry (Power SYBR Green MasterMix, Applied Biosystems). The amplification efficiency of this assay was measured as 99.27% using a 4-log10 dilution series of *P. daedalea* DNA.

We assayed DNA extracted from each sample in duplicate with the *P. daedalea* PaxC assay and with the multiplexed *Symbiodinium* clades C and D assays on a StepOnePlus qPCR platform (Applied Biosystems). Thermal cycling consisted of initial incubation at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s and annealing/extension at 60°C for 1 min. Cycle threshold (CT) values (with the fluorescence threshold set to 0.01) for each target were adjusted to account for differences in fluorescence intensity among assays based on the amplification of copy number standards (following the methods of Cunning & Baker 2013). We then calculated symbiont to host cell ratios using the formula $2^\wedge(Ct_{(host)} - Ct_{(symbiont)})$, and adjusting to account for 1) differences in target locus copy number (see below), 2) differences in symbiont and host ploidy (Santos & Coffroth 2003), and 3) differences in DNA extraction efficiency (Cunning & Baker 2013). We calculated symbiont to host cell ratios using R code available at github.com/jrcunning/steponeR.

Determination of actin copy number in clade C and D Symbiodinium in P. daedalea

We extracted *Symbiodinium* C and D cells from frozen tissue (3 samples C, 3 samples D), counted the number of *Symbiodinium* cells under a microscope using a hemocytometer, and extracted DNA from 3 replicate aliquots of 20,000 cells from each sample. DNA template equivalent to 2,000 cells from each extraction was then assayed along with copy number standards. We generated copy number standards (e.g., known numbers of copies of each qPCR target locus) by the purification of each amplified target (Wizard SV Gel and PCR Clean-up System, Promega), quantified nucleic acid concentration using a Nanodrop-1000, and converted to number of copies based on the length of the amplicon and a conversion factor of 660 Daltons per base pair. We used a log2 dilution series from 64,000 copies to 2,000 copies of *Symbiodinium* C and D targets as a standard curve to quantify the number of copies present in DNA extracted from known numbers of *Symbiodinium* cells.

High-throughput (MiSeq) amplicon sequencing

ITS-2 Amplicon

We chose the ITS-2 amplicon for high-throughput sequencing because it is currently the standard region used for identification and quantification of *Symbiodinium* taxa. ITS-2 is phylogenetically (LaJeunesse 2001), functionally and ecologically (Cunning et al. 2017) informative. To minimize misinterpretation of intragenomic data due to the multi-copy nature of the ITS-2 marker, we conducted 97% within-sample OTU clustering. Instead of combining all sequences together into one fasta file and clustering together, we cluster each sample independently, and then collapse identical taxa across samples. As described in (Cunning et al. 2017), this approach increases the likelihood of collapsing intragenomic variation within a sample, while maintaining what is more likely to be biologically and ecologically relevant interspecific variation among samples.

Library preparation and sequencing

Library preparation for Illumina MiSeq ITS-2 amplicon sequencing was performed by the HIMB Genetics Core Lab following the Illumina 16S Metagenomic Sequencing Library Preparation (Illumina protocol, Part # 15044223 Rev. B) with the following modifications:

- ITS primers (ITS-forward: 5’-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG AAT TGC AGA ACT CCG TC-3’ and ITS-reverse: 5’-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GCC TCC GCT TAC TTA TAT GCT T-3’ (*41*)) were used instead of the 16S primers.
- PCR 1 annealing temperature was 52°C, PCR 1 was performed in triplicate, and PCR product was pooled prior to bead clean.
- 60 µl of SPRI beads were used for PCR 1 clean up.
- PCR 1 bead clean up elution buffer volume varied depending on the Qubit concentration of initial gDNA. Changes were as follows:

- gDNA concentrations with ≤1 ng/µl were resuspended in 12.5 µl elution buffer;
- gDNA concentrations of 2 – 4 ng/µl were resuspended in 42.5 µl elution buffer;
- gDNA concentrations of 5 ng/µl were resuspended in 52.5 µl elution buffer.

Samples were sequenced on the Illumina MiSeq platform with 2x300 paired-end read chemistry. A total of 142 *P. daedalea* samples were successfully amplified, sequenced, and used in downstream analyses.

Bioinformatics

All code for this bioinformatics pipeline is available on GitHub (https://github.com/daniclaar/KI_Platy). We conducted quality filtering of raw Mi-Seq sequence reads (in fastq format) first using the iu-filter-quality-bokulich script implemented in Illumina-Utils (Bokulich et al. 2013, Eren et al. 2013), followed by paired-end sequence merging via the iu-merge-pairs script (also in Illumina-Utils (Eren et al. 2013), with a maximum mismatch of three bases between the forward and reverse reads. After quality filtering, we performed sequence processing and identification following all specifications of (Cunning et al. 2017); chimeric sequences were removed, primers were trimmed, sequences from each sample were clustered independently at 97% similarity using UCLUST (Edgar 2010) implemented in QIIME (Caporaso et al. 2010) and resulting OTUs were collapsed at 100% identity across samples, sequences were aligned using the Needleman-Wunsch global alignment algorithm (Biostrings package, Pagès et al. 2017) in R (R Development Core Team 2008), and *Symbiodinium* sequences were named using a reference database (Cunning et al. 2015, Cunning et al. 2017);

reference database is archived, along with full bioinformatic pipeline at https://github.com/daniclaar/KI_Platy).

We used the phyloseq package (McMurdie & Holmes 2013) in R to store and analyze OTU tables, taxonomic information, and sample metadata. We filtered the phyloseq object to remove OTUs observed <5 times in the entire data set (n = 33 OTUs removed and n = 114 kept). We further filtered the phyloseq object to remove samples with very low sequence abundances due to amplification issues (<200 sequences).

Next, we built a *Symbiodinium* phylogenetic tree by aligning ITS-2 sequences from each clade separately (align_seqs.py from QIIME (Caporaso et al. 2010) using muscle (Edgar 2004)). After sequences were aligned within each clade, we created a distance matrix encompassing all sequences using nr28s-rDNA distances (divergence of the D1–D3 region of the 28S; Pochon & Gates 2010, Putnam et al. 2012) to describe between-clade distances. Using upgma (R package phangorn v.2.2.0; Schliep 2011), we created a phylogenetic tree which we imported into the phyloseq object before statistical analysis.

Statistical analysis

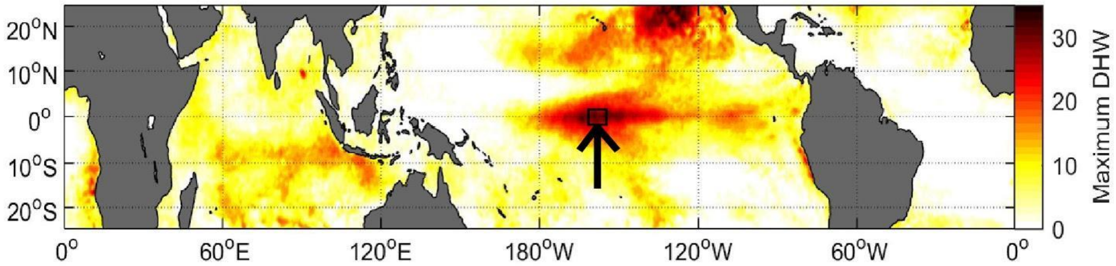
All code for analyses is available on GitHub (https://github.com/daniclaar/KI_Platy).

Constrained ordination

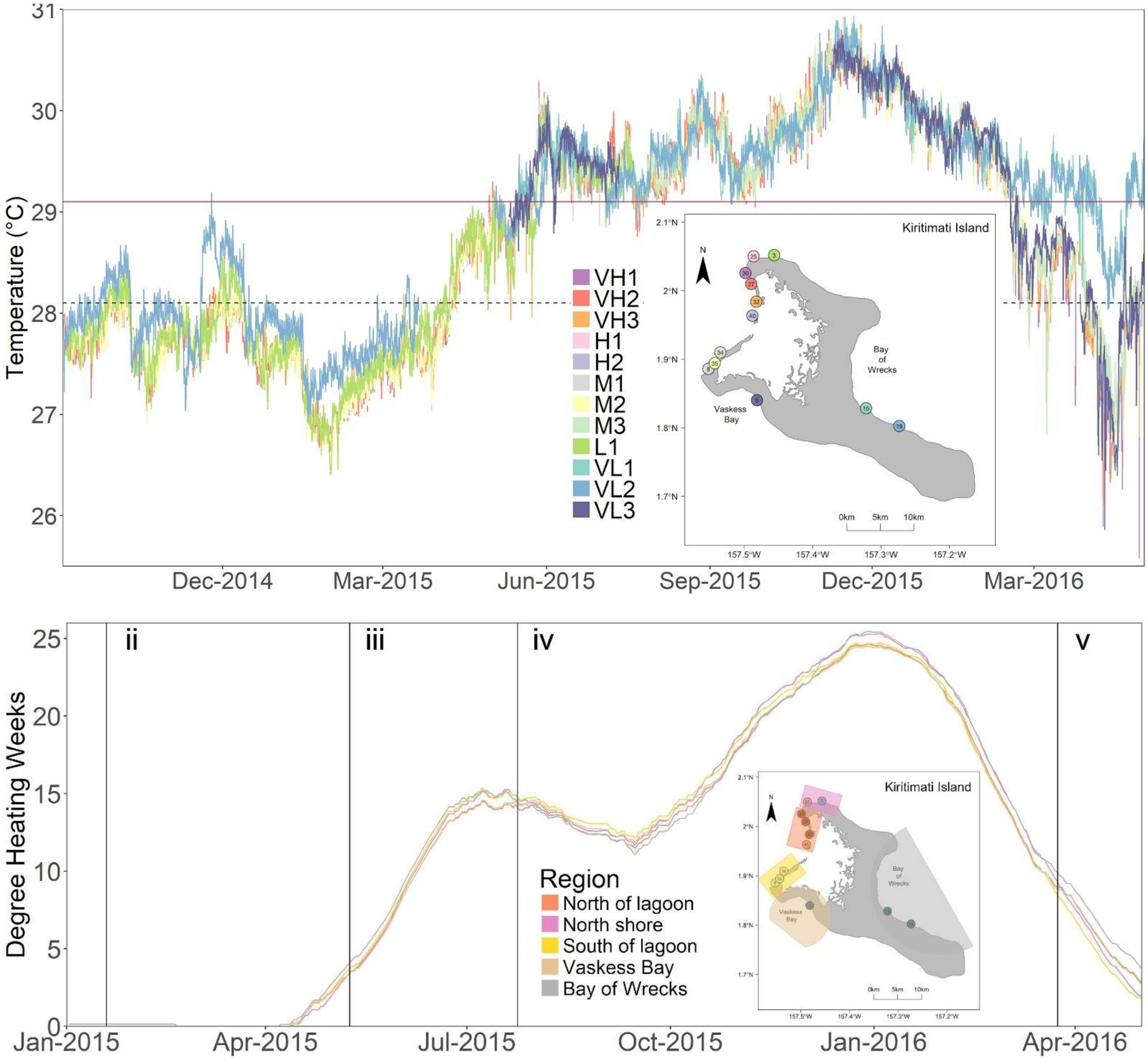
To assess the factors driving differences among *Symbiodinium* communities, we performed a canonical analysis of principal coordinates (CAP) using the MiSeq sequence data. CAP is a constrained ordination method which allows for direct comparison of environmental variables and changes in *Symbiodinium* community composition by constraining ordination axes to linear combinations of the environmental variables. After exhausting all potential constrained axes, residual variability is addressed by fitting additional unconstrained axes (which represent linear variability which is caused by factors not included in the constrained axes). We conducted ordination with the function ‘ordinate’ (phyloseq; McMurdie & Holmes 2013), using weighted unifrac distances (Lozupone et al. 2007), and included field season (time point during which each coral was collected), status (whether the coral survived the bleaching event or died), and local human disturbance level (very high, high, medium, low, and very low). The constrained ordination was visualized (Figure 7.2B), and points from low sites lie directly under visible points from very low and medium sites (*Symbiodinium* communities are so similar that they are not distinguishable from one another). After ordination, we conducted an ANOVA-like permutation test to determine if the defined model was significant (anova.cca, vegan package; Oksanen 2017). We confirmed these results using an automatic stepwise model building tool to build and evaluate the significance of constrained axes using permutation p values (ordistep tool, vegan package). We also computed the variance inflation factors (vif.cca, vegan package) to test for redundant constraints, or for multicollinearity between factor levels. All variance inflation factors were small (< 1.5), so multicollinearity is unlikely to influence our results.

Potential confounding effects

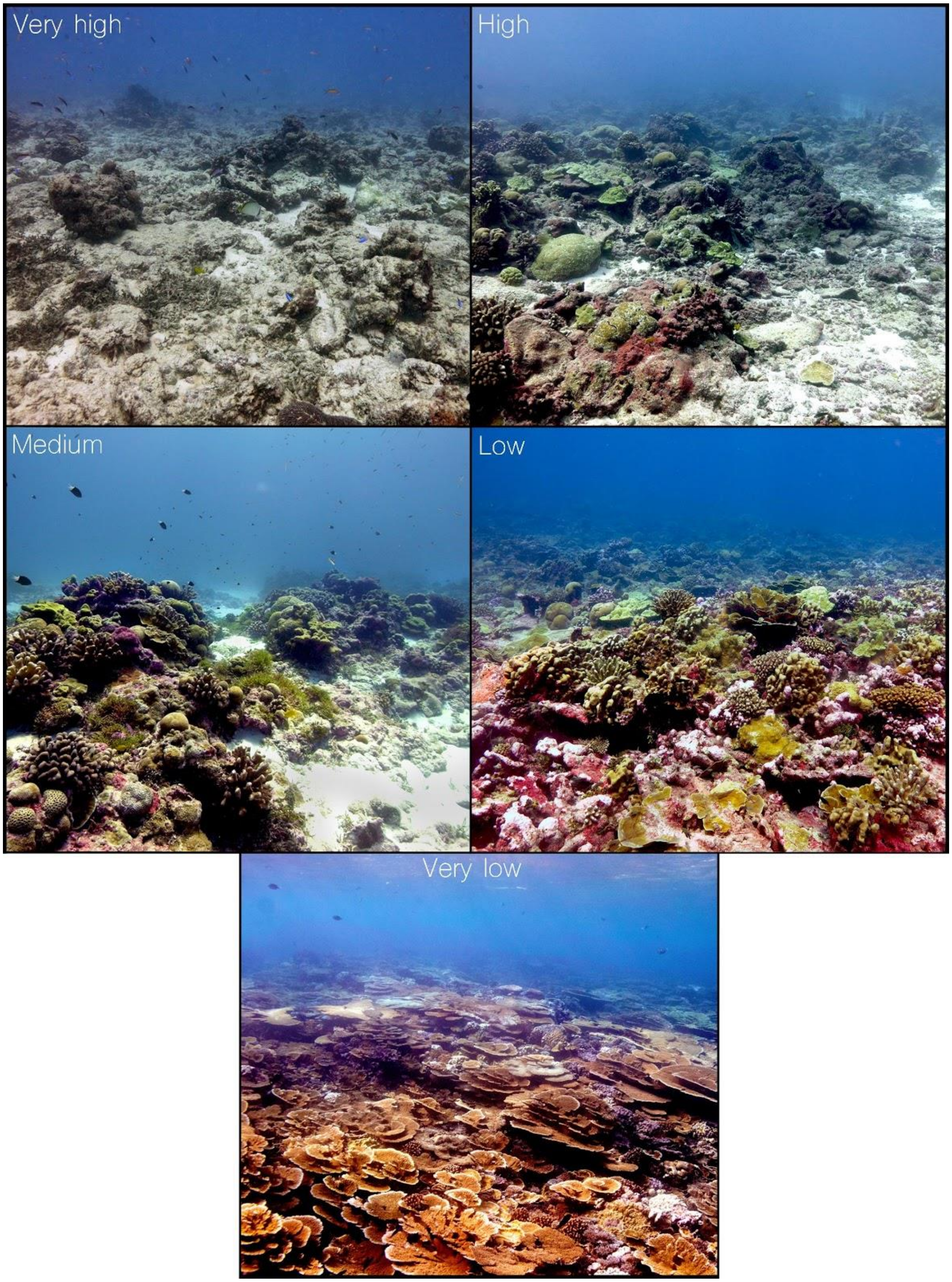
Study sites on different parts of the atoll can experience different exposures and weather conditions. Two lines of evidence indicate, however, that these differences cannot explain the observed patterns of coral survival and *Symbiodinium* community structure. First, despite physical differences amongst fore reefs at the southern edge of the lagoon (medium disturbance), the north shore (high and low disturbance sites), Vaskess Bay (very low disturbance), and the Bay of Wrecks (very low disturbance), coral survivorship and symbiont communities were consistent across all of these areas. We view this as a strength of our study in that, if we had simply compared sites with distinct disturbance levels in two different areas then our results might have been confounded with these environmental variables. Instead, we are able to show that results were consistent across several different reef areas. Second, oceanographic conditions are virtually the same between the fore reefs sites at the northern edge of the lagoon (i.e. the three sites with very high disturbance and one with high disturbance) and the fore reef sites at the southern edge of the lagoon (i.e. the four sites with medium disturbance) (Figure 7.2), but corals at these sites had different symbiont communities and survivorship - the only appreciable difference between these sites is human disturbance.



Appendix D. **_Figure S1._** Global heat stress on coral reefs during the 2015-2016 El Niño event. Color indicates maximum heat stress (Degree Heating Weeks, DHW) measured between May 2015 and June 2016. Black arrow and box indicate Kiritimati's location, at the epicenter of the heat stress during this event.

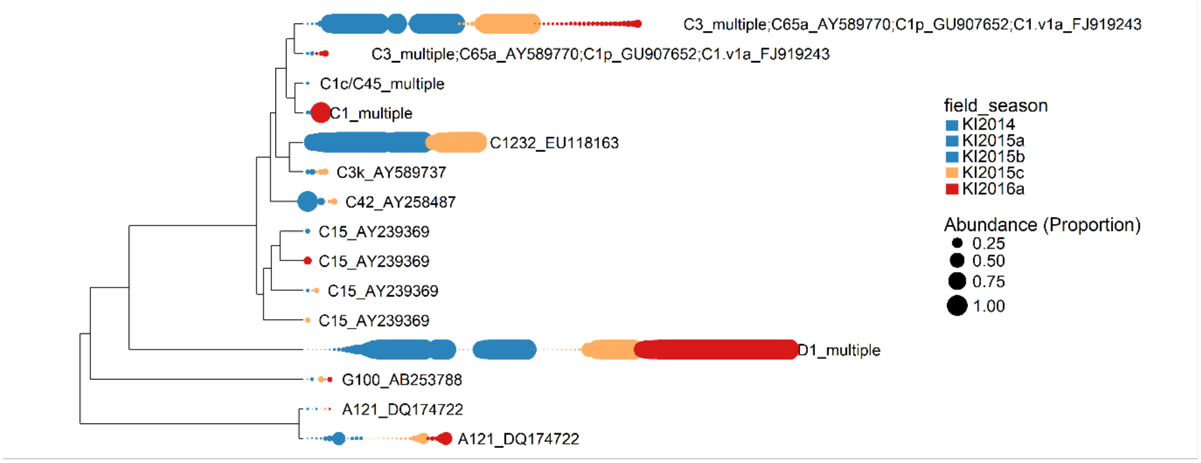


Appendix D. Figure S2. Temperature on Kiritimati throughout the 2015-2016 El Niño event. (A) *In situ* temperature at each site, measured using Sea-Bird Electronics SBE-56 loggers. Dashed horizontal line corresponds to the maximum monthly mean (MMM), and solid maroon line corresponds to the bleaching threshold (MMM +1°C). (B) NOAA's Daily 5-km Satellite Coral Bleaching Heat Stress Monitoring Product (v3) for each region around the atoll. Vertical lines indicate the timing of our expeditions.

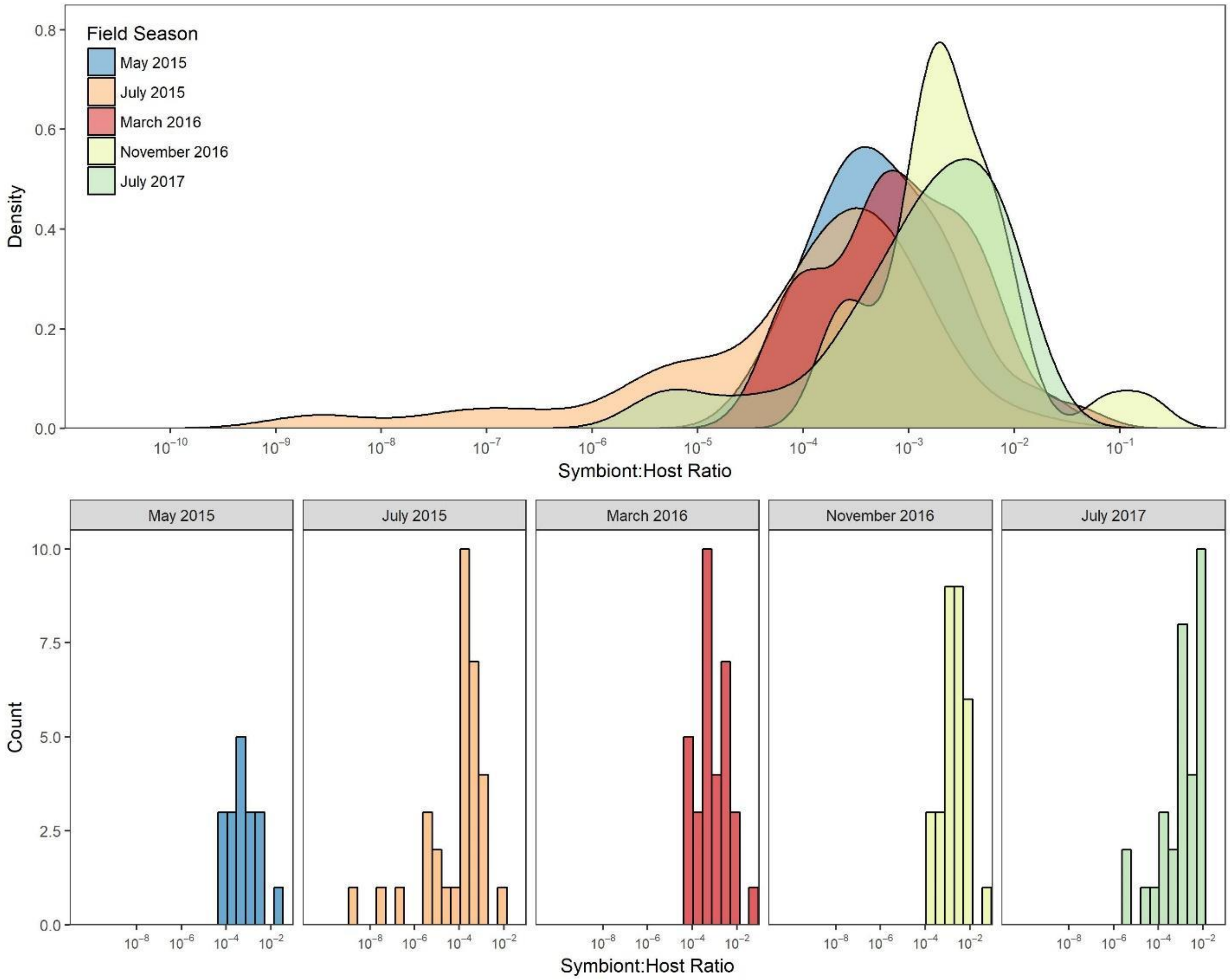


Appendix D. Figure S3. Human disturbance gradient on Kiritimati. Photographs from before the 2015-2016 El Niño, at sites on Kiritimati representing each of the six different levels of local human disturbance.

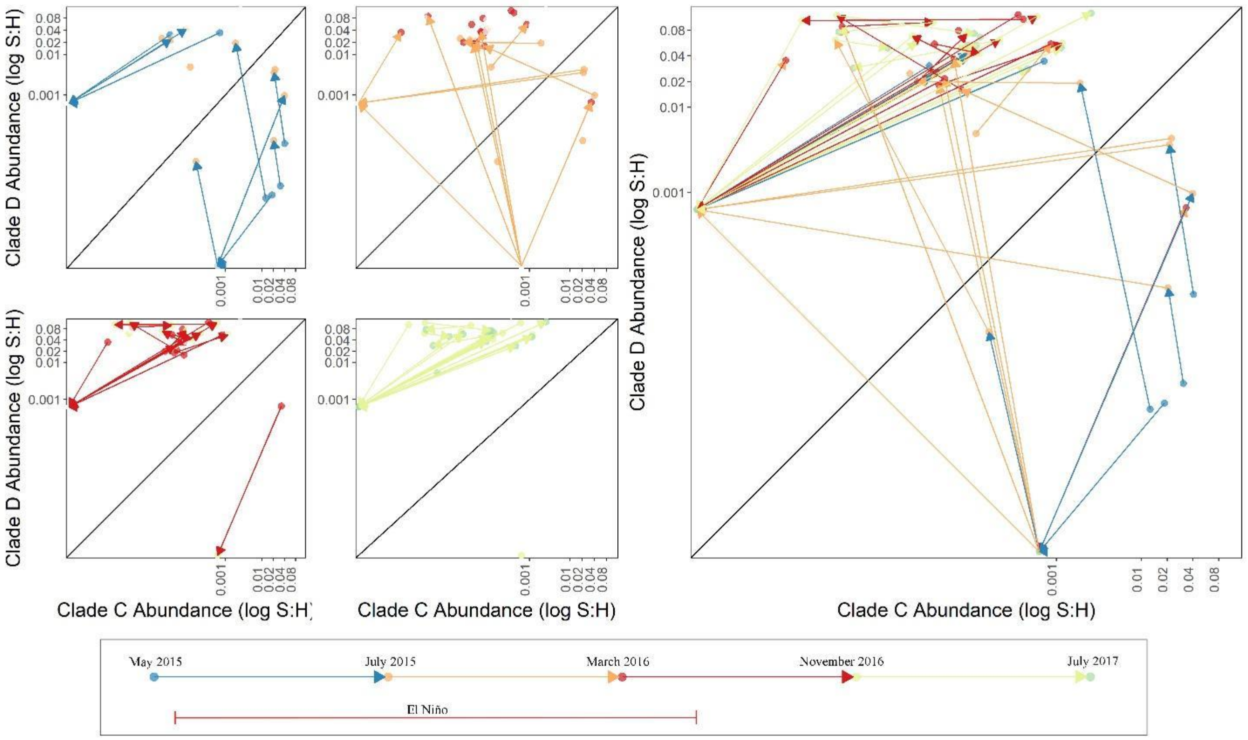
Appendix D. Fig. S4. Transition of individual tagged coral colonies of three different species on Kiritimati from bleached to recovered during the 2015-2016 El Niño event. Photographs of **i-iii.** *Platygyra ryukyuensis*, **iv-vi.** *Favites pentagona*, **vii-ix.** *Favia matthaii* (*Dipsastrea matthaii*) taken two months into the heat stress (July 2015, left column), at the end of the heat stress (March 2016, right column), and after the heat stress (*iii* and *vi* November 2016, *ix* July 2017). Partial visual recovery was observed before the conclusion of the heat stress event in each species, followed by an apparently healthy trajectory (*Platygyra ryukuensis*), partial recovery and persistence (*Favites pentagona),* and partial persistence and extensive tissue mortality (*Dipsastrea matthaii*, note that this colony had small patches of living tissue on the lower sides of the colony).



Appendix D. Figure S5. Phylogenetic tree of ITS2 sequence BLAST hits for all *Platygyra daedalea* samples. OTUs were included if there were at least 100 sequences for that OTU across the entire data set, and samples were included if there were at least 100 sequences within a single sample. Color indicates which field season the sample was collected in, and abundance represents proportional sequence abundance within each sample. Branch tip labels are NCBI BLAST hits with accession numbers based on our reference database, available at https://github.com/daniclaar/KI_Platy.



Appendix D. Figure S6. Total symbiont-to-host cell ratio (S:H) for all *Platygyra daedalea* samples colored by field season. Top: Density plot by field season, showing changes in the distribution of S:H throughout the El Niño event. There is a longer tail to the left during July 2015, with lower S:H during peak bleaching. Bottom: Histograms of S:H cell ratio by field season, showing the same trend.



Appendix D. Figure S7. Trajectories of the *Symbiodinium* communities of all individual *Platygyra* coral colonies through the 2015-2016 El Nino event. Left panels: *Symbiodinium* community changes between single time points (blue arrows, May to July 2015; orange arrows, July 2015 to March 2016; red arrows, March to November 2016; green arrows, November 2016 to July 2017) showing the transition from clade C dominated communities to clade D dominated communities during the El Niño (first two time points), and the stability of clade D dominated communities after the El Niño (last two time points). Right panel: *Symbiodinium* community change for all time periods combined.

Appendix D. Table S1. Similarity in thermal stress (degree heating weeks, DHW) across Kiritimati's disturbance levels. Maximum DHW is the maximum thermal stress reached on Kiritimati during the 2015-2016 El Niño event, measured using NOAA's Daily 5-km Satellite Coral Bleaching Heat Stress Monitoring Product (v3). Difference from maximum DHW is calculated based on the percentage difference between the highest DHW (25.45) and all other DHW measurements.

Disturbance Level	**Maximum DHW**	**Difference from Maximum DHW**
Very High	24.49	-3.8%
High	24.62	-3.3%
Medium	24.71	-2.9%
Low	25.32	-0.5%
Very Low	25.45	--

Appendix D. Data S1. (separate file)

All unique *Symbiodinium* BLAST hits (.csv format). See file allhits_seqs.csv. Denovo name (created during clustering), and accession number of the closest matching sequence from the reference dataset.

Appendix D. Data S2. (separate file)

All unique *Symbiodinium* BLAST hits (.fasta format). Fasta file (phy97_f_c_platy_unique_denovo_ids.fasta) with sequence information for each denovo sequence cluster.