An investigation of the *Symbiodinium* community structure following two consecutive coral bleaching events at Lord Howe Island, eastern Australia

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An investigation of the *Symbiodinium* community structure following two consecutive coral bleaching events at Lord Howe Island, eastern Australia

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A thesis submitted in fulfilment of the requirements for the degree of

**Masters by Thesis**

SOUTHERN CROSS UNIVERSITY

School of Environment, Science and Engineering

23th of March 2016
To my parents,
for their unconditional love
and unfailing support.
I, **Nadine Boulotte** certify that the work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part, for a degree at this or any other university.

I acknowledge that I have read and understood the University's rules, requirements, procedures and policy relating to my higher degree research award and to my thesis.

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Nadine M. Boulotte

23rd of March 2016
ABSTRACT

Global climate change manifests in various ways in the marine environment, including periodically higher than usual summer sea surface temperatures, resulting in thermal stress that affects marine organisms. Mass coral bleaching events induced by thermal stress and high irradiance represent one of the most serious threats to the survival of coral reefs. Assessment of the potential for coral reefs survival in future warmer oceans requires an accurate assessment of their adaptive capacity, which has been shown to be strongly influenced by the identity of their *Symbiodinium* spp. symbionts.

While many studies have focused on the effects of stressors on tropical reefs, few have studied subtropical locations. Lord Howe Island (LHI), the world’s southernmost coral reef, experienced two consecutive widespread coral bleaching events in 2010 and 2011. This thesis aims to investigate the *Symbiodinium* community structure and dynamics following these consecutive coral bleaching events.

Coral samples from four dominant coral species from the LHI lagoon were collected during and after each bleaching event. Using next-generation sequencing analysis of *Symbiodinium* rDNA ITS2 PCR amplicons, the spatio-temporal dynamics of dominant and rare *Symbiodinium* types were assessed. Results from this study provide evidence consistent with *de novo* acquisition of *Symbiodinium* types from the environment (symbiont switching) by adult colonies of *Pocillopora damicornis* and *Stylophora pistillata* following the two consecutive bleaching events. This finding is particularly important given the maternal mode of *Symbiodinium* transmission in these two species, which generally results in high symbiont specificity.

Further investigations (Chapter 3) show that not all coral species respond equally to the same level of disturbance. For instance, *Symbiodinium* community structure was generally
stable over time within another pocilloporid species *Seriatopora hystrix*, but was dynamic in *Porites heronensis* following the bleaching events. Both species-specific patterns were different from the common pattern observed in the two other pocilloporids.

Moreover, in all species, the deep sequencing analysis revealed an elevated Shannon’s diversity, mainly due to a group of rare *Symbiodinium* types that, whilst being below 1% of relative abundance (“the *Symbiodinium* rare biosphere”); was responsible for more than 80% dissimilarity in the community structure over time. In this context, the *Symbiodinium* rare biosphere may have acted as a reservoir which enabled the shuffling of potentially physiologically better adapted *Symbiodinium* types to the new prevailing conditions. Further investigations are needed to elucidate the ecological or functional role of the *Symbiodinium* rare biosphere in the response of coral to environmental changes.

Overall, the results in this thesis are likely to lead to a paradigm shift in our understanding of the flexibility of the coral-*Symbiodinium* symbiosis and the mechanisms of environmental acclimatisation in corals.
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23 of March 2016
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CHAPTER 1
GENERAL INTRODUCTION
Coral reefs ecosystem and their key foundation species

Coral reefs represent one of the most valuable ecosystems in the world for a wide range of ecological, geological, economic and cultural reasons (Sheppard et al., 2009). Scleractinian reef corals are foundation species and as ecosystem engineers, they created the primary structure of coral reefs that have essential roles in the life cycle of at least hundreds of thousands of marine species as well as other functions and values (Birkeland, 1997; Moberg and Folke, 1999; Hoegh-Guldberg, 1999; Harrison and Booth, 2007). This highly diverse and complex ecosystem also supports many local economies in terms of reef-based tourism revenue (Wells and Hanna, 1992; Wilkinson and Buddemeier, 1994; Bryant et al., 1998; Sheppard et al., 2009), seafood as a primary source of protein (Donner and Potere, 2007), pharmaceuticals (Bryant et al., 1998) and coastal protection (Wells and Hanna, 1992; Sheppard et al., 2009; Veron et al., 2009).

Usually found in warm, clear, shallow waters with low levels of nutrients and calcium carbonate supersaturation (Harrison and Booth, 2007), coral reefs can also be found at higher latitudes where major warm currents flow from the tropics (Spalding et al., 2001). For example, the East Australian Current (EAC) which transports warm oceanic waters from the Coral Sea, enabled the development of the world’s southern-most (31°33 S), fringing coral reef at Lord Howe Island off the east coast of Australia (Harrison and Booth, 2007). The success of coral reef ecosystems is largely due to the mutualistic symbiotic relationship between the reef structure engineers, hermatypic scleractinian corals, and unicellular dinoflagellate algae in the genus Symbiodinium (Baker, 2003). The dinoflagellate eukaryote is not the only endosymbiont involved in the coral holobiont, as thousands of species of Bacteria, Archaea, fungi and viruses are known to interact symbiotically within scleractinian corals (e.g., reviewed in Blackall et al., 2015).
**Symbiodinium, the ubiquitous endosymbiont**

*Symbiodinium* spp. or zooxanthellae are phototrophic, unicellular dinoflagellates that live inside the coral gastrodermal cells (Trench, 1979). *Symbiodinium* are also found in a plethora of host species in both tropical and temperate environments, from Cnidaria, (hard/soft corals, jellyfish, box jellyfish and anemones), to Porifera (sponges), Mollusca (snails and clams) and Foraminifera (Hoegh-Guldberg, 1999). In coral hosts, they are usually arranged in a monolayer (Muscatine and Porter, 1977; Stambler, 2011) and are present at a very high density (more than $10^6$ cell/cm$^2$ of coral surface area, Fig. 1). Temporal dynamic in cell density have been shown to occur in response to seasonal abiotic factors such as temperature and irradiance (Fitt *et al.*, 2000).

![Figure 1: Symbiodinium cells under different magnification. Panel (A) represents Symbiodinium cells (brown spots) visible through the tentacle of a coral polyp from a close-up photograph (Credits: Jean-Baptiste Raina). Panel (B) and (C) are confocal microscopy images of Symbiodinium cells within a coral polyp. Photo credits: Marie Strader (B) and Jeong et al. 2013 (C).](image)

Corals and *Symbiodinium* both gain benefits in the symbiosis via transportation and exchange of nutritional resources (Muscatine, 1990; Yellowlees *et al.*, 2008). Indeed, in this relationship, most hermatypic corals rely on *Symbiodinium* for survival and growth, as up to 95% of the corals’ energy needs are provided by photosynthetically-fixed organic compounds (photosynthate) released by *Symbiodinium* cells (Muscatine, 1990). In addition, the endosymbiont recycles the coral nitrogen waste and translocates these compounds back to the host for use in daily metabolic function such as calcification or respiration (Pearse and Muscatine, 1971). In return, *Symbiodinium* cells receive a safe and nutrient-rich habitat for
optimum photosynthesis and nitrogenous waste compounds generated by the host which supports symbiont growth (Pearse and Muscatine, 1971; Muscatine and Porter, 1977). This successful association underpins the vigorous growth of reef-building corals in oligotrophic waters and enables coral reefs to exist (Stamblar, 2011). However, the disruption of this symbiosis is harmful to the entire ecosystem. It was initially thought that all dinoflagellates involved in symbiotic relationships with corals were a single cosmopolitan species, *Symbiodinium microadriaticum* (Freudenthal, 1962). Evidence from morphology, physiology, biochemistry, and more recently, molecular genetics studies have shown, however, that the genus *Symbiodinium* is highly diverse (reviewed in Baker, 2003). The genus *Symbiodinium* is composed of nine phylogenetic lineages or clades, labelled A to I (Fig. 2; Pochon and Gates, 2010), where each clade is divided into several sub-clades, or types, based on the nuclear internal transcribed spacer (ITS) and the domain V of chloroplast large subunit (cp23S) regions (LaJeunesse, 2001; Rodriguez-Lanetty, 2003; Baker, 2003; van Oppen, Mahiny, *et al.*, 2005). Clade A is the most ancestral *Symbiodinium* lineage, with clades C and H the most derived (Tchernov *et al.*, 2004; Pochon *et al.*, 2006).
Specificity and flexibility in the host-symbiont relationship

The coral-\textit{Symbiodinium} symbiosis can be flexible, while some coral species show specificity to a certain type. Hence, \textit{Symbiodinium} in clades A, B, C, D are mostly associated with scleractinian corals (Baker, 2003), while clade F and G are mainly associated with foraminifera, but occasional associations with stony corals have also been reported (LaJeunesse, 2001; van Oppen, Mieog, \textit{et al.}, 2005; Pochon \textit{et al.}, 2006; Goulet, 2007). \textit{Symbiodinium} in clades H and I are specific to the large, benthic foraminiferan sub-family Soritinae (Pochon and Pawlowski, 2006), and representatives in clade E are free-living strains found in the coral reef environment (Pochon \textit{et al.}, 2006). Biogeographic studies of \textit{Symbiodinium} have shown that clade C and D are more abundant in tropical latitudes while
clades A, B, and occasionally F, are common in subtropical or temperate latitudes (Rodriguez-Lanetty et al., 2000; Loh et al., 2001; van Oppen et al., 2001; Savage et al., 2002; LaJeunesse et al., 2003; LaJeunesse, 2005).

The specificity between the coral host and its symbionts varies considerably (Baker, 2003). Some Symbiodinium clades or types are considered as “generalists” because they are found in a wide range of host taxa and locations; while “specialists” are endemic to a specific location or found in a specific coral species (Baker et al., 1997; LaJeunesse, 2002; Baker, 2003; Pochon and Gates, 2010). Generalist and specialist Symbiodinium types can be found within the different clades. For example, ITS2 types C1 and C3 are two of the main generalist Symbiodinium types identified in clade C, and occur throughout the Caribbean and the Indo-Pacific. “Generalist” or “specialist” also applies to the coral host, as some corals can be found associated with many different Symbiodinium types, whereas others appear to be limited to only one (Fig. 3).

![Figure 3: Conceptual framework for symbiosis specificity.](image)

Figure 3: Conceptual framework for symbiosis specificity. Reprinted from Mieog, 2009 (PhD thesis).

It was previously assumed that specificity was very common among reef-building corals, i.e., most coral species were thought to host a single Symbiodinium type (Baker, 2003; Goulet, 2006, 2007). However, recent studies have demonstrated that flexibility was more
ubiquitous than specificity as the majority of coral species were hosting two or more *Symbiodinium* clades (Silverstein *et al.*, 2012).

**Coral bleaching: the breakdown of coral-*Symbiodinium* associations**

Coral bleaching is considered to be the main climate-induced threat to coral reefs (Coles and Brown, 2003; Hoegh-Guldberg *et al.*, 2007). Bleaching refers to the breakdown of coral-*Symbiodinium* symbiosis, which triggers a periodic loss of the pigmented microalgae from the coral gastrodermal tissues, leaving the white calcium carbonate skeleton visible (Glynn, 1993; Berkelmans, 2002). Evidence from field and experimental studies have shown that a mere 1-2°C above average seawater summer temperatures, over a prolonged period of time can be lethal for the coral host (Hoegh-Guldberg, 1999; Douglas, 2003; Berkelmans *et al.*, 2004; Donner *et al.*, 2005) and this phenomenon is predicted to increase in frequency and magnitude (Hoegh-Guldberg *et al.*, 2007).

Bleaching patterns are heterogeneous across host taxa (Marshall and Baird, 2000; Loya *et al.*, 2001; McClanahan *et al.*, 2004) and the threshold temperature at which bleaching or mortality occurs varies among reef locations, regions and latitudes (Berkelmans, 2002). Moreover, bleaching patterns and thresholds have been shown to be contingent with the taxonomic composition of the *Symbiodininum* community harboured by corals (Baker, 2003; Berkelmans and van Oppen, 2006; Howells *et al.*, 2011; Stat *et al.*, 2013). For instance, corals hosting members of *Symbiodinium* clade D are often more bleaching-resistant than when hosting members of clade C (Glynn *et al.*, 2001; Baker *et al.*, 2004; Fabricius *et al.*, 2004; Stat *et al.*, 2006, 2013); however, the association with particular types in other clades has also been shown to result in higher thermal tolerance in specific environments (LaJeunesse *et al.*, 2003; Sampayo *et al.*, 2008; Hume *et al.*, 2013, 2015).
Coral reefs response to climate change: The Adaptive Bleaching Hypothesis

In early 1990s, the Adaptive Bleaching Hypothesis (ABH) provided a framework for exploring the potential for corals to adapt to climate change by “switching” or “shuffling” their symbionts to stress-resistant *Symbiodinium* types (Buddemeier and Fautin, 1993; Baker, 2001, 2003; Fautin and Buddemeier, 2004). Since then, the ABH concept has been controversial and stimulated rapidly growing research of *Symbiodinium* diversity and community structure in recent decades, providing a basis for understanding acclimatisation and the potential adaptation of corals to climate change (Baker, 2003).

The mechanism of switching represents the acquisition of a novel *Symbiodinium* type from the surrounding environment (i.e. from the water column or sediments), but until now, this mechanism has not been demonstrated in adult scleractinian corals (Jones *et al.*, 2008). Shuffling refers to a relative change of the *Symbiodinium* type abundance already present in the coral tissue and has been documented in response to seasonal environmental variation or environmental stress. Symbiont shuffling has been suggested as one mechanism by which corals may be able to respond rapidly to environmental change (Baker *et al.*, 2004; Buddemeier and Fautin, 1993; Berkelmans and van Oppen, 2006).

Several studies have documented the temporary dominance (months to years) by opportunistic *Symbiodinium* types following bleaching events (Berkelmans and van Oppen, 2006; Baker *et al.*, 2004; Thornhill *et al.*, 2006; Jones *et al.*, 2008; LaJeunesse *et al.*, 2009), in transplantation experiments (Baker, 2001; Berkelmans and van Oppen, 2006), and at regional scales (van Oppen *et al.*, 2001; van Oppen, Mahiny, *et al.*, 2005). Changes in the dominant symbiont type are not a widespread response to bleaching events, and indeed, some studies have shown that not all the thermally stressed coral colonies respond through a shuffling mechanism (Berkelmans and van Oppen, 2006; Thornhill *et al.*, 2006; McGinley *et al.*, 2012).
Genetic studies of *Symbiodinium* diversity and community structure

To investigate *Symbiodinium* diversity and taxonomy, several molecular techniques have been used in past decades: from the analysis of Restriction Fragment Length Polymorphisms (RFLP) in early 1990’s, to Denaturing Gradient Gel Electrophoresis (DGGE) (LaJeunesse and Trench, 2000); Single-Stranded Conformation Polymorphisms (SSCP) (van Oppen *et al.*, 2001); cloning (Apprill and Gates, 2007); direct sequencing (Loh *et al.*, 2001); real-time PCR (qPCR) (Ulstrup and van Oppen, 2003; Mieog *et al.*, 2009); microsatellites (Santos *et al.*, 2003; Bay *et al.*, 2009); and, High-Resolution Melting (HRM) analysis (Granados-Cifuentes and Rodriguez-Lanetty, 2011).

Over the last decades, DGGE of the rDNA Internal Transcribed Spacer (ITS1 and ITS2) region has been the most popular technique used to characterise dominant *Symbiodinium* types in cnidarian hosts (Sampayo *et al.*, 2007). However, the most limiting factor of this methodology is the detection threshold that overlooks *Symbiodinium* types occurring below 10% of relative abundance, which are known as background or rare types (Baker, 2003; Ulstrup and van Oppen, 2003; Berkelmans and van Oppen, 2006; Mieog *et al.*, 2007; Silverstein *et al.*, 2012). Recently, the use of next-generation sequencing (NGS) has enabled a deep coverage of *Symbiodinium* diversity within reef-building corals (Quigley *et al.*, 2014) and represents the most sensitive genotyping method to characterise dominant and rare *Symbiodinium* types.
Research overview: Problem statement and thesis aims

To accurately assess the contribution of *Symbiodinium* types to the adaptive capacity of corals, a comprehensive overview and understanding of their diversity is crucial. Whilst many studies of *Symbiodinium* diversity have focused on tropical reef environments, few have explored subtropical reef environments and only a few published studies (Wicks *et al*., 2010; Noreen *et al*., 2015) have focused on the *Symbiodinium* diversity of Lord Howe Island corals (LHI).

LHI is an isolated island 600 km off the east coast of northern New South Wales (NSW), eastern Australia (Harriott *et al*., 1995). Located at latitude 31.5 °S and longitude 159.0 °E, LHI is the world's southern-most true lagoonal coral reef, which was listed on the UNESCO World Heritage in 1982 and classified as a Marine Park in 2002 (Hutton and Harrison, 2004). Although located over 1000 km from the Great Barrier Reef (GBR), LHI supports unique benthic reef assemblages of tropical, subtropical and temperate marine species; where approximately 100 scleractinian coral species have been reported to occur (Harriott *et al*., 1995; P. Harrison unpubl. data). Like South-Eastern Australia, LHI is considered to be a hotspot for climate change, representing a key region of concern with regard to ocean warming (Ayre and Hughes, 2004; Hobday and Pecl, 2013).

During the 2010 and 2011 austral summers, seawater temperature was 1°C higher than average for nineteen and seven weeks respectively (Fig. 4). Combined with high light penetration and calm seas, up to 95% of the coral community in the LHI lagoon displayed signs of bleaching, representing the first extensive, severe coral bleaching events recorded at LHI (Harrison *et al*., 2011; Dalton and Carroll, 2011).
As yet, temporal field based studies investigating thermal adaptation in the coral-algal symbiosis prior, during and following natural bleaching events are lacking. Moreover, no studies have documented these mechanisms following consecutive annual coral bleaching events. My aim in this thesis was to investigate *Symbiodinium* diversity and changes within their community structure (i.e. symbiont shuffling and switching) during and following two consecutive bleaching events at LHI. Using next-generation sequencing and a time series dataset, this study examines how the *Symbiodinium* community responded to consecutive bleaching events within four dominant coral species (*Pocillopora damicornis*, *Stylophora pistillata*, *Seriatopora hystrix* and *Porites heronensis*) collected from two separate locations within the LHI lagoon.

This thesis includes a peer-reviewed publication and a completed manuscript ready for submission. As a result, some repetitions may occur in the Introduction and Methods sections in the different chapters. Each chapter is outlined below:
Chapter Two is a reprint of an article accepted for publication in the ISME journal. It explores and documents the highly diverse “Symbiodinium rare biosphere” within *P. damicornis* and *S. pistillata*; and provides the first evidence for symbiont switching in both species following severe bleaching events.

Chapter Three describes the *Symbiodinium* community dynamics within the two other dominant coral species in the LHI lagoon *S. hystrix* and *P. heronensis* and compares their pattern to the two pocilloporids discussed in Chapter Two. The results highlight that not all species respond equally to the same level of disturbance and that the *Symbiodinium* rare biosphere can exhibit species-specific patterns.

Finally, Chapter Four provides a brief synthesis of the key findings from this research and discusses future research recommended to further assess the contribution of *Symbiodinium* types towards the adaptive capacity of reef corals.
CHAPTER 2:

EXPLORING THE *SYMBIODINIUM* RARE BIOSPHERE PROVIDES EVIDENCE FOR SYMBIONT SWITCHING IN REEF-BUILDING CORALS
Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals

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ABSTRACT

Reef-building corals possess a range of acclimatisation and adaptation mechanisms to respond to sea-water temperature increases. In some corals, thermal tolerance increases through community composition changes of their dinoflagellate endosymbionts (Symbiodinium spp.), but this mechanism is believed to be limited to the Symbiodinium types already present in the coral tissue acquired during early life-stages. Compelling evidence for symbiont switching, i.e., the acquisition of novel Symbiodinium types from the environment, by adult coral colonies, is currently lacking.

Using deep sequencing analysis of Symbiodinium rDNA ITS2 PCR amplicons from two pocilloporid coral species, we show evidence consistent with de novo acquisition of Symbiodinium types from the environment by adult corals following two consecutive bleaching events. Most of these newly detected symbionts remained in the rare biosphere (background types occurring below 1% relative abundance), but one novel type reached a relative abundance of ~33%. Two de novo acquired Symbiodinium types belong to the thermally resistant clade D, suggesting that this switching may have been driven by consecutive thermal bleaching events.

Our results are particularly important given the maternal mode of Symbiodinium transmission in the study species, which generally results in high symbiont specificity. These findings will cause a paradigm shift in our understanding of coral-Symbiodinium symbiosis flexibility and mechanisms of environmental acclimatisation in corals.
INTRODUCTION

The eukaryotic and prokaryotic microbial communities (i.e., the microbiome) associated with animals and plants play essential roles in their health and functioning (McFall-Ngai et al., 2013). Reef-building corals form symbioses with a wide range of microbial symbionts, including phototrophic dinoflagellates in the genus Symbiodinium. As the coral host depends on photosynthate for nutrition, a prolonged breakdown of the symbiosis (referred to as coral bleaching) often leads to coral death (Hoegh-Guldberg et al., 1997; Baker, 2003). Episodes of mass coral bleaching have increased in frequency and intensity due to climate change and have caused a substantial loss in coral cover in many coral reef regions over the last few decades (Hoegh-Guldberg, 1999; Hoegh-Guldberg et al., 2007; De’ath et al., 2012).

The role of Symbiodinium symbionts in acclimatisation of the coral holobiont to environmental changes has been extensively covered in the recent literature (Blackall et al., 2015). The genus Symbiodinium is classified into nine phylogenetic clades (A through I) based on DNA sequence analysis, with a range of different types (putative species) within each clade (Pochon and Gates, 2010). Symbiodinium types can be transmitted directly from parent to offspring via eggs (vertical transmission) or aposymbiotic larvae/early recruits can acquire their symbionts from the environment (horizontal transmission) (Harrison and Wallace, 1990; van Oppen, 2001; Padilla-Gamino et al., 2012). Different Symbiodinium types have distinct physiological optima and stress tolerance levels, which confer different phenotypes to their coral hosts. For instance, corals dominated by Symbiodinium clade D are generally more thermally tolerant compared to those predominantly associating with types in other clades (Berkelmans and van Oppen, 2006).

More than one Symbiodinium type can exist simultaneously within a single coral host (Mieog et al., 2007; Correa et al., 2009; Silverstein et al., 2012); these can occur in high abundance as ‘dominant types’ or in very low abundance known as ‘background types’, i.e.,
the ‘Symbiodinium rare biosphere’. In other microbial ecosystems, the rare biosphere represents a low-abundance, high-diversity group (in terms of numbers of Operational Taxonomic Units [OTUs]) representing less than 1% of relative abundance (Sogin et al., 2006; Reid and Buckley, 2009). Therefore, in the present study, all Symbiodinium types that occurred below this threshold were considered members of the ‘Symbiodinium rare biosphere’.

The capacity of reef-building corals to host different symbionts (symbiotic flexibility) suggests two potential adaptive mechanisms to environmental changes: symbiont ‘shuffling’ and ‘switching’ (Buddemeier and Fautin, 1993; Fautin and Buddemeier, 2004). Some corals have been shown to resist and/or recover from thermal stress through changes in the relative abundance of Symbiodinium types that constitute the in hospite community, i.e., symbiont shuffling (Baker et al., 2004; Rowan, 2004). This acclimatisation response is well documented (Baker et al., 2004; Chen et al., 2005; Berkelmans and van Oppen, 2006; Jones et al., 2008; Baskett et al., 2009), but is believed to be limited to the Symbiodinium types acquired vertically or horizontally in early life stages. Symbiont ‘switching’ refers to a change in the in hospite Symbiodinium community due to the uptake of new Symbiodinium types from the environment, potentially from the water column and sediments (Buddemeier and Fautin, 1993; Fautin and Buddemeier, 2004).

Preliminary studies have indicated that adult corals are unable to form stable symbioses with exogenous algal symbionts, therefore this mechanism is believed to occur only during a relatively short period of the coral larval and early juvenile life stages (Goulet and Coffroth, 2003; Little et al., 2004; Coffroth et al., 2010). Testing of this hypothesis has been hampered, however, by the use of genetic methods that lack sensitivity to detect Symbiodinium types that occur below 5-10% of total relative abundance.
Here we challenge this notion by exploring the *Symbiodinium* rare biosphere using next-generation sequencing (NGS), a cost-effective, high-throughput method which has been recently shown to accurately detect low-abundance *Symbiodinium* types (Quigley *et al.*, 2014; Thomas *et al.*, 2014; Arif *et al.*, 2014; Green *et al.*, 2014; Edmunds *et al.*, 2014).

We assess *Symbiodinium* communities in a time-series sample set to investigate i) the cryptic diversity of the *Symbiodinium* rare biosphere within two common pocilloporid species; ii) possible changes within the *Symbiodinium* community over a period of time that spans two successive bleaching events; and, iii) whether *Symbiodinium* shuffling and/or switching has occurred in pocilloporid corals from a subtropical reef at Lord Howe Island (LHI), eastern Australia.

**MATERIALS AND METHODS**

*Study location*

Lord Howe Island is located 600 km off the east coast of northern New South Wales, Australia, in a dynamic oceanographic transitional region at latitude 31.5 °S and longitude 159.0 °E (Harriott *et al.*, 1995). LHI is the world's southern-most true lagoonal coral reef, which was inscribed on the UNESCO World Heritage list in 1982 and classified as a Marine Park in 2002 (Hutton and Harrison, 2004). This isolated island supports unique benthic reef assemblages resulting from a bio-geographical overlap of tropical, subtropical and temperate marine species, which accounts for the high species diversity present (Harriott *et al.*, 1995).

Although located over 1000 km from the southernmost regions of the Great Barrier Reef (GBR), approximately 100 scleractinian coral species have been reported to occur on its fringing reefs and on rocky substrate in deeper waters, providing habitat for many threatened and protected marine species (Harriott *et al.*, 1995; P. Harrison unpubl. data). The occurrence of tropical coral species at LHI results from the influence of the East Australian Current that
flows southwards from the GBR, enabling the migration of some tropical marine species further south (Harriott et al., 1995; Ayre and Hughes, 2004; Noreen et al., 2009).

Sample collection and DNA extraction

During the 2010 and 2011 austral summers, abnormally high sea surface temperatures, high light penetration and calm seas resulted in the first recorded extensive and severe coral bleaching at LHI (Harrison, 2011; Dalton and Carroll, 2011). Up to 95% of the coral community in the lagoon displayed variable bleaching with 41% and 56% mortality occurring in *Pocillopora damicornis* and *Stylophora pistillata* respectively; two species that dominate the LHI lagoonal coral community (Harriott et al., 1995; Dalton and Carroll, 2011).

Two hundred coral fragments were collected (*P. damicornis* n=110; *S. pistillata* n=90) from two locations within the lagoon (Comet’s Hole and North Bay Wreck) over a two-year period: two and six months after the first bleaching event in 2010, during the second bleaching event in 2011, and 18 months afterwards in 2012. All samples were fixed in absolute ethanol and DNA was extracted following Wayne’s method with slight modifications (Lundgren et al., 2013).

Amplification of the internal transcribed spacer 2 (ITS2) region and preparation for Roche 454 targeted amplicon sequencing

The *Symbiodinium* nuclear DNA ribosomal ITS2 region was amplified by PCR using the specific forward 5’-GTGAATTGCAGAACTCCGTG-3’ and reverse 5’-CCTCCGCTTACTTATATGCTT-3’ primers, which further contained a known 10 bp tag (identifier) allowing the identification of amplicons from different samples after pooling for 454 sequencing.

Each 25 µL PCR reaction contained 1 µL of 1/100 diluted DNA template (from 20 to 50 ng/µl), 12.5 µL of Taq HotStart mix (Bioline), 2 µL of 2 µM of each forward and reverse
primer and 9.5 µL of DNase free water. PCR was performed using the following conditions: 95°C for 5 mins, followed by 30 cycles of 30 sec at 95°C, 40 sec at 52°C and 30 sec at 72°C, with a final extension at 72°C for 3 min. PCR products were run on a 1% TAE-agarose gel, excised and purified using an in house method prior to a second PCR step. For this second PCR, each 35 µL PCR reaction contained 1 µL of purified PCR template, 12.5 µL of Taq HotStart mix (Bioline), 2 µL of 2 µM of each forward and reverse primer and 20.5 µL of DNase free water. PCR was performed using the following conditions: 95°C for 10 mins, followed by 10 cycles of 30 sec at 95°C, 40 sec at 52°C and 1 min at 72°C, with a final extension at 72°C for 10 min. PCR products were purified using Sephadex G-50 Columns (Sigma).

To ensure good coverage per sample, up to 44 PCR products per quarter of plate were pooled. Pooled samples were sent to an external sequencing provider (Macrogen, Korea) for 454 targeted amplicon sequencing using Roche GS FLX platform.

**Bioinformatics analysis of 454 sequencing output**

The raw 454 sequencing reads were demultiplexed and denoised using QIIME (Caporaso et al., 2010). First, demultiplexing and quality control were performed which included filtering sequences with short reads (<150 bp), low read quality (< 20), sequences with more than 6 ambiguous base calls, and sequences that imperfectly matched the priming and the barcoding site. After each read was assigned to one barcode and the reverse primer truncated, sequences were denoised in order to remove noise (errors) generated by the amplification and sequencing process (Reeder and Knight, 2010).

To assign an identity to each read, SymTyper (www.symtyper.com, M. Belcaid in review, see Edmunds et al., 2014; Cunning, Silverstein, et al., 2015), a Symbiodinium-specific bioinformatics pipeline was used. Type level assignment was completed in SymTyper using
BLAST against a *Symbiodinium* reference ITS2 database (www.symtyper.com) that classified sequences into six categories:

1. **Multiple Hits** - alignment with equal similarity and length to multiple target sequences;
2. **Perfect** - unambiguous alignment to only one sequence in the reference database (e.g., 100% similarity to 96% of the length of the target);
3. **Unique** - alignment of >97% similarity over 96% of the target length;
4. **New** - no alignment to a single target with >97% similarity over 96% of the length of the target;
5. **ShortNew** - alignment with high similarity to a reference sequence according to the dynamic similarity threshold (1); and,
6. **Short** - does not meet minimum similarity and length requirements (e.g., <90% similarity to <96% of the length of the target).

To confidently resolve subtypes for reads that are shorter than the reference sequences, SymTyper dynamically adjusts the minimum similarity threshold required to accept a hit such that, as the query length decreases to 90%, the percent similarity required to accept a hit increases. In addition to satisfying the dynamic similarity threshold for short reads, a best hit is also required to be unique (highest raw bit score) for the hit to be valid.

The minimum required similarity threshold is computed as follows:

\[
\text{Required similarity} = 100 - \frac{C - \text{min}_c}{1 - \text{min}_c} \times (100 - \text{min}_s)
\]

Where \(C\) is the actual coverage fraction of the query and the hit sequences; \(\text{min}_c\) is the minimum accepted coverage fraction between the query and the hit sequences, and \(\text{min}_s\) is the minimum similarity threshold between the query and the hit sequences.
Short sequences were removed from the analysis, while New sequences were manually compared using nucleotide BLAST in NCBI and reported to the clade level only (i.e. LHL_C.XX). Sequences with Multiple hits were placed in a phylogenetic tree, assigned to the most recent common ancestor node and reported to the clade level with a node ID (i.e. C_I:52). The raw sequences have been deposited in NCBI under accession number PRJNA311610.

**Statistical analysis**

All statistical analyses were conducted using PRIMER v.6 software (http://www.primer-e.com). To compare the genetic structure of *Symbiodinium* in *P. damicornis* and *S. pistillata*, the log transformed abundance of the *Symbiodinium* types was compared for each pair of samples using the Bray-Curtis similarity coefficient.

A nonmetric multidimensional scaling (nMDS) ordination diagram was produced using the AVERAGE function to visualise the relationship of *Symbiodinium* communities within *P. damicornis* and *S. pistillata* over time and between sites. To test for significant spatial and temporal partitioning of *Symbiodinium* communities within hosts, a PERMANOVA test was performed with ‘host’, ‘time’ and ‘site’ as fixed factors, using type III sums of squares and unrestricted permutation of raw data. A post-hoc pair-wise comparisons test among all pairs of levels of ‘host × time × site’ factor was used to identify where these significant differences occurred. The Similarity Percentages (SIMPER) test was used to identify *Symbiodinium* types contributing toward dissimilarity using a 90% cut-off for low contributions of selected variables between groups. Shannon diversity (H’) and species richness (S) indices were calculated and plotted in SPSS (http://www-01.ibm.com/software/au/analytics/spss).
RESULTS

Symbiodinium diversity within Pocillopora damicornis and Stylophora pistillata using Next Generation Sequencing

The deep sequencing analysis of Symbiodinium rDNA ITS2 PCR amplicons, yielding 5115 ± 189 SEM and 4730 ± 440 SEM reads per coral colony (see Fig. S4) for P. damicornis and S. pistillata respectively, revealed a total of 258 Symbiodinium types belonging to clades A, B, C, D, F and G. Among these, 51 were previously known sequence types while 207 were undescribed Symbiodinium ITS2 sequences. All members newly discovered here (with the exception of a few C types) formed part of the Symbiodinium rare biosphere. A mean of 11 ± 0.17 and 10 ± 1.95 SD Symbiodinium types were simultaneously hosted in each P. damicornis and S. pistillata colony, respectively.

We acknowledge that the multiple-copy nature of the ITS2 region, which may result in pseudogenes or numerous low-abundant functional variants (Thornhill et al., 2007; Sampayo et al., 2009; LaJeunesse and Thornhill, 2011; Arif et al., 2014), can affect the interpretation of NGS data as an individual sequence does not necessarily represent an individual biological entity (Stat et al., 2011). However, while not designed as a species delineation taxonomic approach, the data presented here represent a robust comparative approach to assess genetic variation and new genetic variants in the assemblage of Symbiodinium sequences throughout two consecutives bleaching events.

Symbiodinium community changes throughout two consecutive bleaching events

After the first bleaching event in 2010 and during the second bleaching event in 2011, the two coral species were associated with the dominant type C_I:52, representing an average of 99.3% of the total Symbiodinium abundance in P. damicornis and 96.2% in S. pistillata (Figs. 5A and 5B). While no changes within dominant types were observed during this period, PERMANOVA tests revealed significant spatial and temporal partitioning of Symbiodinium
communities throughout the two thermal stress periods (PERMANOVA $p = 0.001$, Table S1). Indeed, a few shuffling events occurred within the Symbiodinium rare biosphere between May 2010 and September 2010 and between September 2010 and March 2011 (Fig. 5A and 5B).

Several instances of new appearances of Symbiodinium types previously not observed in the coral tissues were also recorded, which we interpret as de novo uptake (i.e. switching events). These new acquisitions resulted in new members in the rare biosphere (Fig. 5A and 5B, and see Figs. S1 and S2 for the complete list of all Symbiodinium rare biosphere members detected).

In contrast, 18 months after the second bleaching event (September 2012), significant changes in the Symbiodinium community composition harboured by the two coral species were observed, with changes occurring in both dominant types and within the rare biosphere (Fig. 6; nMDS post-hoc test $p=0.001$, Tables S3 and S4). Shuffling of type C_I:53, which previously belonged to the rare biosphere (0.03% of the total abundance in 2011) resulted in a mean relative abundance of 47% in 2012, while abundance of the previously dominant C_I:52 was significantly reduced following the second bleaching event (Fig. 5A and 5B).

Furthermore, in five P. damicornis samples, there was uptake of exogenous Symbiodinium LHI_C.28, which reached a mean relative abundance of ~33% in these samples (Fig. S3), and ~ 7% mean relative abundance when averaged across the 20 sampled colonies (Fig. 5A). Additionally, the relative abundance of the previously dominant C_I:52 type declined to ~ 4% (Fig. S3). Of particular importance, our results suggest the de novo acquisition of two members of clade D, types D_I:6 and D1.12 in both coral species. These D types were the most abundant types within the rare biosphere in September 2012 (Figs. 5A and 5B).
Temporal changes in the *Symbiodinium* community composition were mostly due to members in the rare biosphere. The SIMPER test revealed that the two co-dominant types C_I:52 and C_I:53 found in association with both *P. damicornis* and *S. pistillata* in September 2012, explained only 4.52% and 11.47% of the dissimilarity in the *Symbiodinium* community composition respectively. More than 80% of the dissimilarity between the disturbance period ‘2010–2011’ and the recovery period in 2012, was explained by the rare biosphere (Table S2). In addition, the *Symbiodinium* community diversity (using Shannon’s index) was 10 times higher in September 2012 than previously (Fig. 6 and Table S5).
Figure 5: Summary of *Symbiodinium* diversity in *Pocillopora damicornis* (A) and *Stylophora pistillata* (B) from four collection periods spanning May 2010 to September 2012. Pie charts represent the mean relative abundances of *Symbiodinium* types across all sampled colonies detected at each time point. Types that are dominant at any of the time points are represented in orange, brown or yellow and types belonging to the rare biosphere throughout the sampling period are represented in grey. Bar graphs represent the abundances (expressed in number of sequencing reads) of *Symbiodinium* types in the rare biosphere only and black stars represent a switching event. A switching event was deemed to occur when, during any one sampling a type was detected among multiple samples, but was absent from previous sampling times among any sample. Note that only the most abundant types and the ones that shuffled or switched are shown on this figure. See Figures S1 and S2 for the complete figure of all the *Symbiodinium* background types detected for each period of time.
Figure 6: Nonmetric multidimensional scaling ordination (nMDS) representing the *Symbiodinium* genetic structure from the resemblance matrix of *Pocillopora damicornis* and *Stylophora pistillata* centroids belonging to samples collected from 2010 to 2012. The nMDS showed a temporal partitioning within hosts of the *Symbiodinium* types divided into two distinct groups: May 2010, September 2010 and March 2011 are clustered together while September 2012 is widely separated. This highlights a substantial change in the structure of *Symbiodinium* assemblage 18 months after the second bleaching event. The bar chart represents the average of Shannon diversity over time within the two Pocilloporidae coral species. Error Bars represent 95% CI.
DISCUSSION

Extraordinary Symbiodinium diversity and symbiotic flexibility in LHI reef-building corals

In both coral species, the deep sequencing analysis revealed an extraordinary diversity within the Symbiodinium community. In fact, the diversity reported here is almost five times greater than that reported in other recent NGS studies on Symbiodinium diversity (Quigley et al., 2014; Thomas et al., 2014; Arif et al., 2014; Green et al., 2014; Edmunds et al., 2014). For example, a study on Acropora coral species (Thomas et al., 2014) at another high latitude reef (Abrolhos Island, Western Australia) found a Shannon diversity of 0.145 (vs. 0.620 at LHI in September 2012).

The high Symbiodinium diversity as well as the endemicity of LHI coral-algal symbioses (mostly composed of previously undescribed ITS2 Symbiodinium types) support previous studies showing that the LHI Symbiodinium community is genetically and physiologically distinct (Wicks, 2009; Wicks et al., 2010; Noreen et al., 2015). Our results highlight a high level of symbiont diversity within LHI subtropical corals, with a mean of 11 symbiont types per coral host. While only Symbiodinium belonging to clade C have been previously detected in LHI corals using a gel electrophoresis based method (Wicks et al., 2010), here we detected Symbiodinium types from clades A, B, C, D, F and G. The association of Symbiodinium clade B with S. pistillata and clade G with both P. damicornis and S. pistillata found here have not been previously observed.

Nevertheless, the majority of the symbionts detected here were members of Symbiodinium clade C, which explains the high level of specificity to clade C reported previously (Wicks et al., 2010). Further research is needed to investigate whether different Symbiodinium clade C types simultaneously hosted by a single colony can provide different
physiological performance and potentially enable acclimatisation, as previously suggested for clade C types in Caribbean corals (Sampayo et al., 2008).

*Temperature anomalies may drive fine-scale changes within the Symbiodinium community*

During the two bleaching events, we did not observe any changes within dominant types; however, the *Symbiodinium* rare biosphere showed a dynamic pattern where both shuffling and switching events were quite common during thermal stress and recovery periods (Figs. S1 and S2). For instance, we observed the new appearance of 104 and 80 *Symbiodinium* types in *P. damicornis* and *S. pistillata*, respectively, over all sampling periods. The substantial changes observed in the *Symbiodinium* community of both coral species following each of the two bleaching events suggest that environmental disturbance drives symbiont community changes in LHI corals (Buddemeier and Fautin, 1993; Fautin and Buddemeier, 2004; Berkelmans and van Oppen, 2006; Jones et al., 2008; Silverstein et al., 2015) and that symbiotic associations in species that show maternal symbiont transmission are more flexible than previously thought. This concurs with a recent study showing that corals that are sensitive to environmental conditions display high intra- and inter-species flexibility (Putnam et al., 2012). Interestingly, 18 months after the two bleaching events, the recovered coral colonies harboured a completely different *Symbiodinium* assemblage with new dominant and background types. We hypothesise that the newly acquired dominant *Symbiodinium* type (LHI_C.28), and the type that was already present in the rare biosphere at the first sampling time point (C_I:53), may be better adapted to cope with temperature anomalies and the potentially altered environmental conditions following such events. Notably, we observed a switching event to *Symbiodinium* clade D and 90% of the *Symbiodinium* rare biosphere members were also newly acquired in 2012, which may provide more options to cope with
future bleaching events. These findings overthrow the notion that the period for uptake of algal endosymbionts is narrow and only limited to early life stages in these reef-building corals.

Role and importance of members of the ‘Symbiodinium rare biosphere’

There is increasing evidence to suggest that members of Symbiodinium clade D can confer enhanced thermal tolerance to the coral holobiont compared to other clades (Stat and Gates, 2011). Repopulation of recovering bleached coral hosts with clade D types has been reported as a survival mechanism for elevated sea temperatures (Chen et al., 2005; Berkelmans and van Oppen, 2006; Mieog et al., 2007; Jones et al., 2008; Stat et al., 2013; Silverstein et al., 2015).

This mechanism has, however, been primarily attributed to shuffling of Symbiodinium D pre-existing in the rare biosphere rather than de novo acquisition. While the newly acquired D types in LHI corals occurred at low relative abundance in our results, studies on other microbial communities have demonstrated that rare species can be metabolically very active (Campbell et al., 2011; Logares et al., 2014). It has also been shown that rare functionally important species can become dominant to maintain the integrity of functional processes when environmental conditions change (Shade et al., 2014). Moreover, a network theoretic modelling approach on coral-Symbiodinium associations under climate change (Fabina et al., 2013) predicts that both elevated symbiont diversity as well as types occurring at low abundance, which provide redundant or complementary symbiotic function, can significantly increase community stability in response to environmental change. Hence, following these predictions, our results indicate that the switch to clade D in the Symbiodinium rare biosphere and the increase in symbiont diversity documented here in LHI corals may enhance the ability of these corals to resist and/or recover from future bleaching events.
The repopulation with previously undetectable clade D was also documented in an experimental study following two induced bleaching events (Silverstein et al., 2015). Even though the source of these newly dominant types could not be identified (from the rare biosphere or from the environment), the authors found an increase in the host thermotolerance and concluded that members of the *Symbiodinium* rare biosphere can be critical components of coral recovery (Silverstein et al., 2015). Similarly, the newly acquired *Symbiodinium* clade D documented here could increase their hosts’ thermotolerance during future bleaching events.

It is now well-established that the rare biosphere plays significant ecological roles in ecosystems such as diazotrophic bacteria in seawater, bacterial and archaeal ammonia oxidisers in soils, methanogens in intestines (Shade et al., 2014), marine planktonic microeukaryotes in the ocean (Logares et al., 2014) and, our findings suggest the same is true for reef-building corals.

*Implications of symbiont switching for reef-building coral community structure*

Climate change is responsible for changes in species composition and population structure (Ateweberhan et al., 2013). In coral reef ecosystems in particular, the general trend is the loss of stress-sensitive coral species and replacement by stress-tolerant species that survive the disturbance. For example, a study conducted over a 14 year period that included two thermal stress events (in 1998 and 2001) at the high latitude reef of Sesoko Island (Okinawa, Japan), reported a complete change in the coral community structure (van Woesik et al., 2011). The stress-sensitive branching pocilloporid corals were replaced by stress-tolerant massive corals such as poritids and brain corals.

Our study suggests that symbiont switching to more thermally tolerant symbionts in the two pocilloporid coral species has the potential to assist the persistence of these environmentally-sensitive coral species over time. Given that the frequency of thermal stress
events is predicted to increase (IPCC 2014), these findings have important implications for predicting coral assemblage recovery after mass bleaching events and will also help to refine evolutionary models that predict the future of coral reefs.
CHAPTER 3:

THE *SYMBIODINIUM* COMMUNITY STRUCTURE EXHIBIT A SPECIES-SPECIFIC RESPONSE TO SEVERE CORAL BLEACHING
ABSTRACT

Global climate change drives a number of changes in the marine environment, including periods of higher than usual seawater temperature. Mass coral bleaching induced by thermal stress and high irradiance represents one of the most serious threats to coral reef survival; however, the adaptive capacity of corals to climate warming is not well understood. One of the corals’ adaptive responses involves changes in the community composition of the *Symbiodinium* spp. symbionts. Detailed understanding of this mechanism has been hampered by genotyping methodologies with low sensitivity and a lack of temporal field based studies encompassing pre and post bleaching data. Here, we combined next-generation sequencing analysis with a time series sample set spanning two severe bleaching events, to investigate the temporal dynamics of prevalent and rare *Symbiodinium* types within two dominant brooding coral species at Lord Howe Island (LHI), eastern Australia.

First, our results reveal that, as opposed to recent findings, not all LHI coral-*Symbiodinium* associations responded equally to one of the most extensive and severe bleaching events. Instead, we observed a species-specific pattern where, in some coral species, the *Symbiodinium* community structure was dynamic and responsive to environmental stochasticity; while being mostly stable and persistent over time in another. Particularly, our results show that symbiont shuffling and switching within the *Symbiodinium* rare biosphere were more common than changes in dominant types. Most of these minor changes within the *Symbiodinium* community structure occurred during bleaching episodes, which suggests that the *Symbiodinium* rare biosphere acted as a reservoir ready to respond during or following coral bleaching and may support specific acclimatisation mechanisms. Although our results provide some insights on the *Symbiodinium* rare biosphere dynamics following consecutive bleaching events, further research is needed to elucidate their ecological roles within reef-building corals acclimatisation mechanisms.
INTRODUCTION

Coral reefs are one of the most valuable ecosystems on Earth, yet are characterised among the most vulnerable ecosystems to climate change (Hoegh-Guldberg, 1999; Hughes et al., 2003; Pandolfi et al., 2011). Indeed, coral reefs have been widely documented to be threatened by climate change impacts such as changes in seawater chemistry/pH and temperature (Ateweberhan et al., 2013; van Hooidonk et al., 2013). Triggered by higher sea surface temperatures, mass coral bleaching events lead to the breakdown of the association between reef-building corals and their phototrophic dinoflagellate endosymbionts, *Symbiodinium* spp. (Brown, 1997). As bleaching events deprive the coral host of key metabolic needs obtained from its symbionts, bleaching often provokes coral death if it persists for a prolonged period of time (Baker, 2003).

The survival of coral reefs in warmer oceans will depend on their ability to increase their thermal tolerance. Coral thermal tolerance has been widely discussed in the literature (e.g., reviewed in Blackall et al., 2015) and the identity of the *Symbiodinium* spp. symbionts has been shown to be a key component influencing upper limits of coral thermal tolerance (Howells et al., 2011; Cunning and Baker, 2014). The *Symbiodinium* genus is composed of nine different clades (clade A to I) within which many putative species (referred to as types) occur, some of which are physiologically and ecologically distinct (Pochon and Gates, 2010; LaJeunesse et al., 2014). For example, types within clade D are known to generally perform better than other types at high temperatures (Berkelmans and van Oppen, 2006; Jones et al., 2008; Stat and Gates, 2011), while a C3 type supports coral growth in the Persian/Arabian Gulf, one of the world’s hottest seas (Hume et al., 2013, 2015).

Several *Symbiodinium* types can cohabit within the same coral host and changes in their community structure can occur in response to environmental disturbances, a phenomenon called “shuffling” (Buddemeier and Fautin, 1993; Fautin and Buddemeier, 2004). Shuffling
refers to a temporal change in the abundance of *Symbiodinium* types already present within the coral tissue (Chen *et al.*, 2005; Mieog *et al.*, 2007; Jones *et al.*, 2008; Silverstein *et al.*, 2015; Ross Cunning *et al.*, 2015). In contrast, symbiont switching involves the acquisition of exogenous types present in the coral environment (i.e. sediments or water column, Nitschke *et al.*, 2015). As yet, the ecological importance of symbiont switching is still being questioned (Hoegh-Guldberg, 2014) and symbiont shuffling is not ubiquitous as some coral species exhibit temporally stable *Symbiodinium* communities (Goulet and Coffroth, 2003; Thornhill *et al.*, 2006; LaJeunesse *et al.*, 2008; Klepac *et al.*, 2015). Moreover, these mechanisms are believed to be influenced by the severity of bleaching, the environmental conditions during recovery or by biological factors such as host flexibility and specificity to certain types (Silverstein *et al.*, 2015; Ross Cunning *et al.*, 2015).

To accurately assess the contribution of *Symbiodinium* symbionts to corals’ adaptive capacity, a comprehensive overview and understanding of their diversity as well as spatiotemporal field based studies encompassing before, during and after bleaching data are crucial. The recent use of next-generation sequencing (NGS) has enabled a deep coverage of *Symbiodinium* diversity within reef-building corals and this has revealed the existence of a very diverse group of low abundance types, also known as the ‘*Symbiodinium* rare biosphere’ (Chapter 2). Using NGS and a time-series dataset spanning annual bleaching events, a recent study (Chapter 2) highlighted that shuffling and switching events were common within the *Symbiodinium* rare biosphere of two dominant coral species from subtropical Lord Howe island (LHI, eastern Australia). For instance, in this study, *Pocillopora damicornis* and *Stylophora pistillata* which are documented as stress-sensitive, brooding coral species, were shown to be more flexible in their symbiosis than previously thought.

LHI is part of a globally important subtropical climate change hotspot, where ocean temperature increases at an accelerated rate (Ayre & Hughes, 2004; Hobday & Pecl, 2013);
making it an excellent location to investigate corals’ adaptive mechanisms when facing temperature-induced bleaching events. Here, we investigate the structure and temporal dynamics of the *Symbiodinium* community within the dominant reef brooding corals *Porites heronensis* and *Seriatopora hystrix* during and after two severe bleaching events at LHI (Harrison *et al*., 2011; Dalton and Carroll, 2011). We compare their symbiont community pattern with that of two previously documented coral species (Chapter 2) and explore whether types present in the rare biosphere were transient or persistent over time and how much they contribute to the temporal variability of the community structure.

**MATERIALS AND METHODS**

*Study location*

Located 600 km off the east coast of Australia, Lord Howe Island (LHI) is the world’s southern-most lagoonal true coral reef (31.5500° S, 159.0833° E). It supports an assemblage of tropical, subtropical and temperate marine species (Harriott *et al*., 1995). During the 2010 and 2011 austral summers, the seawater temperature at LHI was 1°C higher than average for a period of nineteen and seven weeks respectively, resulting in severe bleaching in the lagoon (Harrison *et al*., 2011; Dalton and Carroll, 2011).

*Sample collection, DNA extraction, amplification of the ITS2 region and preparation for Roche 454 amplicon sequencing*

A total of 147 coral colonies nubbins of *Porites heronensis* and *Seriatopora hystrix* were collected at two locations within the LHI lagoon. Samples were collected over a two-year period: during (March 2010), 2 months (May 2010) and 6 months (September 2010) after the first bleaching event in 2010, during the second bleaching event (March 2011) and 18 months after the second bleaching event (September 2012).
DNA extraction and the *Symbiodinium* nuclear DNA ribosomal ITS2 region was amplified as described in Chapter 2. Up to a maximum of 44 PCR products per quarter of a plate were pooled and sequenced using 454 sequencing (Roche, GS FLX Titanium chemistry).

**Bioinformatic analysis of 454 sequencing output**

Using Qiime (Caporaso et al., 2010), the raw 454 sequencing reads were subjected to quality control, demultiplexed and denoised as indicated in Chapter 2. The resulting sequences were run thought SymTyper (www.symtyper.com), a *Symbiodinium*-specific bioinformatic pipeline, for OTU mapping (Edmunds et al., 2014; Ross Cunning et al., 2015).

**Statistical analysis**

To visualise the relationship between *Symbiodinium* communities within *P. heronensis* and *S. hystrix* over time, a nonmetric multidimensional scaling (nMDS) ordination diagram was produced using the AVERAGE function in PRIMER v.6 (http://www.primer-5e.com). Then to test for significant temporal partitioning of *Symbiodinium* communities within hosts, the log transformed abundance of the *Symbiodinium* types was compared for each pair of samples using the Bray-Curtis coefficient of similarity and a PERMANOVA test was performed with ‘host’ and ‘time’ as fixed factors, using type III sums of squares and unrestricted permutation of raw data. A post-hoc pairwise comparisons test among all pairs of levels of ‘host × time’ factor was used to identify which type contribute the most to the variability. Shannon diversity (H’) and species richness (S) indices were also calculated for each period of time.
RESULTS

The deep sequencing analysis of *Symbiodinium* rDNA ITS2 PCR amplicons returned 1,059,504 sequences with a mean of 7,593 sequence reads (±1448 SD) per coral colony. A total of 183 *Symbiodinium* types belonging to the clades B, C, D, and G were detected, including 58 existing *Symbiodinium* types and 125 undescribed *Symbiodinium* ITS2 types (*P. heronensis* N=79, *S. hystrix* N=46).

*Symbiodinium community structure throughout two consecutive bleaching events*

The visualisation of the *Symbiodinium* genetic structure within *Porites heronensis* and *Seriatopora hystrix* over time visualised by the nMDS revealed two distinct patterns between the two species (Fig. 7). Moreover, the PERMANOVA and the pairwise test revealed a significant temporal partitioning of *Symbiodinium* communities within each coral species (*P=0.001*, Table 6, 7).

**Figure 7: Nonmetric multidimensional scaling ordination (nMDS) representing the Symbiodinium genetic structure from the resemblance matrix of Porites heronensis and Seriatopora hystrix centroids belonging to samples collected from 2010 to 2012.** The grey bubble plots indicate the partitioning of the Symbiodinium communities revealed by the pairwise test.
Within *S. hystrix*, the *Symbiodinium* community was partitioned in two groups (Fig. 7) with the first group comprising samples from March and May 2010, as no significant differences were detected 2 months after the thermal stress in May 2010 (*P*=0.287, Table 7). However, a change in the *Symbiodinium* genetic structure occurred 6 months after the first bleaching event in September 2010, which formed the second group along with March 2011 and September 2012 samples. In *P. heronensis*, a similar pattern was observed with no significant differences between March and May 2010 (*P*=0.161, Table 7), however significant differences (*P*=0.002 and *P*=0.032, Table 7) were detected between May 2010 and September 2010, as well as between September 2010 and the second bleaching event in March 2011 (Fig. 7). No significant differences were detected by the pairwise test between the disturbance period in March 2011 and the recovery period in September 2012 (Table 7).

Furthermore, there were no significant differences in the Shannon diversity between the two species (*P*=0.54, Table 8) but significant differences appeared over time (*P*=0.002, Table 8). Indeed, the diversity was higher during the disturbance periods than during the recovery periods (September 2010 and 2012) in both species. Conversely, the species richness was higher during the recovery period in *S. hystrix*, but higher during the disturbance periods in *P. heronensis* (Fig. 11 and Table 9).

**Temporal dynamics of prevalent and rare Symbiodinium types within *P. heronensis***

During the first bleaching event, *P. heronensis* was dominated by C_I:53, C_I:52 and LHI_C.28 reaching 58.27%, 38.77% and 1.10% of relative abundance respectively, while in the rare biosphere, D_I:6 and LHI_C.16 types were the most abundant (Fig. 8). Two months after the thermal stress, we observed a shuffling of a previously rare type G_I:5 (0.03% in March 2010) reaching 3.6% of relative abundance in May 2010. In addition, several switching events were detected resulting in new *Symbiodinium* members within the rare biosphere in May.
2010. Six months after the first bleaching event (September 2010), the relative abundance of
the prevalent type C_I:53 was 13% higher while G_I:5 type abundance decreased below the
rare biosphere threshold (<1%, Fig. 8). Within the rare biosphere, 11 Symbiodinium new types
were detected and a few shuffling events occurred (see C1cstar, LHI_C.16, LHI_C.101 and
LHI_C.10 types; Fig. 8). During the second bleaching event in March 2011, G_I:5 re-emerged
in the prevalent types and C_I:53 relative abundance increased by ~15% while the relative
abundance of C_I:52 decreased by ~41%. Interestingly, of the 31 switching events detected,
18 Symbiodinium types were previously observed to switch in May 2010 (see types with a
white star in Fig. 8). When the coral colonies appeared to have fully recovered (i.e. displayed
normal pigmentation coloration) from the two consecutive bleaching events (September 2012),
the prevalent types followed the same trend as in September 2010: an increase in C_I:53
relative abundance, a decreasing abundance of C_I:52 while G_I:5 became a member of the
rare biosphere. Within the rare biosphere, the relative abundance of LHI_C.28 was lower and
11 new Symbiodinium new types were detected. In addition, the SIMPER test showed that the
two dominant types C_I:52 and C_I:53 explained an average of 31.2% of the community
structure dissimilarity (Table 10); which supports the view that the temporal changes in the
Symbiodinium community composition were mostly due to members in the rare biosphere.
Figure 8: Summary of Symbiodinium diversity in Porites heronensis from four collection periods spanning May 2010 to September 2012. Pie charts represent the mean relative abundances of Symbiodinium types across all sampled colonies detected at each time point. Bar graphs represent the abundances (expressed in number of sequencing reads) of Symbiodinium types in the rare biosphere only. Black stars represent a switching event and white stars represent types that have previously switched.
Temporal dynamics of prevalent and rare Symbiodinium types within *S. hystrix*

During the first bleaching event in May 2010, C_I:53 (73.5%), C_I:52 (20.1%) and LHI_C.21 (2.9%) were prevalent types in *S. hystrix*, while LHI_C.10 and C3ff were the most abundant types in the rare biosphere (Fig. 9). Two months after the thermal stress, we observed the shuffling of the previously background types LHI_C.10 and C3ff reaching 1.7% and 1.8% of relative abundance, respectively, and a total of 15 switching events were recorded in the rare biosphere where C1043 was the most abundant type.

Six months after the first bleaching event (September 2010), we observed an important decline of ~80% in relative abundance of C_I:52 (4% in September 2010) as well as a gain of 16% for C_I:53, while the relative abundance of LHI_C.21, LHI_C.10 and C3ff remained approximately constant. Within the rare biosphere, 20 new *Symbiodinium* new types were detected and C1043 along with C_I:6 were the most abundant background types (Fig. 9).

During the second bleaching event in March 2011 and 18 months after in September 2012, LHI_C.21 was no longer detected in the colonies sampled, while C_I:52, LHI_C.10 and C3ff relative abundances remained stable. C_I:53 remained the most abundant dominant type reaching a relative abundance of 91.2% in September 2012. Within the rare biosphere, a few switching events were observed (2 in 2011 and 6 in 2012) while the relative abundance of C1043 and C_I:6 remained stable over this period time.

Moreover, the SIMPER analyses showed that, during the bleaching events, temporal changes in the *Symbiodinium* community structure were mostly due to members in the rare biosphere (representing ~80% of the community dissimilarity, Table 10) while the contribution of dominant types increased during recovery periods (average ~42%, Table 10).
Figure 9: Summary of *Symbiodinium* diversity in *Seriatopora hystrix* from four collection periods spanning May 2010 to September 2012. Pie charts represent the mean relative abundances of *Symbiodinium* types across all sampled colonies detected at each time point. Bar graphs represent the abundances (expressed in number of sequencing reads) of *Symbiodinium* types in the rare biosphere only. Black stars represent a switching event.
DISCUSSION

To better understand the contribution of *Symbiodinium* symbionts toward their coral host thermal tolerance, well-designed time series datasets combined with a sensitive genotyping methodology are crucial. Here we use NGS to investigate the temporal dynamics of prevalent and rare *Symbiodinium* types in two coral species throughout two severe bleaching events at LHI. Overall, the results reveal that changes within the *Symbiodinium* rare biosphere were more common than changes in dominant types within *P. heronensis* and *S. hystrix*. These minor changes within the *Symbiodinium* community structure that occurred during bleaching episodes may have an adaptive value. For instance, a previous study found that fine scale changes in the *Symbiodinium* community structure of *Orbicella faveolata* have impacted the host photochemical efficiency (Cunning, *et al.*, 2015).

*Symbiont community shifts exhibit a species-specific response to environmental disturbance*

The 2010 and 2011 bleaching events at Lord Howe Island were the most extensive and severe recorded at LHI to date (Harrison *et al.*, 2011). Although shifts in the *Symbiodinium* assemblage can depend on the severity of the bleaching (Ross Cunning *et al.*, 2015), our results reveal that species-specific patterns are also important; the symbiosis dynamics differed dramatically between LHI coral host species during these severe environmental disturbances.

For instance, the *Symbiodinium* assemblage within *P. heronensis* displayed a dynamic pattern where most of the shuffling and switching events occurred during the bleaching episodes. More interestingly, we observed a type belonging to clade G moving in and out of the rare biosphere during the bleaching events. In addition, in March 2011, we observed the re-emergence of several types (white stars, Fig. 8) that had already been observed switching in May 2010. Conversely, within *S. hystrix*, the symbiont shuffling and switching was observed only after the first bleaching event in 2010, and the *Symbiodinium* community structure
remained stable after September 2010 despite further environmental disturbance. This concurs with several reports showing similarly stable patterns of *Symbiodinium* assemblages facing environmental stochasticity (Thornhill *et al.*, 2006; McGinley *et al.*, 2012; Putnam *et al.*, 2012; Kenkel *et al.*, 2013; Klepac *et al.*, 2015).

However, the stability of the *S. hystrix*-Symbiodinium symbiosis in response to the repetitive bleaching differed from its pocilloporid counterparts investigated during the same bleaching events at LHI (Chapter 2). As shown on the nMDS (Fig. 10), the *Symbiodinium* assemblages of the three Pocilloporidae species and *P. heronensis* (Poritidae) exhibit a species-specific pattern. Indeed, after the second bleaching event, both *Pocillopora damicornis* and *Stylophora pistillata* harboured a different *Symbiodinium* assemblages with substantial changes observed within dominant and rare types (Chapter 2).

![Figure 10: Nonmetric multidimensional scaling ordination (nMDS) representing the Symbiodinium genetic structure from the resemblance matrix of the four coral species centroids belonging to samples collected from 2010 to 2012.](image)
Furthermore, in *P. damicornis* and *S. pistillata*, the diversity (using Shannon index) was ten times higher in September 2012 once the corals had recovered from the second bleaching event (Fig. 11). Conversely, there was less variation within *S. hystrix* mean symbiont diversity and species richness throughout the two bleaching events and in *P. heronensis*, the indexes were higher during the disturbance periods than the recovery periods (Fig. 11).

**Figure 11:** Mean species richness and Shannon diversity within the four coral species over time. Error Bars represent 95% CI.
Overall, these results highlight that not all coral species and their *Symbiodinium* community respond equally to the same level of disturbance. Although coral thermal tolerance has been shown to be contingent on specific *Symbiodinium* types, the interaction between the coral host and its symbionts can be complex (Baird *et al.*, 2009). For instance, in some coral species (*P. heronensis*, *P. damicornis* and *S. pistillata*), the repetitive bleaching events may have caused substantial changes in their *Symbiodinium* community structure and may have supported mixed assemblages 18 months after the second bleaching events, whereas persistent assemblages were observed in *S. hystrix* after the first bleaching event. Moreover, a previous study (Abrego *et al.*, 2008) found that *Acropora tenuis* juveniles acquired an enhanced thermal tolerance when associated with *Symbiodinium* type C1 than with *Symbiodinium* type D; however, the opposite has been demonstrated within *Acropora millepora* (Berkelmans and van Oppen, 2006). Therefore, it is of particular importance to consider both the coral host and its symbiont assemblage when investigating coral responses to bleaching events.

*Dynamism or stability: what is the role of the Symbiodinium rare biosphere following coral bleaching?*

Although the *Symbiodinium* rare biosphere represented less than 1% of the total *Symbiodinium* community, overall, it contributed to 70% of the temporal variability in the community structure within *P. heronensis* and more than 80% in *S. hystrix, P. damicornis* and *S. pistillata* (Chapter 2 and Table S1). In *P. damicornis* and *S. pistillata*, environmental stressors such as higher than average seawater temperature may have triggered two responses: 1) the growth of a member of the rare biosphere (C_I:53) that is potentially metabolically and physiologically better adapted to the new prevailing conditions; and 2) the decline of a previously dominant type (C_I:52) that is no longer adapted to the new environmental conditions. In that case, we suggest that the *Symbiodinium* rare biosphere acted as a reservoir bank ready to respond during or following coral bleaching. Moreover, previous research came
to the same conclusion when investigating the temporal dynamics of microbial communities such as Proteobacteria within coastal sands (Campbell et al., 2011), bacterial taxa in grass prairie soil (Elshahed et al., 2008), sulfur-metabolizing bacteria lineages in spring sediments (Coveley et al., 2015), diverse microbial communities from the air, ocean, human gut and skin, brewery wastewater, freshwater lake and stream, (Shade et al., 2014) and fast-growing bacteria within soil from different ecosystems (Aanderud et al., 2015).

Corals in the genus *Porites* have previously been thought to be specialists with low flexibility and narrow *Symbiodinium* assemblages that are stable and persistent over time despite environmental disturbance (Stat et al., 2009; Barshis et al., 2010; Putnam et al., 2012). However, here we show that the *Symbiodinium* community within *P. heronensis* is dynamic in response to bleaching events, which may assist its host to balance trade-offs between metabolic performance and thermal tolerance (Cunning et al., 2015).

However, while it may be argued that the rare biosphere can be ephemeral or composed of opportunistic types that occupy empty niches after coral bleaching, our results revealed that in some coral species like *S. hystrix*, the *Symbiodinium* rare biosphere can also be persistent over time despite severe environmental disturbances. It has been suggested that the retention of the rare biosphere members in a specific ecosystem could be due to their putative essential ecological functions (Coveley et al., 2015). Thus, it is possible that the persistence of the *Symbiodinium* rare biosphere within *S. hystrix* may support other acclimatisation mechanisms such as enhanced capacity for photosynthesis and carbon fixation that have been previously observed in subtropical *S. hystrix* colonies when exposed to fluctuating temperatures (Mayfield et al., 2012).

So far, the ecological roles and dynamics of the *Symbiodinium* rare biosphere within reef-building corals are still unknown. Further temporal investigations coupled with NGS are
needed to elucidate and better understand the contribution of these rare symbionts toward their host thermal tolerance in a changing ocean.
CHAPTER 4: SYNTHESIS
Major findings and limitations

The microbiome associated with reef-building corals plays an essential role in holobiont functioning. Therefore, dissecting the contribution of each component of the coral microbiota is crucial to understand and predict coral responses to environmental changes. To advance our insight of the role of *Symbiodinium* symbionts in their coral host thermal tolerance, time-series datasets combined with a sensitive genotyping approach is essential. In this thesis, next-generation sequencing (NGS) was used to investigate the temporal dynamics of prevalent and rare *Symbiodinium* types in four coral species throughout two severe bleaching events at Lord Howe Island.

The analysis of *Symbiodinium* rDNA ITS2 PCR amplicons from four different species shows that thermal anomalies in the subtropical reef environment at LHI may have driven symbiont shuffling and switching during or following severe bleaching events, although these mechanisms did not always occur after each disturbance or among all coral species (Chapter 3). Moreover, the evidence of symbiont switching following thermal stress presented here (Chapter 2), supports the Adaptive Bleaching Hypothesis and challenges the accepted paradigm that reef-building corals can only acquire *Symbiodinium* types early in life. Not only do these temporal changes observed within the *Symbiodinium* community structure show that some coral species can be more flexible following environmental changes than previously reported, but they also highlight the complexity of the coral-*Symbiodinium* association.

The majority of the *Symbiodinium* types detected during this research were present below 1% of relative abundance and were referred to as the “*Symbiodinium* rare biosphere”. In *Pocillopora damicornis*, *Stylophora pistillata* and *Porites heronensis*, the *Symbiodinium* rare biosphere may have acted as a reservoir that enabled the shuffling of potentially physiologically better adapted *Symbiodinium* types to the new prevailing conditions. In contrast, in *Seriatopora*...
hystrix, the retention and temporal stability of the Symbiodinium rare biosphere suggests that these low abundance types might also be metabolically active and may support other acclimatisation mechanisms.

Furthermore, the high level of symbiont diversity and species richness observed in the four coral species may assist community stability that may, in turn, enhance the ability of the coral host to resist or recover from future bleaching and other stress events. Although experimental evidence is needed to support such a hypothesis, results from a modelling approach on coral-Symbiodinium associations under future climate change scenarios have shown similar results (Fabina et al., 2013).

While the NGS approaches are rapidly advancing our understanding of coral-Symbiodinium symbioses, it is also important to note there are some limitations to the interpretation of NGS data due to the multiple-copy nature of the ITS2 region, and numerous low abundant functional variants are often present within a single coral colony or Symbiodinium cell (Thornhill et al., 2007; Arif et al., 2014). Therefore, an individual sequence does not necessarily represent an individual biological entity (Stat et al., 2011). However, the data presented in this thesis were not designed to be a taxonomic work, but rather a robust comparative approach to assess genetic variation and new genetic variants in the spatio-temporal assemblage of Symbiodinium sequences. Several Symbiodinium genome sequences are now available or underway (Shinzato et al., 2011; Bayer et al., 2012; Shoguchi et al., 2013; Shinzato et al., 2014), and it is hoped that highly variable single-copy markers can be developed in the near future.

In addition to the limitation in interpreting NGS data, some concerns can be raised about the high sensitivity of this methodology. For instance, there is a possibility that a certain proportion of the Symbiodinium rare biosphere detected here, may be representative of the
corals’ environment as the dinoflagellate is known to be found free-living in the water column or in the sediments (Gou et al., 2003; Coffroth et al., 2006; Manning and Gates, 2008; Littman et al., 2008; Pochon et al., 2010; Takabayashi et al., 2012; Sweet, 2014; Nitschke et al., 2015). Preferably, water and sediments surrounding the coral colonies should be sampled at the same time in order to characterize the *Symbiodinium* community of both the corals and its environment. Furthermore, cross contamination during sample preparation prior to sequencing can arise. Therefore, negative controls should be performed during the DNA extraction phase and should also be amplified and sequenced alongside with negative PCR controls. Moreover, a subset of the samples should be re-amplified and re-sequenced to reduce the chance of including sequencing artefact in the analysis and to create a better understanding of the total diversity in a sample.

**Future directions**

Until now, the ecological roles and dynamics of the *Symbiodinium* rare biosphere within reef-building corals remain unknown. Future investigations should aim to:

- Explore the phylogenetic affiliations of each member of the *Symbiodinium* rare biosphere, i.e., investigate whether most background rare types are unique or whether the rare biosphere comprises novel types that are closely related to an existing dominant *Symbiodinium* type among different coral species.

- Study the biogeography of the *Symbiodinium* rare biosphere and investigate spatial and temporal dynamics across different region and latitudes within several coral species.
• Experimentally, investigate the effect of key environmental stressors (such as changes in seawater temperature and light conditions, pH, salinity, nutrients or polluants) on the temporal dynamics of the *Symbiodinium* rare biosphere.

• Explore the metabolic activity of members in the *Symbiodinium* rare biosphere. For instance, using the rRNA:rDNA sequences ratio to highlight whether the *Symbiodinium* rare biosphere contains mainly dormant or metabolically active cells that may potentially play key a role in coral functioning.

• Investigate the functional diversity of the *Symbiodinium* rare biosphere through comparison of the differential gene expression between each member of the rare biosphere; as recently done with members of clade B (Parkinson *et al*., 2016).

• Compare *Symbiodinium* communities at Lord Howe Island with coral phenotypes to gain further insight into the functional roles of *Symbiodinium* in the coral holobiont.

Once these knowledge gaps in the ecological roles of the *Symbiodinium* rare biosphere have been addressed, we will gain a better understanding of the adaptive capacity of corals to environmental changes and therefore, may better predict their resilience to impending global change.
APPENDICES
Appendix 1

Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals

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**Supplementary Information:**

**Supplementary figures and tables**

Figures S1-S4

Tables S1-S5
Fig. S1. Symbiodinium diversity in *Stylophora pistillata* during, and subsequent to, the two consecutive bleaching events. The pie charts represent the overall *Symbiodinium* type’s relative abundance detected for each period of time. The dominant types are represented in orange, brown or yellow and the background types are represented in grey. The bar charts represent only the *Symbiodinium* rare biosphere abundance (expressed in number of sequencing reads). Black stars represent a switching event. A switching event was deemed to occur when, during any one sampling a type was detected among multiple samples, but was absent from previous sampling times among any sample.
Fig. S2. *Symbiodinium* diversity in *Pocillopora damicornis* during and subsequent to the two consecutive bleaching events. The pie charts represent the relative abundance of the *Symbiodinium* types detected for each period of time. The dominant types are represented in orange, brown or yellow and the background types are represented in grey. The bar charts represent only the *Symbiodinium* rare biosphere abundance (expressed in number of sequencing reads). Black stars represent a switching event. A switching event was deemed to occur when,
during any one sampling a type was detected among multiple samples, but was absent from previous sampling times among any sample.

Fig. S3. *Symbiodinium* diversity within five samples of *Pocillopora damicornis* in September 2012, 18 months after the second bleaching event. The pie charts show relative abundances of the dominant *Symbiodinium* types detected. Dominant types are represented in brown, yellow, orange or blue; types that are a member of the rare biosphere are represented in grey. The bar charts represent the abundances (expressed in number of sequencing reads) of *Symbiodinium* types in the rare biosphere for each sample.
Fig. S4. Number of sequencing reads per samples within (A) *Pocillopora damicornis* and (B) *Stylophora pistillata* after quality control with Qiime and removal of short sequences with SymTyper.
**Table S1:** PERMANOVA of *Symbiodinium* types among hosts, over time and between sites

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<td>Host x Time x Site</td>
<td>3</td>
<td>3.87</td>
<td>0.001*</td>
</tr>
<tr>
<td>Residual</td>
<td>172</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant values (p<0.05)

**Table S2:** SIMPER test of the two dominant *Symbiodinium* types in September 2012.

<table>
<thead>
<tr>
<th>C_I:52</th>
<th>Site</th>
<th>Av.Value</th>
<th>Av.Sq.Dist</th>
<th>Sq.Dist/SD</th>
<th>Contribution %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pocillopora damicornis</em></td>
<td>CH</td>
<td>7.08</td>
<td>1.93</td>
<td>0.53</td>
<td>3.65</td>
</tr>
<tr>
<td></td>
<td>NBW</td>
<td>7.01</td>
<td>2.62</td>
<td>0.50</td>
<td>7.32</td>
</tr>
<tr>
<td><em>Stylophora pistillata</em></td>
<td>CH</td>
<td>7.02</td>
<td>1.99</td>
<td>0.54</td>
<td>5.12</td>
</tr>
<tr>
<td></td>
<td>NBW</td>
<td>7.53</td>
<td>0.77</td>
<td>0.52</td>
<td>2.00</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>4.52</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C_I:53</th>
<th>Site</th>
<th>Av.Value</th>
<th>Av.Sq.Dist</th>
<th>Sq.Dist/SD</th>
<th>Contribution %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pocillopora damicornis</em></td>
<td>CH</td>
<td>6.40</td>
<td>5.86</td>
<td>0.53</td>
<td>3.65</td>
</tr>
<tr>
<td></td>
<td>NBW</td>
<td>6.92</td>
<td>5.03</td>
<td>0.48</td>
<td>14.05</td>
</tr>
<tr>
<td><em>Stylophora pistillata</em></td>
<td>CH</td>
<td>6.85</td>
<td>3.85</td>
<td>0.48</td>
<td>9.90</td>
</tr>
<tr>
<td></td>
<td>NBW</td>
<td>6.80</td>
<td>7.03</td>
<td>0.54</td>
<td>18.28</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>11.47</strong></td>
</tr>
</tbody>
</table>
Table S3: Pair-wise test of *Symbiodinium* types for pairs of levels of factor ‘Time’.

<table>
<thead>
<tr>
<th>Groups</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 2010, September 2010</td>
<td>2.6371</td>
<td>0.001*</td>
</tr>
<tr>
<td>May 2010, March 2011</td>
<td>2.3349</td>
<td>0.001*</td>
</tr>
<tr>
<td>May 2010, September 2012</td>
<td>5.3955</td>
<td>0.001*</td>
</tr>
<tr>
<td>September 2010, March 2011</td>
<td>1.9821</td>
<td>0.002*</td>
</tr>
<tr>
<td>September 2010, September 2012</td>
<td>5.7733</td>
<td>0.001*</td>
</tr>
<tr>
<td>March 2011, September 2012</td>
<td>5.9503</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

* Significant values (*p*<0.05)

Table S4: Average similarity between/within groups from the Pair-wise test

<table>
<thead>
<tr>
<th></th>
<th>May 2010</th>
<th>September 2010</th>
<th>March 2011</th>
<th>September 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 2010</td>
<td>48.985</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>September 2010</td>
<td>47.892</td>
<td>54.898</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>March 2011</td>
<td>48.420</td>
<td>50.940</td>
<td>50.855</td>
<td>-</td>
</tr>
<tr>
<td>September 2012</td>
<td>33.607</td>
<td>37.005</td>
<td>34.059</td>
<td>49.313</td>
</tr>
</tbody>
</table>

Table S5: PERMANOVA of *Symbiodinium* diversity (Shannon indices) among hosts, over time and between sites.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Pseudo-F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>1</td>
<td>3.45</td>
<td>0.063</td>
</tr>
<tr>
<td>Time</td>
<td>3</td>
<td>72.03</td>
<td>0.001*</td>
</tr>
<tr>
<td>Site</td>
<td>1</td>
<td>2.18</td>
<td>0.132</td>
</tr>
<tr>
<td>Host x Time</td>
<td>3</td>
<td>1.16</td>
<td>0.323</td>
</tr>
<tr>
<td>Host x Site</td>
<td>1</td>
<td>0.14</td>
<td>0.720</td>
</tr>
<tr>
<td>Time x Site</td>
<td>3</td>
<td>2.09</td>
<td>0.106</td>
</tr>
<tr>
<td>Host x Time x Site</td>
<td>3</td>
<td>1.21</td>
<td>0.335</td>
</tr>
<tr>
<td>Residual</td>
<td>172</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant values (*p*<0.05)
APPENDIX 2

**Table 6:** PERMANOVA of *Symbiodinium* types among host (*S. hystrix* and *P. heronensis*) and over time.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Pseudo-F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>1</td>
<td>47.74</td>
<td>0.001*</td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>2.41</td>
<td>0.001*</td>
</tr>
<tr>
<td>Host x Time</td>
<td>4</td>
<td>2.62</td>
<td>0.001*</td>
</tr>
<tr>
<td>Residual</td>
<td>144</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant values (p<0.05)

**Table 7:** Pair-wise test of *Symbiodinium* types for pairs of levels of factor ‘Host x Time’.

<table>
<thead>
<tr>
<th>Groups</th>
<th><em>S. hystrix</em></th>
<th></th>
<th><em>P. heronensis</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t</td>
<td>P</td>
<td>t</td>
<td>P</td>
</tr>
<tr>
<td>March 2010, May 2010</td>
<td>0.49867</td>
<td>0.951</td>
<td>1.2166</td>
<td>0.161</td>
</tr>
<tr>
<td>March 2010, September 2010</td>
<td>1.5502</td>
<td>0.047*</td>
<td>0.89763</td>
<td>0.59</td>
</tr>
<tr>
<td>March 2010, March 2011</td>
<td>3.0587</td>
<td>0.002*</td>
<td>1.388</td>
<td>0.041*</td>
</tr>
<tr>
<td>March 2010, September 2012</td>
<td>2.7404</td>
<td>0.002*</td>
<td>1.1159</td>
<td>0.274</td>
</tr>
<tr>
<td>May 2010, September 2010</td>
<td>1.8452</td>
<td>0.01*</td>
<td>1.9321</td>
<td>0.002*</td>
</tr>
<tr>
<td>May 2010, March 2011</td>
<td>2.8099</td>
<td>0.001*</td>
<td>1.8775</td>
<td>0.001*</td>
</tr>
<tr>
<td>May 2010, September 2012</td>
<td>2.738</td>
<td>0.001*</td>
<td>2.0264</td>
<td>0.003*</td>
</tr>
<tr>
<td>September 2010, March 2011</td>
<td>1.3305</td>
<td>0.146</td>
<td>1.4166</td>
<td>0.032*</td>
</tr>
<tr>
<td>September 2010, September 2012</td>
<td>1.1791</td>
<td>0.227</td>
<td>1.0114</td>
<td>0.379</td>
</tr>
<tr>
<td>March 2011, September 2012</td>
<td>1.2177</td>
<td>0.176</td>
<td>0.99525</td>
<td>0.413</td>
</tr>
</tbody>
</table>

* Significant values (p<0.05)
Table 8: PERMANOVA of *Symbiodinium* diversity (Shannon indices) among hosts and over time for *P. heronensis* and *S. hystrix*.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Pseudo-F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>1</td>
<td>0.36072</td>
<td>0.54</td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>5.5647</td>
<td>0.002*</td>
</tr>
<tr>
<td>Host x Time</td>
<td>4</td>
<td>2.4335</td>
<td>0.055</td>
</tr>
<tr>
<td>Residual</td>
<td>148</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant values (p<0.05)

Table 9: PERMANOVA of species richness indices among hosts and over time for *P. heronensis* and *S. hystrix*.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Pseudo-F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host</td>
<td>1</td>
<td>58.192</td>
<td>0.001*</td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td>0.41441</td>
<td>0.814</td>
</tr>
<tr>
<td>Host x Time</td>
<td>4</td>
<td>3.5432</td>
<td>0.015*</td>
</tr>
<tr>
<td>Residual</td>
<td>148</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant values (p<0.05)
Table 10: SIMPER test of dominant *Symbiodinium* types within *Seriatopora hystrix* and *Porites heronensis* over time.

<table>
<thead>
<tr>
<th>March 2010</th>
<th>Types</th>
<th>Av.Value</th>
<th>Av.Sq.Dist</th>
<th>Sq.Dist/SD</th>
<th>Contribution %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Seriatopora hystrix</em></td>
<td>C_I:52</td>
<td>6.59</td>
<td>2.58</td>
<td>0.57</td>
<td>4.05</td>
</tr>
<tr>
<td></td>
<td>C_I:53</td>
<td>8.34</td>
<td>2.83</td>
<td>0.56</td>
<td>4.45</td>
</tr>
<tr>
<td></td>
<td>LHI_C.21</td>
<td>3.18</td>
<td>13.5</td>
<td>0.68</td>
<td>21.19</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>29.69</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Porites heronensis</em></td>
<td>C_I:52</td>
<td>6.02</td>
<td>2.27</td>
<td>0.51</td>
<td>6.78</td>
</tr>
<tr>
<td></td>
<td>C_I:53</td>
<td>6.74</td>
<td>3.18</td>
<td>0.44</td>
<td>9.51</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>16.29</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>May 2010</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Seriatopora hystrix</em></td>
<td>C_I:52</td>
<td>6.16</td>
<td>3.07</td>
<td>0.53</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>C_I:53</td>
<td>7.52</td>
<td>5.96</td>
<td>0.50</td>
<td>9.69</td>
</tr>
<tr>
<td></td>
<td>LHI_C.21</td>
<td>2.62</td>
<td>11.5</td>
<td>0.60</td>
<td>18.66</td>
</tr>
<tr>
<td></td>
<td>G_I:5</td>
<td>2.33</td>
<td>6.48</td>
<td>0.52</td>
<td>14.57</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>50.36</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Porites heronensis</em></td>
<td>C_I:52</td>
<td>6.41</td>
<td>3.36</td>
<td>0.44</td>
<td>7.55</td>
</tr>
<tr>
<td></td>
<td>C_I:53</td>
<td>6.4</td>
<td>6</td>
<td>0.51</td>
<td>13.48</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>35.6</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>September 2010</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Seriatopora hystrix</em></td>
<td>C_I:52</td>
<td>5.1</td>
<td>1.09</td>
<td>0.40</td>
<td>3.18</td>
</tr>
<tr>
<td></td>
<td>C_I:53</td>
<td>8.83</td>
<td>0.265</td>
<td>0.44</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>LHI_C.21</td>
<td>1.39</td>
<td>7.59</td>
<td>0.46</td>
<td>22.20</td>
</tr>
<tr>
<td></td>
<td>G_I:5</td>
<td>4.97</td>
<td>0.634</td>
<td>0.38</td>
<td>1.85</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>46.23</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Porites heronensis</em></td>
<td>C_I:52</td>
<td>5.7</td>
<td>7</td>
<td>0.45</td>
<td>19.47</td>
</tr>
<tr>
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<td>6.54</td>
<td>8.75</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
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<tr>
<td></td>
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<td>Types</td>
<td>Av.Value</td>
<td>Av.Sq.Dist</td>
<td>Sq.Dist/SD</td>
</tr>
<tr>
<td>----------------</td>
<td>------------</td>
<td>-------------</td>
<td>----------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td><em>Seriatopora hystrix</em></td>
<td></td>
<td>C_I:52</td>
<td>4.5</td>
<td>0.4</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
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<td>C_I:53</td>
<td>8.63</td>
<td>5.6 E-2</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>C3ff</td>
<td>4.93</td>
<td>5.59 E-2</td>
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</tr>
<tr>
<td></td>
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<td>LHI_C.10</td>
<td>5.69</td>
<td>2.38 E-2</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Porites heronensis</em></td>
<td></td>
<td>C_I:52</td>
<td>6.79</td>
<td>1.82</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_I:53</td>
<td>7.99</td>
<td>3.98</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>G_I:5</td>
<td>2.26</td>
<td>5.4</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>September 2012</em></td>
<td></td>
<td>C_I:52</td>
<td>4.62</td>
<td>0.19</td>
<td>0.54</td>
</tr>
<tr>
<td><em>Seriatopora hystrix</em></td>
<td></td>
<td>C_I:53</td>
<td>8.7</td>
<td>9.79 E-2</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C3ff</td>
<td>4.79</td>
<td>0.257</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LHI_C.10</td>
<td>5.09</td>
<td>2.45</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Porites heronensis</em></td>
<td></td>
<td>C_I:52</td>
<td>6.75</td>
<td>1.55</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C_I:53</td>
<td>7.58</td>
<td>6.54</td>
<td>0.43</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


to climate change: Shifting to new algal symbionts may safeguard devastated reefs from

Protein expression and genetic structure of the coral *Porites lobata* in an environmentally

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Microarray analysis reveals transcriptional plasticity in the reef building coral *Acropora

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**Prog Ser** 244: 17–26.


