Persistence of corals in marginal habitats: the role of the environment, and symbiont diversity and ecophysiology

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Abstract

Many corals live in marginal habitats, close to their survival thresholds of water temperature, light penetration and aragonite saturation. Living under these highly variable and extreme conditions is likely facilitated by specific physiological adaptations and/or the presence of unique species of coral and their symbionts but data on these factors are limited.

The specific objectives of the study were to: (1) examine the diversity and distribution patterns of corals in marginal environments, (2) investigate the diversity, distribution patterns and host specificity of symbionts in corals in marginal environments, (3) assess the influence of environmental variables on host and symbiont distribution in marginal environments, in comparison to 'optimal' environments, and (4) examine the physiological responses to changing environmental conditions and stress of corals and their symbionts in marginal environments.

Surveys of coral community patterns were conducted at the Kermadec Islands (KI), New Zealand, and Palmyra Atoll, USA, with local scale environmental parameters (i.e. wave exposure and sedimentation) found to control the diversity and distribution of the coral communities. *Symbiodinium* types were identified to sub-cladal level in a range of coral species at each of the survey sites, using ITS2-DGGE. A high diversity of C type symbionts (19 types in 13 host genera), and reduced host specificity was observed at the high latitude site of Lord Howe Island (LHI), Australia, with similarly high diversity at the KI (10 types in 9 genera). Thirteen novel clade C types were identified in corals at LHI, with two of these types also present in hosts at the KI. The reduced host specificity of symbionts at LHI,

compared to tropical sites, implies that the evolution of novel holobionts may be an important mechanism whereby corals can cope with variable and stressful conditions. Further, physiological assessment of the novel LHI symbionts led to the suggestion that *Symbiodinium* at LHI may be specialised for cooler and more variable temperatures, so contributing to the success of corals at this marginal location.

In contrast, a low diversity of generalist symbionts (C and D types) were uncovered at the equatorial site of Palmyra Atoll (10 types in 13 genera), attributed to the stressful environmental regime resulting in a reduced population of stress-tolerant symbionts. The variation in environmental parameters, particularly sedimentation, around Palmyra Atoll has led to diversification of coral communities, however this environmental variation has not affected the symbiont communities.

While it has been suggested that marginal coral communities might be better adapted for survival in an environment modified by global climate change, the local scale environmental factors are also important drivers of both coral and symbiont distributions, and should be considered when making predictions for the future. Further, assessment of the physiological tolerance ranges of both the multiple, novel symbionts at high latitudes, and the few, potentially stress-tolerant symbionts at Palmyra should be conducted, to help determine whether they have the ability to adjust to new environmental conditions.

Contributions and publications

This thesis is written as a series of manuscripts, which are in the review process or to be submitted in the near future. All field-work, lab-work and writing were conducted by the Author, with expections as below.

Chapter 2

This chapter has been submitted for publication to the Marine Ecology Progress Series, and is in review. Sample collection was undertaken by JPA Gardner and J Long, of Victoria University of Wellington.

Chapter 3

The molecular work in this chapter was assisted by Dr Eugenia Sampayo, of The University of Queensland. Figure 3.3 was designed by Dr Sampayo, and made editorial comments on the text.

Chapter 5

The design and implementation of this study was aided by Dr Ross Hill of The University of Technology, Sydney. Dr Hill also made editorial comments on the text

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List of abbreviations

ΦPSII= Effective photosynthetic yield

18S or SSU = Small subunit of the rDNA

23S = Chloroplast large subunit

28S or LSU = Large subunit of the rDNA

APX = ascorbate peroxidase

ATP = Adenosine triphosphate

chl-*a* = chlorophyll a

COX1 = Cytochrome oxidase subunit 1

cprDNA = Chloroplast ribosomal DNA

DGGE = Denaturing gradient gel electrophoresis

DNA = Deoxyribonucleic acid

FIC = Fast induction curve

FTU = Formazin turbidity unit

F_v/F_m = Dark adapted photosynthetic yield

GBR = Great Barrier Reef

ITS = Internal transcribed spacer region of the rDNA

KI = Kermadec Islands

LHC = Light harvesting complexes

LHI = Lord Howe Island

MAA = mycosporine-like amino acids

NADPH = Oxidized nicotinamide adenine dinucleotide phosphate

NPQ = Non photochemical quenching

PAM = Pulse amplitude modulation

PCOM = Percent organic matter

PCR = Polymerase chain reaction

PEA = Plant efficiency analyser

POM = Particulate organic matter

PSII = Photosystem II

rmANOVA = Repeat measures analysis of variance

Q_A = Primary electron acceptor

Q_B = Secondary electron acceptor

Q_m = Maximum excitation pressure over PSII

rDNA = Ribosomal DNA

ROS = Reactive oxygen species

SOD = superoxide dismutase

SSCP = Single strand conformation polymorphism

SST = Sea surface temperature

TPM = Total particulate matter

UVR = Ultraviolet radiation

Glossary of terms

Environmental specialist: A symbiont specialised for a particular environment, recognisable by their patterns of distribution

Flexibility: Ability of a host to associate with multiple *Symbiodinium* types

Holobiont: Unit defined by the host species and symbiont type.

Host generalist: A symbiont which associates with a range of host species or genera

Host specialist: A symbiont that only associates with one host species or genera

ITS2 type: Genetic ITS2 variant, lower taxonomic level than a clade. Taxonomic status as yet unresolved but may represent a species or a genetic variant within a species (strain).

Marginal reefs/communities: those that occur where conditions are close to the environmental thresholds for coral survival (Kleypas et al 1999, Harriott & Banks, 2002). These thresholds include high or low temperatures, salinities or nutrient levels, low light penetration or low aragonite saturation state (Kleypas et al 1999).

Selective sweep: reduction or elimination of variation among the nucleotides in neighbouring DNA of a mutation as the result of recent and strong positive natural selection.

Specificity: Members of the same host taxa harbouring specific symbiont types, e.g. *Pocillopora damicornis* with C41

Chapter 1

General Introduction

Marginal coral communities are viewed as those that exist close to the environmental thresholds for coral survival (Kleypas et al. 1999). Given that most of the world's coral reefs will become marginalised as our climate rapidly changes (Hoegh-Guldberg et al. 2007), study of marginal corals and their dinoflagellate symbionts (zooxanthellae) may provide useful analogues for understanding the ecology of coral reefs subjected to deteriorating conditions (Perry & Larcombe 2003). Moreover, it has been suggested that marginal coral communities might be better adapted for survival in an environment modified by global climate change, as a result of their existence in already stressed conditions (Glynn 1996, Riegl & Piller 2003). Knowledge of the environmental parameters driving coral and symbiont distributions, and the physiological tolerances of these symbionts to environmental stressors, are central to understanding how reefs or coral communities persist in marginal conditions.

1.1 Corals and symbiosis

Dinoflagellates of the genus *Symbiodinium* are phototrophic unicellular algae ('symbionts') that form mutualistic relationships with a wide variety of marine invertebrates and protists ('hosts'), in both tropical and temperate waters (Trench 1979, 1987, Banaszak et al. 1993). These hosts include members of various phyla,

including the Cnidaria, Platyhelminthes, Mollusca, Porifera, and Foraminifera (Trench 1979, Pawlowski et al. 2001). In the relationship between scleractinian (hard) corals and *Symbiodinium* spp., the algae live intracellularly, residing in host-derived vacuoles in the endodermal cells of the host that line the gastrovascular cavity (Fig 1.1a, Colley & Trench 1983, Lewin & Chang 1989, Farmer et al. 2001). These microscopic golden-brown algae (size ~5 μm, Fig. 1.1b), commonly known as zooxanthellae, are present at extremely high densities in their host (greater than 10^6 cm^{-2}, Muscatine & Porter 1977). Densities of symbionts may fluctuate over time in response to seasonal variables such as irradiance and temperature (Fagoonee et al. 1999, Fitt et al. 2000). Internally, *Symbiodinium* cells possess a large nucleus containing permanently condensed chloroplast(s) with a spiked pyrenoid (housing enzymes for CO_2 fixation), and pigments including chlorophylls *a* and *c*, peridinin and diadinoxanthin. Zooxanthellae reproduce asexually, by mitotic division when in the coccoid state only; growing evidence also suggests that sexual recombination can occur (Stat et al. 2006).

The host relies on its dinoflagellate symbionts for survival; they release substantial amounts of photosynthetically-fixed organic compounds ('photosynthate'), meeting up to 95% of the hosts energy requirements (Muscatine 1990, Wang & Douglas 1998). Photosynthate consists largely of low molecular weight, energy rich compounds such as glycerol and glucose, almost all essential amino acids, organic acids, and, arguably, lipids (Markell & Trench 1993, Trench 1993, Muscatine et al. 1994). In return, the host provides carbon dioxide for photosynthesis and supplies metabolic waste products (containing nitrogen and other elements including phosphorus) to the symbiont. The symbionts recycle the nitrogen by incorporating it into amino acids, which are then translocated back to the coral for

use in respiration. Additionally, by hosting *Symbiodinium* cells, the host conserves nitrogen by preferentially using the low molecular weight nitrogen produced by the symbiont for its own respiration, rather than host-derived amino acids (Wang & Douglas 1998). These nutritional interactions promote the growth and development of coral reefs in nutrient-poor tropical seas (Muscatine & Porter 1977), with energy from the symbiont also assisting in coral calcification and hence formation of the reef framework (Pearse & Muscatine 1971, Hoegh-Guldberg et al. 2004). The lack of coral reefs in temperate waters, and the reduced framework of coral communities at high latitudes, is in a large part related to the reduced capacity of the dinoflagellate symbionts to photosynthesise in cooler waters, leading to a reduced capacity for net reef accretion (Glynn & Stewart 1973, Crossland 1981).

(a) **(b)**

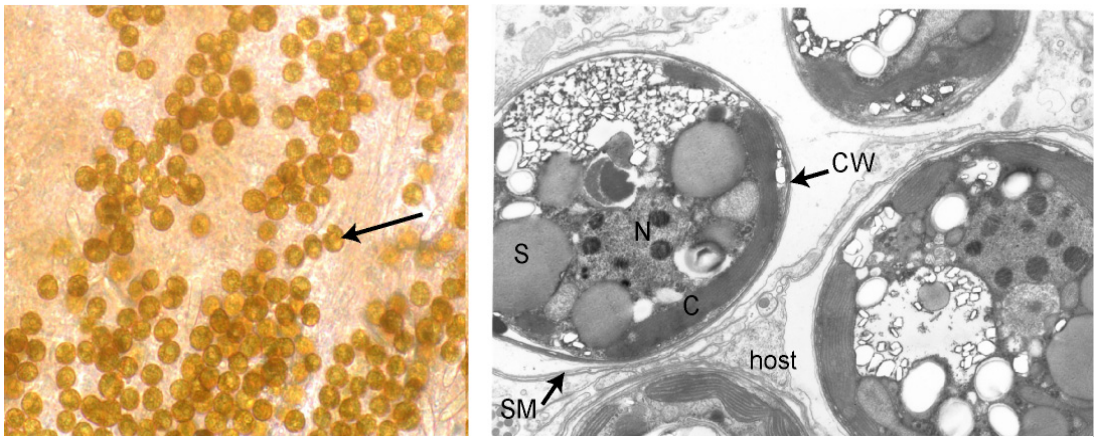


Figure 1.1 Freshly isolated zooxanthellae (*Symbiodinium* sp.). (a) light microscope of zooxanthellae cells, each cell ~5 µm. Arrow points to single zooxanthella. Courtesy of Ove Hoegh-Guldberg. (b) transmission electron micrograph of zooxanthellae in a thin section of the sea anemone *Aiptasia pallida* showing cellular structure and host symbiont arrangement. CW, cell wall; SM, symbiosome membrane; S, starch storage granule; C, chloroplast; N, nucleus containing dark, permanently condensed chromosomes. Courtesy of Giselle Muller-Parker and Kit Lee.

1.2 Genetic diversity of *Symbiodinium*

The lack of distinct morphological differences between *Symbiodinium* types led to the initial belief that the genus consisted of a single species, *Symbiodinium microadriaticum*. However, over the past several decades, molecular, microscopic and physiological analyses of various *Symbiodinium* spp. have repeatedly shown that considerable genetic, morphological, biochemical and physiological diversity exists within the genus (Freundenthal, 1962, Schoenberg & Trench 1980a, b, c, Chang et al. 1983, Tytler & Trench 1986, Trench & Blank 1987, Markell & Trench 1993, Trench 1993, reviewed by Coffroth & Santos 2005).

Current knowledge on the evolution, biogeography and ecology of *Symbiodinium* stems from our ability to, with the aid of molecular techniques, distinguish members of this highly diverse group of microorganisms. Thus far, 8 clades (A-H) and numerous sub-cladal types of *Symbiodinium* have been identified, with six of these clades (A-D, F and G) known to form associations with scleractinian corals (reviewed in Rowan 1998, Baker 2003, Coffroth & Santos 2005). *Symbiodinium* clades represent divergent lineages, with the sub-clades representing closely related molecular 'types' (Fig. 1.2). A 'type' refers to the genetic lineage of *Symbiodinium* defined by a unique ITS2 sequence. The greatest number of types has thus far been identified in clade C, which is found in association with most marine invertebrate hosts that harbour *Symbiodinium* cells and is particularly prevalent in the Pacific Ocean (Baker & Rowan 1997, Veron 2000, LaJeunesse et al. 2003, 2004a, b). Symbiont types C3 and C1 (identified by the internal transcribed spacer region, ITS2) have been called the 'living ancestors' of the clade C lineage (LaJeunesse 2005), from which all other known types diverge. The diversity within clade C *Symbiodinium* has been postulated to have resulted from a selective sweep of these

types or their ancestor before the closure of the Central American Isthmus. This was followed by allopatric evolution, resulting in different molecular types found in conspecific hosts between the two Oceans (LaJeunesse 2001, 2005). Recently it has been proposed that these multiple sub-cladal types represent intraspecific sequence variations, as they are only minimally divergent from each other (i.e. 1 basepair) (Correa & Baker 2008), however issues have arisen with that study (see LaJeunesse et al 2008, Sampayo et al 2008). The lack of agreement on sub-cladal distinction remains an ongoing issue, with attempts to develop multiple, independent markers continuing (Coffroth & Santos 2005, Correa & Baker 2009).

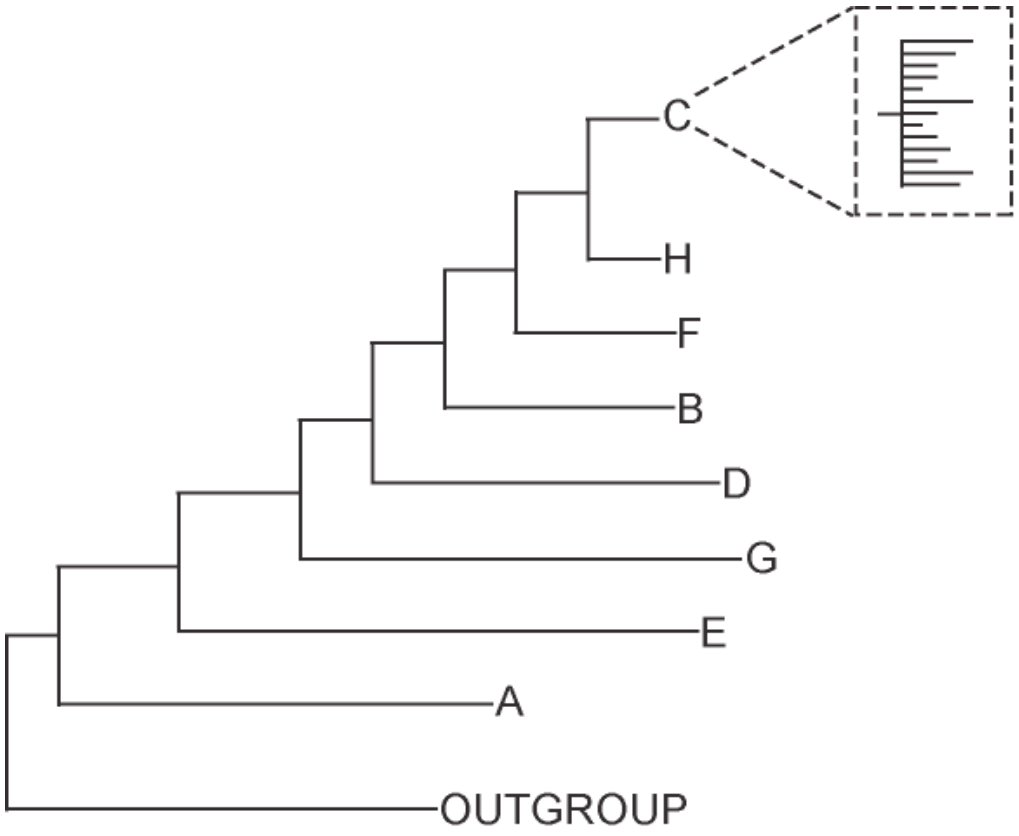


Figure 1.2 *Symbiodinium* clade phylogeny inferred using rDNA. The subcladal diversity is represented for clade C only. Adapted from Pochon et al. (2004).

Although initial studies used the small subunit (n18s) rDNA for taxonomic identification of *Symbiodinium* (Rowan & Powers 1991), recent studies have largely been based on multi-copy ribosomal RNA genes, including the large subunit region (28S) (Rowan 1998, Baker 1999), and the more rapidly evolving, noncoding ITS1 and ITS2 (Hunter et al. 1997, Baillie et al. 2000, LaJeunesse 2001, van Oppen et al. 2001), in addition to the chloroplast large subunit (23S) (Santos et al. 2002), and mitochondrial protein-coding gene (cytochrome oxidase subunit I (COX1); Takabayashi et al. 2004).

For the rapid resolution of distinct *Symbiodinium* types, molecular screening techniques have been used, including denaturing gradient gel electrophoresis (DGGE; e.g. LaJeunesse et al. 2003, 2004a), restriction fragment length polymorphism (RFLP) analysis of small (SSU) and large subunit (LSU) nrDNA (Baker 1999), temperature gradient gel electrophoresis (TGGE, Carlos et al. 2000), single strand conformational polymorphism (SSCP) analysis of ITS nrDNA (van Oppen et al. 2001, Ulstrup & van Oppen 2003, van Oppen 2004), length heteroplasmy of LSU cprDNA (Santos et al. 2003) and phylogenetic analysis of microsatellite flanking regions (Santos et al. 2004). Although efforts to uncover the true diversity of *Symbiodinium* communities have been confused due to the application of different screening techniques in different laboratories, several independent approaches have been shown to uncover the same level of diversity within a community survey (Sampayo et al. 2009). Population level studies of *Symbiodinium* diversity and distribution have recently been developed, using microsatellites, with this technique likely increase in usage in the future.

Over the past 5 years, DGGE of the ribosomal ITS1 and ITS2 regions has been widely used to characterise the symbionts found in cnidarian hosts (Diekmann

et al. 2002, LaJeunesse 2002, LaJeunesse et al. 2003, LaJeunesse et al. 2004a, Sampayo et al. 2007). Both ITS1 and ITS2 have provided insight into sub-cladal classification ('types'), with ITS2 being the most widely used marker. DGGE can be used to distinguish differences in the primary sequence (e.g. ITS2) based on disparities in electrophoretic mobility of the sequence. DGGE was originally developed to detect specific mutations within genomic genes due to one base mismatch (Myers et al. 1985). Since Muyzer et al. (1993) applied this method to environmental microorganisms, analyses of microbial communities using DGGE have become increasingly popular.

The use of DGGE in cnidarian hosts began with LaJeunesse and Trench (2000) and has since been used with ITS1 and ITS2 rDNA to distinguish genetically and ecologically distinct symbionts (e.g. LaJeunesse 2001, Sampayo et al. 2007, LaJeunesse et al. 2008). The advantages of DGGE are that it allows the simultaneous analysis of multiple samples and the comparison of *Symbiodinium* communities both within a single coral colony and within an environment. Further, excision of the bands enables sequence data to be obtained on dominant *Symbiodinium* types. As with all molecular screening techniques, disadvantages also exist, mainly in relation to sequence co-migration and heteroduplexes (Apprill & Gates 2007, LaJeunesse & Pinzon 2007, Thornhill et al. 2007, Sampayo et al. 2009). However, when applied correctly and interpreted conservatively, ITS1 and ITS2 DGGE remain the most appropriate techniques available at present for identifying *Symbiodinium* diversity among cnidarian hosts. The benefits and disadvantages of multiple screening techniques of *Symbiodinium* communities are outlined in Table 1.1.

Table 1.1 Overview of cost, limitations and solutions to limitations of various analysis techniques used to detect diversity on the dinoflagellate genus *Symbiodinium*. Adapted from Sampayo et al. (2008).

	Direct sequencing	**Cloning**	**DGGE**	**SSCP**	**RFLP**
Cost	Moderate-High	High	Moderate	Low-Moderate	Low
Technical expertise	Moderate	Moderate-high	Moderate-high	Moderate	Low
Limitations	• Sequencing ambiguities • Expensive	• Cloning/sequencing errors • Repeatability • Intragenomic variants • Expensive	• Sequence co-migration • Heteroduplexes	• Sequence co-migration • Two bands created for every sequence	Low Resolution (analyses small component of sequence)
Solutions to limitations	Ambiguities limited by direct sequencing of conserved rDNA regions or homogenous nuclear or organelle genes	• Sequence substantial number of clones per sample • Use isoclonal cultures as controls	• Examine and compare entire fingerprint to identify potential co-migrating sequences • Commonly perform band excision and sequencing	Analyses can be performed on SSCP apparatus with unlabelled primers	Increase no. of different restriction enzymes used

1.3 Host-symbiont specificity and flexibility

The current understanding of *Symbiodinium* diversity and host specificity reveals a great deal of complexity in the relationship. The host symbiont partnership can exhibit specificity and flexibility i.e. some members of the same host species generally harbour the same *Symbiodinium* type, or clade at least (Rowan and Powers 1991, Baker et al. 1997), whereas some host species can harbour numerous types of *Symbiodinium* (LaJeunesse 2002, Baker 2003). Single coral colonies can also harbour multiple *Symbiodinium* types, on both a cladal (Baker 2003, Mieog et al. 2007) and sub-cladal level (Ulstrup & van Oppen 2003). Typically, one or two types are numerically dominant (Baker 2003, LaJeunesse 2005, Baker & Romanski 2007, Goulet 2007), with a recent study, using highly sensitive real-time PCR, showing that the presence of multiple cryptic types at low densities within a single colony is common (Mieog et al. 2007).

Specificity also varies between symbiont types; many are known to associate with numerous host taxa ('host generalists'), whilst copious others are specialized to particular host species or genera ('host specialists'; LaJeunesse et al. 2003). For example, type C8/a has only been found to associate with the scleractinian pocilloporid *Stylophora pistillata (*Sampayo et al. 2007), whereas C3 associates with a broad array of coral families, including Acroporidae, Faviidae and Pocilloporidae (e.g. LaJeunesse 2001, LaJeunesse et al. 2003, LaJeunesse 2005).

Although a much debated issue, studies have suggested that the presence of a host-generalist *vs.* a host-specialist symbiont may be strongly linked to the mode of symbiont transmission in the host. Symbionts are acquired by their hosts in one of two ways; either from the water column (horizontal transmission) or via maternal inheritance (vertical transmission). Corals that brood their larvae reproduce by

internal fertilisation and develop their planktonic larvae before release into the water column. As these larvae already contain their symbionts, faithful transmission of symbionts from parents to the next generation is maintained (Barneah et al. 2004). Additionally, corals that reproduce asexually directly pass their symbionts to the new coral colony in the parental tissues. Vertical transmission can be beneficial since it maintains fidelity with a well-suited symbiont, however horizontal transmission allows a shift to a new symbiont pool (Douglas 1998, Rowan 1998). Alternatively, corals that broadcast spawn their larvae, by releasing their gametes into the water column for external fertilisation (Harrison et al. 1984, Harrison & Wallace 1990), can acquire their symbionts in a number of ways, including from the surrounding water column and by infection of the ova prior to their release (Davy & Turner 2003). Broadcast spawning is the dominant reproductive mechanism of corals, (~85%) (Schwarz et al. 2002) and occurs as a synchronous event (Harrison et al. 1984).

The specificity of the host-symbiont relationship has further been suggested to result in co-evolution of specific symbionts types with their vertically transmitting hosts (LaJeunesse 2002, LaJeunesse 2002, Diekmann et al. 2003, LaJeunesse 2005), evident in the proportionally high number of specific, unique symbiont types in vertical transmitters. However there are conflicting data on whether this symbiont acquisition strategy does influence the final host–symbiont assemblage (Loh et al. 2001, van Oppen 2004, Stat et al. 2009). Recent comparative analysis by Stat et al. (2009) found that the majority of vertical transmitting corals associated with distinct species- or genus-specific *Symbiodinium* types, but that some could also associate with the generalist types more common to horizontal transmitters. Host-specific lineages have so far only been detected in one horizontal strategist, *Fungia scutaria*,

which hosts specialist C1f in Hawaii (Weis et al. 2001, Rodriguez-Lanetty et al. 2004).

Specificity between a host and its symbiont also depends heavily on the host, particularly in regards to recognition mechanisms involved in the establishment of symbiosis. While much remains to be learnt of the mechanisms involved (Weis 2008), it is understood that the identity of the symbiont is a key factor in the onset of symbiosis, with variation in the algal cell structure affecting phagocytosis triggers in the host (Markell et al. 1992, Lin et al. 2000). After a symbiont enters a host, the host will have some way to regulate the symbiont population, either through controlling reproduction rates of, selectively destroying, or expelling unwanted symbionts (Gates et al. 1992, Falkowski et al. 1993, Baghdasarian & Muscatine 2000, Dunn et al. 2002, Dunn & Weis 2009). Furthermore, within the host cell, competition between symbionts will determine which symbiont types will be found in the host, with certain types competing more successfully within a particular host or region within a host (Sachs & Wilcox 2006, Fay et al. 2009). Finally, the fitness of the holobiont (host and symbiont; Rohwer et al. 2002), will determine the abundance of a particular host-symbiont pair; as the fitness interests of both partners are aligned, the holobiont will have a fitness advantage over holobionts with mismatched fitness interests.

1.4 Biogeographic and ecological variability of Cnidarian-*Symbiodinium* partnerships

Variability in the *Symbiodinium* type harboured by a host is seen on both a local and biogeographic scale, in response to the environment in which the holobiont lives (e.g. LaJeunesse & Trench 2000, Rodriguez-Lanetty et al. 2004). For instance, clade C tends to dominate in 'normal' stable conditions, while clade B tends to be more prevalent in harsher colder conditions (Baker 2003, LaJeunesse et al. 2003). Latitudinal shifts in host-symbiont pairings have been observed for a number of host species, with a transition from clade C in tropical zones to clade A, B or D recorded by different studies on invertebrate hosts from Japan, the north-eastern Atlantic and Mediterranean Sea (Loh et al. 2001, Rodriguez-Lanetty et al. 2001, Savage et al. 2002). Specifically, LaJeunesse & Trench (2000) found that the sea anemone *Anthopleura elegantissima* from the temperate northern sites of Washington and Oregon contained a different symbiont (clade B) in comparison to southern Californian sites (clade E). This latitudinal pattern was mirrored in the coral species *Plesiastrea versipora* (Rodriguez-Lanetty et al. 2001) along the East Australian coast, whereby a cladal transition occurs from clade C in the tropics and sub-tropics, to clade B in the cooler, high-latitude communities. A recent study in southeast Africa indicates that similar patterns of latitudinal zonation between clades exist at the sub-cladal level, with a transition of clade C *Symbiodinium* types in *Stylophora pistillata* in relation to a latitudinal temperature gradient (Macdonald et al. 2008).

Niche partitioning of host-symbiont partnerships can be seen on a local scale, with irradiance and temperature the two most common environmental variables to correlate with their distribution. Initial observations of the zonation of symbionts according to depth, and associated irradiance, were on the coral *Montastraea* spp.

located on reefs in the Caribbean (Rowan & Knowlton 1995, Rowan et al. 1997, Baker 2003). Clade A *Symbiodinium* was found in *Montastraea* spp. in shallow waters (< 6 m) while clade C *Symbiodinium* was restricted to deeper colonies. Further studies of the zonation of symbionts within a single colony of *Montastraea* sp. found that clades A and B were present in the top of the colony subject to high irradiance, clades A to C at intermediate depths within the colony, and clade C only in the most shaded portions of the colony (Rowan et al. 1997). Depth-related patterns were also observed in scleractinian corals on the GBR: LaJeunesse et al. (2003) identified nine coral species in which the symbiotic partner varied according to host depth. Many of the patterns identified by LaJeunesse et al. (2003) featured variation at a sub-cladal level and these have been corroborated by numerous other studies (LaJeunesse et al. 2004a, LaJeunesse et al. 2004b, Sampayo et al. 2007, Macdonald et al. 2008). In the Caribbean, Frade et al. (2008a,b) found that the depth stratification of clade B *Symbiodinium* types in *Madracis* sp. had associated functional variability: type B15, which was predominantly found at deeper sites, occurs with lower cell densities, larger cell sizes, higher photosynthetic pigment concentrations, and higher peridinin to chlorophyll (chl) *a* ratios than does type B7. These attributes would make B15 better adapted to darker environments. Type B7, on the other hand, was found in shallow colonies and exhibited functional differences consistent with the high irradiance environment, such as a larger dinoxanthin to chl *a* ratio and larger xanthophyll-pool size, suggesting that it is probably more competent in quenching excess of energy.

In terms of the impact of irradiance of symbiont distribution, sedimentation in the water column is a further influential factor. Toller et al. (2001a) found that clade D dominated hosts in areas subject to high sedimentation and suggested that this

clade was more 'stress tolerant' than other clades. Similarly, Garren et al. (2006) found a lack of clade B *Symbiodinium* in *Montastraea annularis* in environments characterised by high sedimentation in Panama where irradiance levels were reduced, whereas clade B was dominant in the waters of Belize with enhanced irradiance levels. Finer scale patterns were suggested to exist, with further examination required using higher resolution molecular techniques. To date, no studies have addressed the sub-cladal distribution of symbionts in response to sediment regime.

The ability of a coral to associate with different symbiont types has ecological implications and has been suggested to delineate the tolerance range of the host. In a genetic and physiological study, Iglesias-Prieto et al. (2004) showed that symbionts may directly influence the vertical distribution of a host species. These authors found that, in two coral species on eastern Pacific reefs, the efficiency of light capture by their symbionts correlated with the depth at which they were found and, following depth transplantation, photosynthetic measurements of the symbionts continued to show the same 'sun-loving' or 'shade-loving' qualities. However, the symbionts may have evolved with the host, to live in the hosts' restricted distribution. The fact that the external environment can lead to specific pairings of hosts and physiologically distinct symbionts will play a key role in the evolutionary success of coral reefs.

1.5 Physiological variability and susceptibility to environmental stress

The physiological variability between *Symbiodinium* types is evident in their local and biogeographic distributions, as has been described in the previous sections, and importantly, can also dictate the tolerance of the holobiont to environmental stress. Coral bleaching involves the breakdown of the symbiotic relationship, whereby the

symbionts and/or their pigments are lost as a stress response (see next section for details of the mechanism). Stressors that induce bleaching include high or low seawater temperature or light levels, reduced salinity, and sedimentation (e.g. Rowan 2004, Tchernov et al. 2004, Berkelmans & van Oppen 2006, Jones 2008). Elevated sea surface temperature (SST) is the main driver of mass coral bleaching and has led to the decimation of reefs worldwide (Hoegh-Guldberg 1999, Donner et al. 2005).

Highly variable patterns of bleaching have been observed between and within geographic regions (Hoegh-Guldberg 1999, Marshall & Baird 2000), as well as between single coral species (Edmunds 1994, Glynn et al. 2001, Brown et al. 2002). This variation has been linked to both host-related factors (i.e. tissue behaviour, Brown et al. 2002; tissue thickness, Fitt et al. 2009), and/or the presence of physiologically distinct symbionts (Rowan et al. 1997, Glynn et al. 2001, Baker et al. 2004, Rowan 2004, Berkelmans & van Oppen 2006). For example, *Pocillopora damicornis* colonies which harboured clade D *Symbiodinium* were less susceptible to bleaching than those containing clade C (Rowan 2004). Clade D *Symbiodinium* have been suggested to be more resistant to thermal stress than other clades (Rowan 2004) and evidence has shown that some coral reefs which have been subject to repeated bleaching episodes harbour a greater proportion of D-type symbionts (Glynn et al. 2001, Toller et al. 2001a, Baker et al. 2004, Fabricius et al. 2004, Rowan 2004). Variability in thermal tolerance has also been observed between sub-cladal types. For example, colonies of the coral *Stylophora pistillata* hosting C78 and C8/a were more thermally tolerant than those containing C79 and C35/a, which suffered significant bleaching and post-bleaching mortality (Sampayo et al. 2008).

It has been hypothesised that some coral species have the potential to adapt to a particular environment by 'switching' or 'shuffling' their stress-vulnerable

symbionts to stress-resistant *Symbiodinium* types (Buddemeier & Fautin 1993, Baker 2001, Baker 2003, Fautin & Buddemeier 2004). Switching refers to the uptake of a novel symbiont from the external environment as an adaptation to the new conditions. Conversely, shuffling refers to the increase in abundance of a 'cryptic' symbiont in the host, better adapted to the new conditions. This much debated issue is known as the 'Adaptive Bleaching Hypothesis' (ABH: Buddemeier & Fautin 1993). Berkelmans and van Oppen (2006) found that transplanting *Acropora millepora* from cooler to warmer sites on the Great Barrier Reef resulted in a change in their symbiont communities to favour *Symbiodinium* in clade D and that this transition increased their thermal tolerance by up to 1.5°C. Jones et al. (2008) corroborated this evidence of a change in symbiont populations in *A. millepora,* with shuffling of pre-existing symbiont types observed following a natural bleaching event.

It is important to note that coping with thermal or other stressors is also dependent on the flexibility of a host to associate with multiple physiologically distinct symbiont types, whether by shuffling or switching its symbionts. It is still unknown if the specific host–symbiont combinations found in nature can switch or shuffle with time, and do so rapidly enough to keep pace with the changing conditions associated with climate change. To understand how and why bleaching occurs, and how this varies between different coral-host partnerships, first we must understand the photosynthetic system involved.

1.6 Photophysiology of *Symbiodinium*

Under normal conditions, *Symbiodinium* cells can exhibit very high rates of photosynthesis, assimilating large amounts of carbon dioxide and generating large quantities of dissolved oxygen (Lesser 2006). Maximum photosynthesis, and hence productivity, is dependent upon the efficiency of the light harvesting complexes (LHC) photosystem 1 (PSI) and photosystem 2 (PSII), in capturing and utilising light energy (Fig. 1.3). The two photosystems, present on the thylakoid membrane, pass light energy to the dark reactions where CO_2 is fixed by the enzyme ribulose biphosphate carboxylase oxygenase (Rubisco). The amount of light energy flowing to the dark reactions is regulated by the inter-conversion of the two pigments diatoaxanthin and diadinoxanthin. Any active oxygen (O_2^-) is quenched by the superoxide dismutase (SOD) and ascorbate peroxidise (APX) enzyme systems (Fig. 1.3).

As the light environment experienced by corals can be highly variable, the symbionts must be capable of adjusting to changes in light quality and quantity. The symbionts must dissipate excess excitation energy absorbed by the photosynthetic apparatus, when the light absorbed is in excess of that required for CO_2 assimilation (Falkowski & Raven 1997, Lesser & Farrell 2004). Increasing light levels lead to the over-reduction of the light reactions and the production of potentially harmful products such as reactive oxygen species (ROS, Lesser 2006). Photoprotective measures are in place in corals and their symbionts to limit the effects of daily and seasonal stressors associated with high light intensities. These include ultraviolet radiation (UVR)-absorbing mycosporine-like amino acids (MAAs) (Shick & Dunlap 2002), excess excitation energy dissipation in PSII via the xanthophyll cycle (i.e.

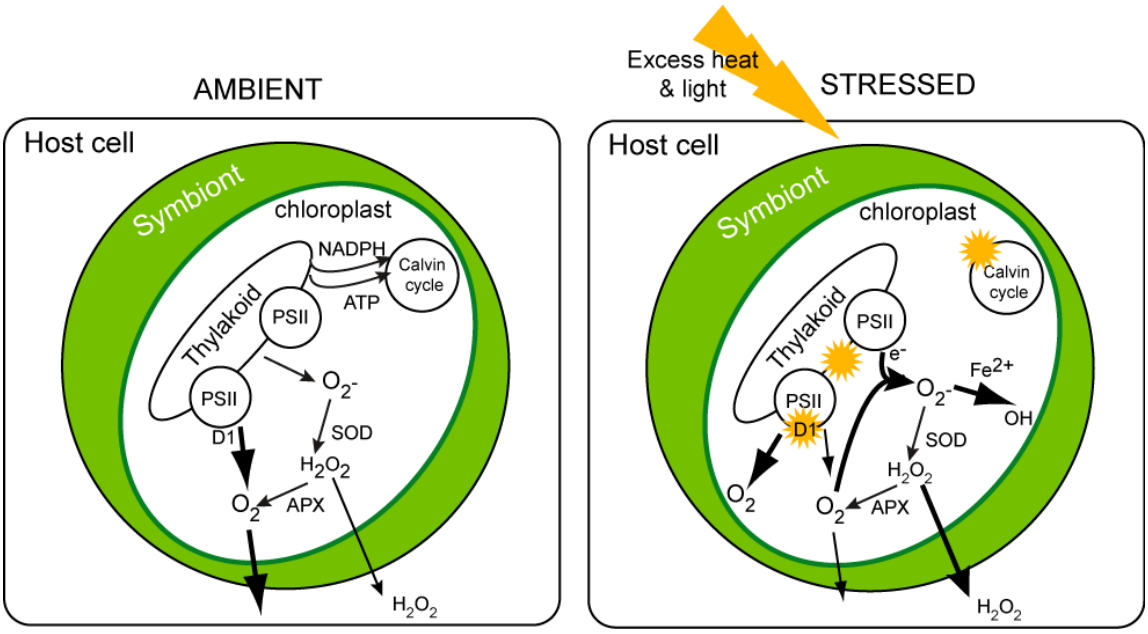


Figure 1.3 Schematic representation of photosynthesis under ambient conditions and elevated temperature and light conditions (stressed). Under ambient conditions, photosystems I (PSI) and II (PSII) on the thylakoid membrane operate normally, producing large amounts of oxygen which diffuse into the host. Any reactive oxygen species produced (ROS) are converted back to oxygen by superoxide dismutase (SOD) and ascorbate peroxidase (APX). Under stressed conditions, damage occurs to the photosystem in at least three places (depicted as yellow flashes): the D1 protein, the Calvin Cycle, and on the thylakoid membrane. This damage produces large amounts of ROS (in the form of $^{\cdot}O_2$ and $O_2^{\cdot}$) that overwhelm the oxygen handling pathways. $O2^{-}$ is converted to both the most highly reactive hydroxyl radical (-OH) and the more stable and highly diffusible hydrogen peroxide (H_2O_2), which can move into host tissues. Figure adapted from Weis (2008) and Venn et al. (2008).

non-photochemical quenching, NPQ, Demmig-Adams et al. 1990, Brown et al. 1999, Gorbunov et al. 2001), the expression of heat-shock proteins and other stress markers (Black et al. 1995, Downs et al. 2002, Lesser & Farrell 2004), the upregulation of antioxidant enzymes (Lesser 1996, 2004, 2006), host energy reserve utilization (Porter et al. 1989, Grotolli et al. 2006), and heterotrophic plasticity (Grotolli et al. 2006).

The primary cause of bleaching is thought to occur as a result of photosynthetic damage to *Symbiodinium* cells by thermal stress (either high or low) and high irradiance (Fitt & Warner 1995, Warner & Fitt 1999, Yakovleva & Hidaka 2004), in at least three inter-related ways (Fig. 1.3). Firstly, damage to the D1 protein and its repair mechanism can occur, which results in a 'backup' of excitation energy and consequent dysfunction of PSII (Warner & Fitt 1999). Secondly, energetic uncoupling in the thylakoid membranes can result, due to damage to the thylakoid membrane (Tchernov et al. 2004). Finally impairment of the Calvin-Benson Cycle can occur, with the site of damage suggested to be Rubisco, the enzyme responsible for primary carboxylation (Lesser 1996). This results in reduced consumption of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) coming from the light reactions (referred to as sink limitation) that in turn leads to the dysfunction of photosystem II *via* backup of excitation energy as described above (Jones et al. 1998, Venn et al. 2008a). In all cases, excess energy is passed to oxygen, causing a build-up of ROS that damage cellular components such as proteins and nucleic acids, and induce physiological malfunction. Furthermore, ROS may diffuse from the symbiont into the coral cytoplasm, where they cause further oxidative damage (Lesser 2006), and may trigger the coral to expel its algal symbionts (Downs et al. 2002, Tchernov et al. 2004, Weis 2008). As even moderate increases of 1 – 2°C can result in reef-wide bleaching events, especially when combined with seasonal high visible and/or UV radiation (Hoegh-Guldberg et al. 2007), understanding why some host-symbiont partnerships bleach whilst others can tolerate the stress is an area of great interest.

1.7 Corals in marginal environments

Central to understanding how corals may cope in the future, with increasing SST and changing chemistry of the oceans, is knowledge of how corals persist in marginal environments, already subject to variable and stressful conditions. Coral reefs are most commonly associated with shallow, clear water environments at low-latitudes. In these locations, environmental conditions are commonly termed as 'optimal', with coral communities growing under favourable conditions and mild human impacts, and build well-defined reef structures. However, many reefs are associated with sub-optimal conditions and hence are termed as 'marginal'. Here, corals exist close to their survival thresholds of water temperature, light penetration and aragonite saturation (Kleypas et al. 1999, Table 1.2). These marginal coral communities generally occur outside the tropical belt or at its edge (Fig 1.4) and are commonly characterised by limited accretion, depth distribution and biological diversity (Harriott & Banks 2002). Living under these highly variable and extreme conditions is likely facilitated by specific physiological adaptations and/or the presence of unique species of corals and dinoflagellate symbionts, but data on these patterns and processes are limited.

High latitude or temperate reefs, usually occurring above 25°S and 25°N, represent marginal environments due to wide ranges in seasonal temperatures (widest in Arabian Gulf, 18-36°C, Riegl 2003), low annual minimum temperatures (lowest 13.3°C, Japan, Yamano et al. 2001) and relatively low irradiance levels (e.g. light penetration of 250 μmol photons m^{-2} s^{-1} seasonally less than 10 m, Japan, Kleypas et al. 1999). Examples of high latitude coral communities include the Persian Gulf, Bermuda, Japan

Table 1.2 Characteristics of coral communities existing under optimal environmental settings (warm, clear water setting) and those associated with two distinct marginal environments. *Symbiodinium* types noted for typical optimal, turbid and high latitude sites.

	Warm, clear water setting	**Turbid setting**	**High latitude setting**
Environment	Clear waters, optimal temperatures, low nutrient availability	Reduced light penetration, high nutrient load	Cooler waters, reduced light penetration
Coral community	High species diversity High coral cover extending to depths of 60-70 m	Restricted species diversity Coral growth spatially and bathymetrically restricted (generally < 15 m)	Reduced species diversity and cover Soft coral dominated communities, bathymetrically restricted to < 30 m
Coral physiology	Optimal photosynthesis	Depressed photosynthesis, enhanced respiration and mucus production	Reduced rates of photosynthesis
Symbiodinium	Clade C dominant in Pacific, A, B and C in Caribbean (Baker 2004)	Clade D (Toller et al. 2001) and C (Garren et al. 2006)	Clades A, B and/or F (Rodriquez Lanetty et al. 2002, Savage et al. 2002)
Framework	Thick (5-10 m) coral dominated framework	Detrital coral-rubble framework set with mixed carbonate:clastic sediments	Non-framework coral community common
Examples	Northern GBR (van Woesik & Done 1997) Jamaica (Liddell & Ohlhurst 1981)	Inhaca Is., Mozambique (Perry 2003) Inshore central GBR (Smithers & Larcombe 2003)	Kermadec Islands (Schiel et al. 1986) S. Africa (Riegl 2003) Lord Howe Island, Australia (Veron & Done 1979)

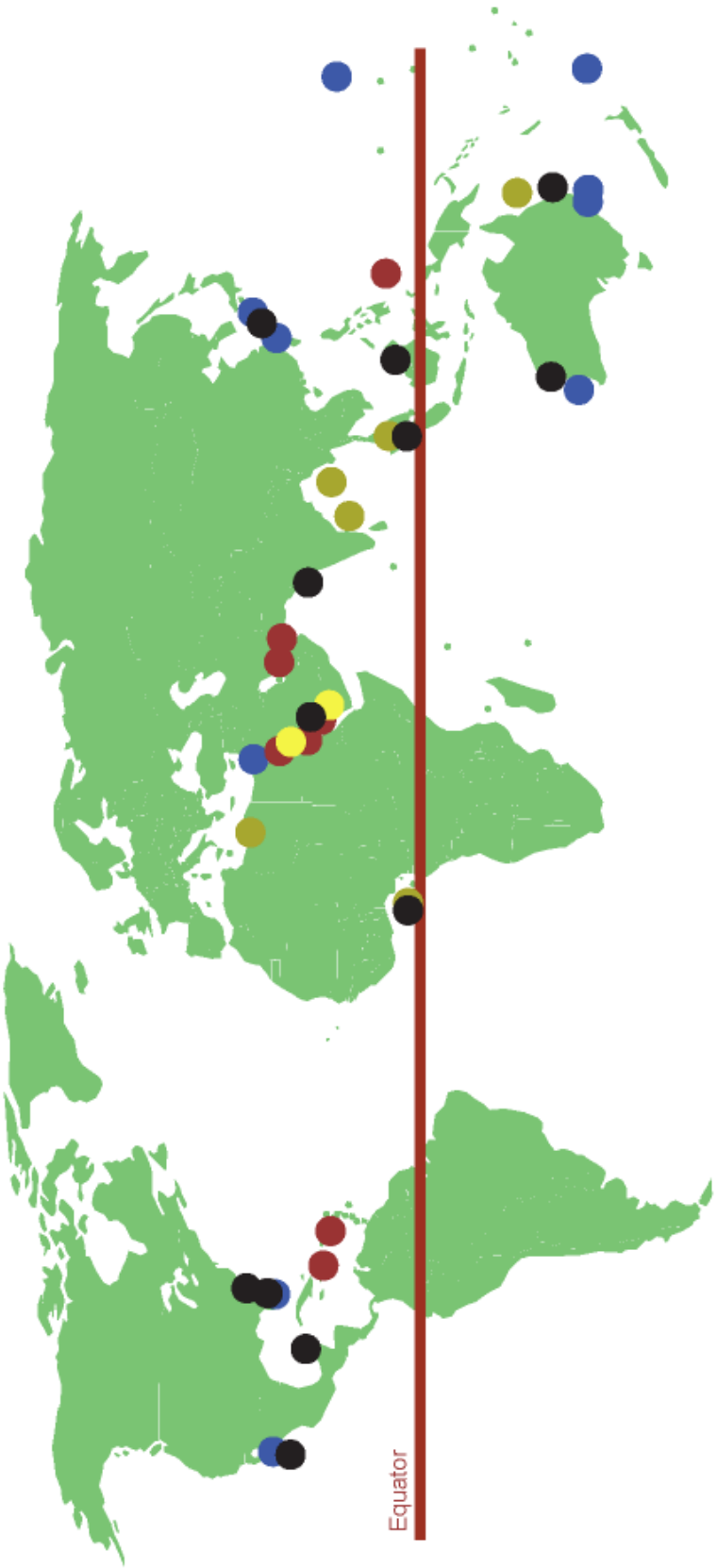


Figure 1.4 Distribution of marginal coral reefs and communities, as determined by Kleypas et al. (1999). ● low temperature, ● high temperature, ● low salinity ● high salinity ●low light penetration. Other environmental variables omitted for ease of interpretation

and Lord Howe Island, Australia. Under these challenging environmental conditions, coral diversity and cover are generally low and existing corals have reduced growth rates in comparison to their tropical counterparts (Crossland 1988, Veron 1995, Lough & Barnes 2000). Total reef accretion is reduced in high latitude areas and in the extremes of hermatypic coral growth, corals form so-called non-reef building communities (van Woesik & Done 1997, Perry & Larcombe 2003).

Many reef-building species cannot grow and survive outside the tropics, and are replaced at high latitudes by subtropical non-reef building species, which are rare or absent on tropical reefs. For example, on the eastern Australian coast, massive *Porites* and staghorn *Acropora* species decline in abundance with increasing latitude, replaced by foliose and encrusting corals such as *Turbinaria* and *Montipora* species (van Woesik & Done 1997). The absence of the dominant components of tropical reefs at high latitudes suggests that they are unable to establish and survive at these cooler temperatures and sub-optimal light levels. Lower temperatures and high nutrient concentrations, common to high latitude sites, favour the growth of macroalgae over corals at many subtropical reefs (Johannes et al. 2003, Harriott & Banks 2002; Fig 1.5).

Marginal communities can also occur within the tropics, where localised conditions are 'sub-optimal', such as areas of high sedimentation or upwelling (Perry & Larcombe 2003). For example, an increase in sediment load in the waters surrounding coral reefs can lead to reduced irradiance, compressed biotic zones, reduced species richness, and reduced coral cover, colony size and recruitment (Brown & Howard 1985, Rogers 1990, Brown 1997a, Fabricius 2005). Marginal coral reefs and communities

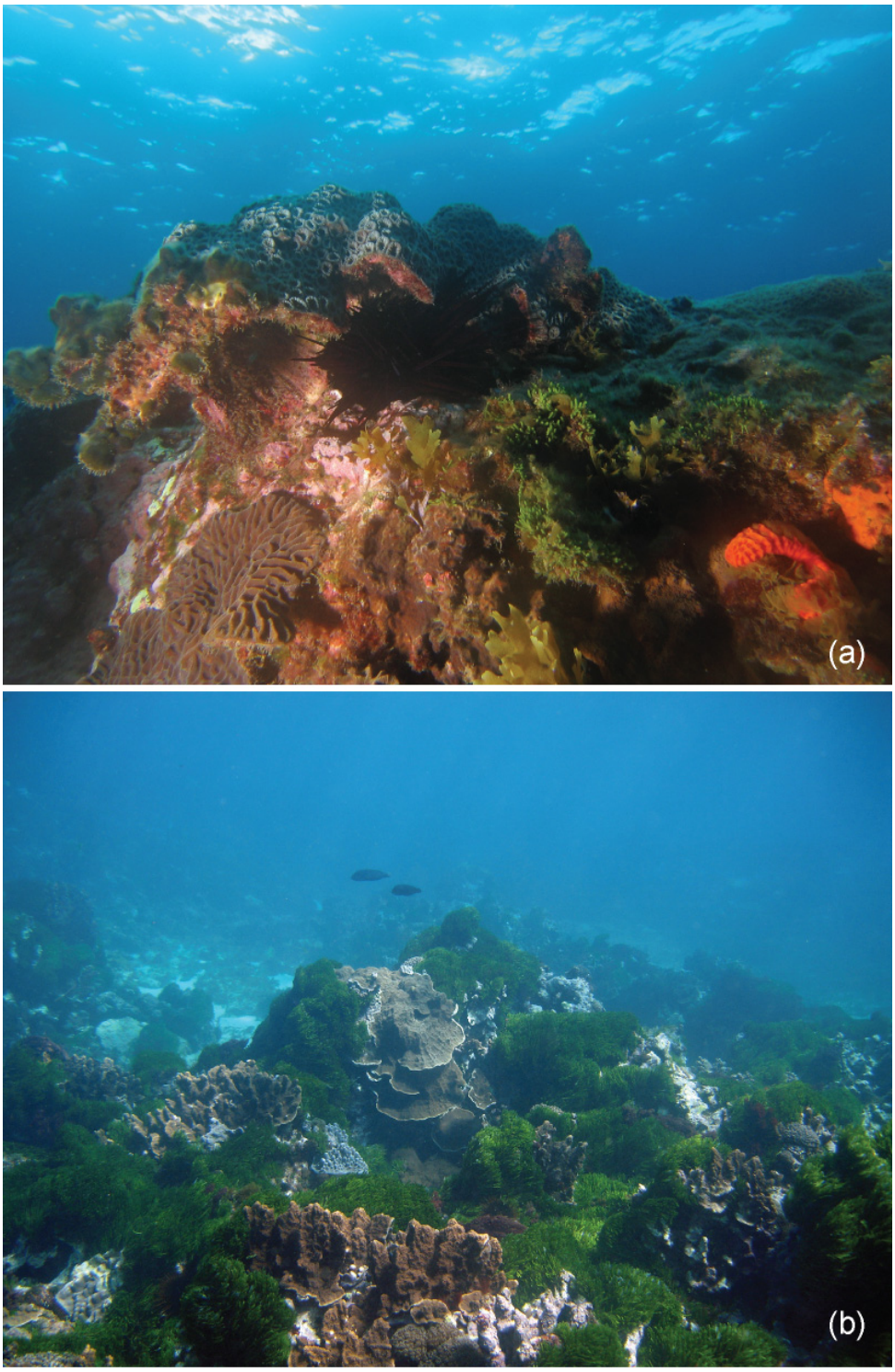


Figure 1.5 Landscape of typical high latitude coral reefs (a) Kermadec Islands, New Zealand, and (b) Lord Howe Island, Australia. Kermadec photo courtesy of Jo Long.

predisposed to environmental variability have been suggested as potential refuges for their resident populations or immigrant species in a future of warming seas and ocean acidification (Glynn 1996, Riegl 2003). If these communities will indeed act as coral refuges, then it is essential that we understand the dynamics of the coral and symbiont communities, and their physiological ability to survive under these variable conditions.

1.8 *Symbiodinium* in marginal environments

From the few studies conducted on marginal coral communities (both reefal and non-reefal), it has been shown that coral and *Symbiodinium* partnerships in these environments differ from their tropical counterparts. For example, marginal reefs in the Persian Gulf and marine lakes in Palau both experience extremes of temperatures and salinity, and are dominated by corals hosting clade D *Symbiodinium* (Baker et al. 2004, Fabricius et al. 2004, Mostafavi et al. 2007). Furthermore, whilst clade D is generally thought to be thermally tolerant, it is also found in high latitude colder regions (Chen et al. 2003, Lien et al. 2007) and limited light environments (Glynn et al. 2001, Toller et al. 2001b, Baker et al. 2004, Fabricius et al. 2004, Rowan 2004). Clades A, B, C and D have all been found in coral and/or anemone hosts at high latitudes and temperate regions, including Japan (Loh et al. 2001), Korea (Rodriguez-Lanetty et al. 2002), USA (LaJeunesse & Trench 2000), and Europe (Savage et al. 2002). The discrepancy in cladal distributions highlights increasing evidence that physiological tolerance ranges overlap poorly at the cladal level, and that the wide variety of sub-cladal *Symbiodinium*

types plays an important role in the distribution, ecology, and physiology of reef corals (Iglesias-Prieto & Trench 1997a, Tchernov et al. 2004, Sampayo et al. 2007, Frade et al. 2008a, Macdonald et al. 2008, Sampayo et al. 2008).

While it is assumed that corals and their symbionts have inadequate phenotypic/genotypic capabilities for rapid adaptation in response to environmental change (Hughes et al. 2003, Hoegh-Guldberg et al. 2007, Edmunds & Gates 2008), the presence of physiologically distinct symbionts and host-symbiont partnerships in marginal habitats may be measures whereby hosts have adapted to the variable and stressful conditions. Alternatively, they may be on the boundary limits of survival, and thus any change in environmental conditions may lead to local extinction. Given the importance of the symbiotic partner, comparing the symbiont communities of 'optimal' and 'marginal' reefs may be crucial to understanding how coral-*Symbiodinium* partnerships persist under marginal conditions.

1.9 Objectives

This study investigated the role that environmental parameters and stressors play in determining the distribution of corals and their dinoflagellate symbionts in marginal environments, as well as the role of the symbionts in facilitating survival under these conditions.

In this study, the marginal environments examined were the high latitude sites of Lord Howe Island, Australia, and the Kermadec Islands, New Zealand, and a site of varying sediment regimes, Palmyra Atoll, USA. The specific objectives of the study were to:

1. Examine the genus and species diversity, and distribution patterns of corals in marginal environments. It is hypothesised that the diversity of corals in marginal environments will be reduced because of the stressful and/or variable environmental conditions.

2. Investigate the genetic diversity, distribution patterns and host specificity of dinoflagellate symbionts (*Symbiodinium* spp.) in marginal environments. It is hypothesised that symbiont diversity will be reduced, host-specificity will be high, and symbionts will be specific to those marginal sites, as a result of symbionts evolving to cope with the stressful and/or variable conditions.

3. Assess the influence of environmental variables on host and symbiont distribution in marginal environments, in comparison to 'optimal' environments. It is hypothesised that both coral and symbiont diversity will be reduced in areas of increased environmental stress.

4. Assess the photo-physiological responses of *Symbiodinium* spp. from a marginal location to thermal and light stress. It is hypothesised that the symbionts will be able to withstand variable/stressful conditions.

The four data chapters are divided into papers, which have either been submitted or are in the process of being submitted, each dealing with a different aspect of the specific objectives (hence there is a degree of repetition between chapters, especially in their introductions).

Chapter 2 addresses the first and third objectives, by investigating the spatial patterns in coral community structure in the Kermadec Islands, with the aim of understanding the processes that control their growth and development. Additionally, the biogeographic affinities of marginal coral communities at high latitude sites and on a wider scale were identified, in order to better understand global patterns of coral biodiversity, the biogeographical limits of corals, and the factors that contribute to these patterns.

Chapter 3 outlines the diversity and host specificity of *Symbiodinium* types in corals at two high latitude locations, the Kermadec Islands and Lord Howe Island, focusing on the role that the symbiont community plays in regulating these high latitude coral reefs. This addresses the second and third objectives.

In chapter 4 the influence of sediment regimes on the diversity and distribution of both corals and their symbionts was investigated in reference to objectives 1 - 3. This part of the study was conducted at Palmyra Atoll, USA, a variable reef ecosystem with a sediment regime gradient. This gradient enabled assessment of variability in community structure among 'marginal' and 'optimal' environments.

Chapter 5 outlines the photo-physiological responses to thermal and light stress of the distinct host-symbiont partnerships at Lord Howe Island, discovered in chapter 3. By elucidating the relationship between *Symbiodinium* photo-physiology and biogeography, this chapter aimed to further our understanding of the mechanisms involved in local adaptation to thermal and solar conditions. This chapter addresses the final objective.

Finally, chapter 6 provides an overview of the major findings, and considers the ecological significance and implications of the research, both in terms of contributing to

our understanding of the structure and dynamics of coral ecosystems, and what the future will hold for ‘marginal’ sites.

Chapter 2

Spatial patterns and regional affinities of coral communities at the Kermadec Islands Marine Reserve (New Zealand), a marginal high-latitude site

ABSTRACT

The Kermadec Islands Marine Reserve (29 - 31°S), New Zealand, harbours one of the world's highest latitude scleractinian coral communities, situated close to the lower thermal limits for coral survival. An understanding of how and why similarities and differences exist among so-called 'marginal' coral communities can provide insight into the physical and biological factors that influence coral distributions. In this study, a depth-stratified survey of benthic community structure and coral species assemblages was conducted at 9 sites around the Kermadec Islands. All coral species identified were subtropical non-reef building species. Scleractinian coral cover and diversity was low and variable among sites and depths (16 spp., 5.3 ± 13.9%) with macroalgae contributing between 36 - 95% of the benthic cover. Multivariate analyses revealed small-scale spatial variability among closely located sites, with no distinct latitudinal gradient in coral community structure. Resulting data on coral species diversity were compared to published data of coral communities from other 'marginal' locations. On a global scale, the Kermadec Islands community structure exhibits greatest affinity with south-eastern Australian sites. In comparison, there was a lack of affinities for other high latitude sites, both in

Australia and globally. Our analyses suggest that the low diversity, highly variable coral communities of the Kermadec Islands are likely shaped by their isolation and high wave energy, rather than the low annual temperatures experienced.

2.1 INTRODUCTION

Marginal coral reefs exist near or beyond the normal limits of reef distribution, close to the environmental thresholds for coral survival (Kleypas et al. 1999). These reefs frequently occur at high latitudes i.e. above 25°S and 25°N, with well known examples being those of the Persian Gulf, Bermuda, Japan and Lord Howe Island, Australia. Structural development and coral species diversity of these high-latitude reefs, at both biogeographic and local scales, are largely regulated by low mean annual and seasonally variable temperatures and irradiances, and the low aragonite saturation state (Buddemeier 1997, Kleypas et al. 1999); other parameters with local importance include wave exposure (Harriott et al. 1994, Benzoni et al. 2003, Coles 2003).

At even higher latitudes, where minimum annual temperatures fall below 18°C, reefs are replaced by coral communities that do not develop reef framework (Wells 1957, Johannes et al. 1983) due to the lack of significant accretion of calcium carbonate (Buddemeier & Smith 1999). The corals in these communities attach to hard substrata, appearing as isolated colonies growing on exposed bedrock (e.g. Macintyre 2003). They can have similar species diversities to true coral reefs (>100 spp.; Sheppard & Sheppard 1991, Harriott et al. 1999), though in most cases species diversities are much lower (less than 100 spp.; Dai 1988, DeVantier & Pilcher 2000, Moyer et al. 2003). Macroalgae play a dominant role in many of these high latitude

coral communities (Fig. 2.1), their rapid growth out-competing corals for space (Johannes et al. 1983). Furthermore, many high latitude coral communities are small and isolated (e.g. Babcock et al. 1986, Brook 1999, Hughes et al. 2002, Gardner et al. 2006), meaning that they are largely self-seeding in terms of larval supply (Ayre et al. 1997).

As most of the world's coral reefs will become marginalised as our climate rapidly changes (Hoegh-Guldberg et al. 2007), study of marginal coral communities may provide useful analogues for understanding the ecology of coral reefs subjected to deteriorating conditions (Perry & Larcombe 2003). Moreover, it has been suggested that high latitude coral communities might be better adapted for survival in

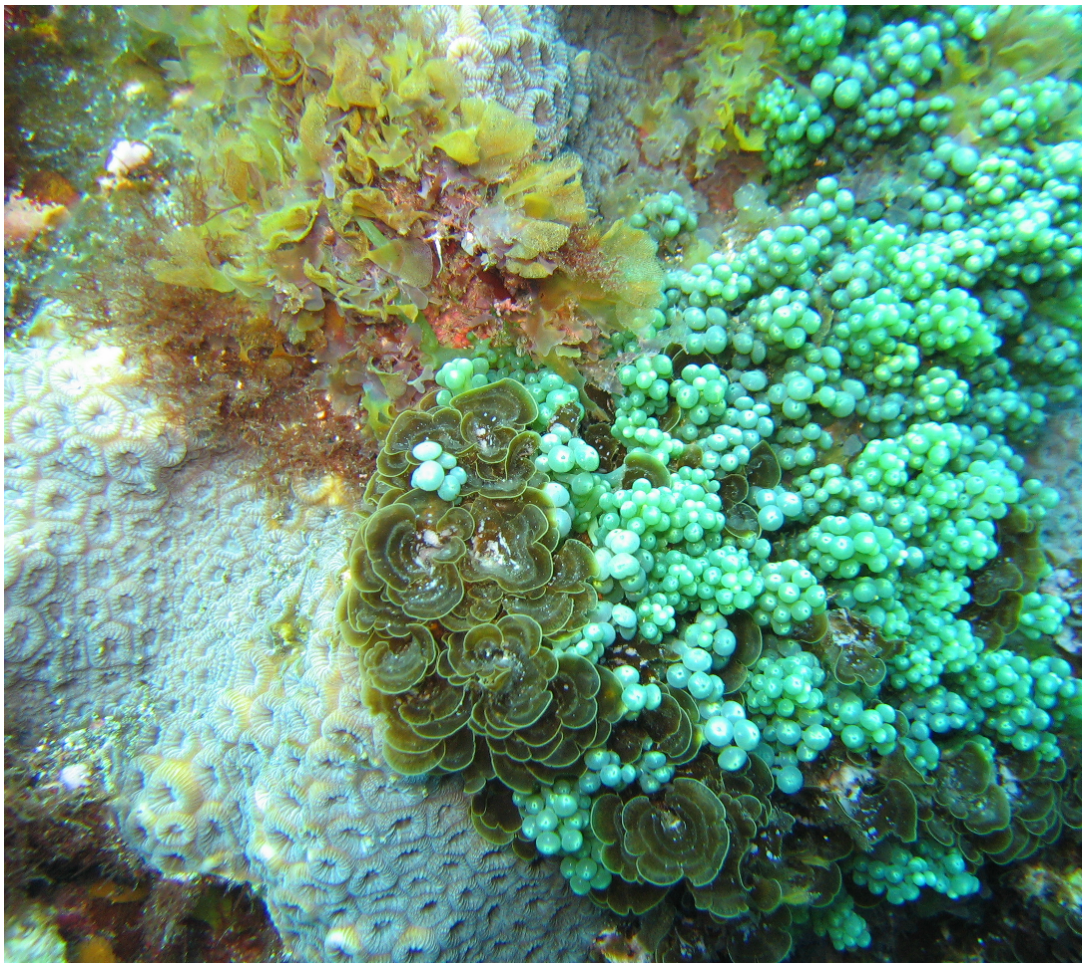


Figure 2.1 Algae competing for space with encrusting coral (*Montastraea curta*), Kermadec Islands, northern New Zealand (photo: J. Long).

an environment modified by global climate change, as a result of their existence in already stressed conditions (Glynn 1996, Riegl & Piller 2003). If these communities will indeed act as coral refuges, then it is essential that we understand the processes controlling their growth and development so that they can be appropriately managed and conserved for the future; it is also important that we have a more comprehensive knowledge of their relatedness to coral communities and reefs elsewhere.

To address this issue we focused on one of the South Pacific's high-latitude coral community sites, the Kermadec Islands Marine Reserve, 750 km off the northeast coast of New Zealand's North Island (Fig. 2.2) and approximately 3000 km east of the Great Barrier Reef (GBR). This study aimed to: (1) assess, in terms of cover and species diversity and richness, the spatial patterns of coral distribution at this high latitude location, (2) determine if different ecological communities exist among neighbouring sites at the Kermadec Islands and how these communities vary with latitude, and (3) identify similarities among coral diversity at the Kermadec Islands and other coral communities and reefs to assess the contribution of latitude in governing coral species distributions. The latter point is developed in analyses of published data of coral biodiversity at marginal and optimal sites throughout the world. It is hypothesised that coral diversity will be low at the Kermadec Islands, due to the high latitude nature of the site, and that environmental parameters will drive coral distribution patterns. Furthermore, coral diversities and community structure will be similar among sites of comparable annual temperature ranges.

Equidistant between temperate New Zealand and tropical Tonga, the Kermadec Islands are one of the few subtropical island groups in the western South Pacific Ocean, and form New Zealand's largest marine reserve (748,000 ha). The islands are volcanic in origin, with steeply sloping rock substrata dominant in the

shallow sub-tidal zone down to 10 m, and gently sloping reef areas of *in situ* rock and large boulders common to depths of 20 - 30 m. The main oceanographic influence on the Kermadec Islands is poorly known (Gardner et al. 2006), but is suggested to be either from the East Australian Current (EAC), flowing from New South Wales (eastern Australia) to Norfolk Island and on to the Kermadec Islands (Marshall 1979, Schiel et al. 1986), or from the northwest route, passing by New Caledonia, Fiji and Tonga (Fig 2.2; Gardner et al. 2006). Little is known of localised oceanographic patterns.

Previous surveys of benthic species diversity at the Kermadec Islands have reported a marine biota that is a mix of tropical, subtropical and temperate species of Southwest Pacific origin (Schiel et al. 1986, Cole et al. 1992, Brook 1999, Gardner et al. 2006), with corals generally restricted to depths of < 30 m (Brook, 1999). The isolation of the Kermadec Islands, coupled with the lack of terrestrial inputs or a resident human population, means that they are free from many anthropogenic stressors to which other reefs are exposed, such as overfishing and terrestrial runoff. However, little is known of the biodiversity variability within the marine reserve (among islands, or among sites within islands), which spans a range of latitudes (29 – 31°S) and sea surface temperatures (SST; 14 - 18°C annual minima, 23 - 26°C annual maxima; Francis et al. 1987). The lack of a comprehensive study of temporal and spatial variation in community structure of the benthic habitats (coral and non-coral benthic taxa) at the Kermadec Islands is due to their inaccessibility associated with their isolation, and problems of wave exposure once on site (Gardner et al. 2006).

2.2 MATERIALS AND METHODS

2.2.1 Study area

The study was conducted at two locations at the Kermadec Islands, 120 km apart and situated between the latitudes of 29° and 30°S, in November 2004. The 11 islands form three discrete groups (Fig. 2.2), the northern group around Raoul Island (29°12'S, 177° 55'W), the middle group around Macauley Island (30°14'S, 178°25'W), and the southern group of Curtis and Cheeseman Islands (31°21'S, 178°41'W). Although corals are present at the southern group, the area was not surveyed, because it was inaccessible at the time of study. Eight of the nine sites surveyed are situated in the northern group, and one site was located close to Macauley Island in the middle group. Sites were chosen on the basis of accessibility and repeatability.

2.2.2 Sampling

At each of the Kermadec sites, quantitative benthic surveys were carried out at four depth strata, 3 - 6 m, 6 - 9 m, 9 - 12 m and 12 - 15 m, consistent with previous surveys (Schiel et al. 1986, Gardner et al. 2006). Sampling was conducted by photographing 16 haphazardly located quadrats of 50 x 50 cm (total area sampled = 4 m^2 per stratum) using a Canon S60 camera. From the digital images, percent cover (± SD) was estimated using random point counts (100 points) of nine benthic groups, including hard and soft corals. Corals present were identified to family and, where possible, to species; their percent cover was recorded.

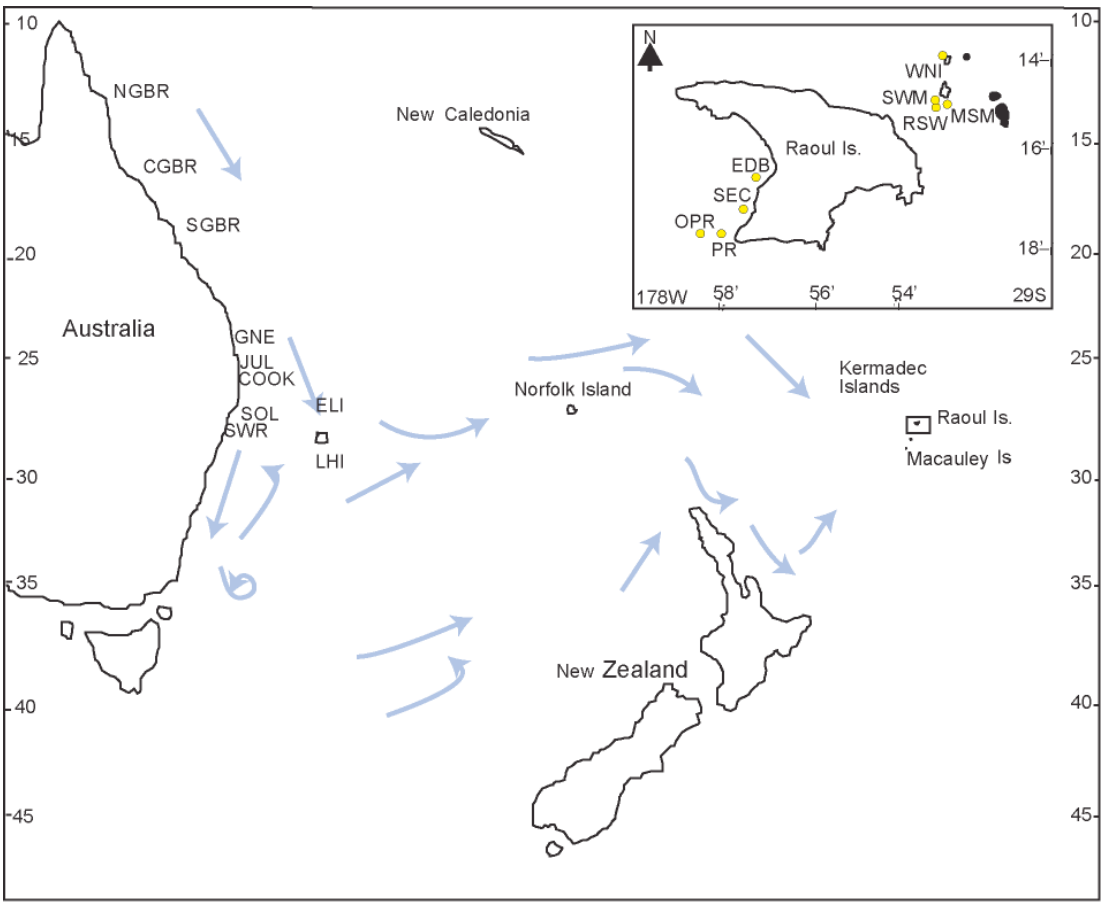


Figure 2.2 New Zealand and Australian sites. Geographic location of Kermadec Islands, northeastern New Zealand. Inset shows location of 8 sites within the Kermadec Islands Marine Reserve. Major oceanic currents indicated by arrows, from Schiel et al. (1986). Australian site abbreviations: GBR, Great Barrier Reef (N, North; C, Central; S, South); GNE, Gneering Shoals; JUL, Julian Rocks; COOK, Cook Island; SOL, Solitary Islands; SWR, Southwest Rocks; LHI, Lord Howe Island; ELI, Elizabeth Reef. Kermadec Islands site abbreviations: EDB, East Denham Bay; SEC, South-east Denham Bay; OPR, Outer Parsons Rock; PR, Parsons Rock; WNI, West Napier Island; SWM, South-west Meyer; RSW, Rock South-west Mayer; MSM, Mid-south Meyer.

2.2.3 Regional affinities

For multivariate analysis of coral community structure in relation to other geographic locations, data on coral species richness in the form of number of species per family was collated from 39 sites. Twelve Australian regions (Fig. 2.2; Ten published by Veron (1993a) cited in Harriott & Banks (2002), others in Veron & Marsh 1988, Oxley et al. 2004)); 19 Central and Eastern Pacific sites from French Polynesia in the east to Colombia in the west (Glynn et al. 2007) and 7 other marginal/isolated sites: Tsushima Is. and Iki Is. Japan (Sugihara & Yamano 2004, Yamano et al. 2004 respectively), the Persian Gulf and the Red Sea (DeVantier & Pilcher 2000), the Gulf of Aqaba (Sheppard & Sheppard 1991), American Samoa (Lamberts 1983) and South Africa (Riegl 1996) were used in the study. For areas in the Central and Eastern Pacific, a biogeographic dataset of zooxanthellate corals was sourced from Glynn et al. (2007), which was combined with the present survey data and the aforementioned data sets (thus excluding azooxanthellate species) for further multivariate analysis. Coral species counts from the present study for the Kermadec Islands were supplemented with records from Brook (1999), who identified seven additional species. Where possible, percent coral cover (when recorded in aforementioned published records), survey depth, and average minimum and maximum annual temperatures for the sites were noted. Most temperature data were collated from ReefBase GIS information (http://reefgis.reefbase.org), with data from Japan from Yamano et al. (2001).

2.2.4 Data analysis

Kermadec Islands

Mean (± 1 SD) of percent cover for each benthic category was estimated for the 16 replicate quadrats at each site. Percent cover of each coral species identified was estimated for each quadrat, and means calculated for each site/depth stratum. Indices used to assess diversity of corals per site were total number of species (S), Hill numbers N1 and N2 (to assess the influence of rare and dominant species on community diversity respectively), and the modified Hill's ratio (N21′) (see Clarke & Gorley 2006 for a summary of each index). The modified Hill's ratio was used as a measure of equitability (spread between taxa) as it has an advantage over traditional measures of evenness by its non-dependency on the number of species (Rogers et al. 1999). Significance of differences in coral cover (hard and soft combined) and species diversity among sites and depths were determined using either ANOVA (coral cover – normally distributed) or Kruskal-Wallis (species diversity - non-normal data) tests.

Multivariate analyses of similarities and differences among sites and depths were performed on percent cover of (a) all benthic categories and (b) coral species only. Data analysis was performed using the PRIMER package v6.0 with PERMANOVA (Multifactorial permutational analysis of variance) add-on (Plymouth Marine Lab.). All benthic group and coral species percent cover values were pre-treated with a dispersion weighting transformation in which the abundances of the different species were differentially weighted on the basis of their observed variability in replicate samples. This method compensates for the spatial clustering common to coral community studies (Clarke et al. 2006a). Similarities were

calculated using the zero-adjusted Bray-Curtis similarity measure (Bray & Curtis 1957) to account for samples with depauperate assemblages (see Clarke et al. 2006b). PERMANOVA with 9999 permutations was used to determine differences in community structure among sites and depths within sites. PERMANOVA is unconstrained by the assumptions of normality and homogeneity of variance that limit the use of traditional MANOVA, and relies on comparing the observed value of a test statistic (pseudo *F*-ratio) against a recalculated test statistic generated from random re-ordering (permutation) of the data (Anderson 2001). Patterns in the data were visualised using a canonical analysis of principal coordinates (CAP), and SIMPER analysis established the species causing the dissimilarities between most sites and outlying site/depth strata.

Regional affinities

To gain insight into the regional affinities of the coral species and diversity patterns seen at the Kermadec Islands, multivariate analyses of similarities and differences were conducted on the total number of species in each family present at each of the aforementioned Pacific, Australian and marginal locations, using the PRIMER software package. Analysis was conducted on untransformed and 4^{th} root transformed data, to identify the role of dominant and rare species respectively. Following calculation of similarities using the Bray-Curtis similarity measure, significant groupings in the multivariate space at the 95% level were identified using the similarity profile permutation test SIMPROF, with SIMPER analyses used to assess the contribution of each family to the similarities within clusters

2.3 RESULTS

2.3.1 Kermadec Islands

Seventeen coral species were identified in this survey (Table 2.1): 16 hard (scleractinian) corals, and one soft coral (*Sinularia* sp.). The most common coral species observed were *Montastraea curta, Hydnophora pilosa* and an encrusting *Montipora* sp. Small spatial scale geographical divisions were observed for some coral species, with one species only found at the southern sites (*Rhizopsammia* sp), and three limited to the northeast region (*H. pilosa, Tubastrea* sp. and *Dendrophyllia* sp). Coral colonies were generally small, ranging from 20 mm diameter (e.g. *Cyphastrea serailia*), to 1 m^2 colonies of soft corals. Total number of species was greatest at the northernmost site of West Napier Island (10), and lowest at the southernmost site of Macauley Island (4).

Coral species diversity was significantly different among sites (Kruskal-Wallis; H = 53.48, DF = 8, $p < 0.01$) and depths within sites (H = 14.59, DF = 3, $p < 0.01$). Diversity of both rare and dominant species (N1, N2; Table 2.1) were greatest at West Napier Island (N1 = 6.6, N2 = 5.1), with a high diversity of rare species also observed at South West Meyer (N1 = 5.4). Minimum species counts and diversity of both rare and dominant species were at the most southerly site of Macauley Island (4 spp., N1 = 1.7, N2 = 1.4) N1 and N2 were also low at some sites in the northern group; Mid South Meyer and East Denham Bay (N1 = 1.9, N2 = 1.4 at both sites). Evenness (N21') was variable among sites (0.4 - 0.9), with the greatest value recorded at Outer Parsons Rock (0.9) and the minimum at East Denham Bay (0.4).

Table 2.1 Mean percent cover ± SD of the benthic categories and coral species at nine sites at the Kermadec Islands, New Zealand. Site codes are in brackets.

	W. Napier Island (WNI) 29°14'S	Mid S. Meyer (MSM) 29°14'S	Rock SW Meyer (RSW) 29°15'S	SW Meyer (SWM) 29°15'S	SE Denham Bay (SEC) 29°17'S	East Denham (EDB) 29°18'S	Outer Parsons Rk (OPR) 29°18'S	Parsons Rk (PR) 29°18'S	McCauley Is. (MCI) 30°17'S
RUBBLE/SAND	3.4 ± 4.0	4.8 ± 8.1	14.1 ± 22.6	13.3 ± 17.0	10.4 ± 13.8	23.0 ± 25.6	5.2 ± 3.9	7.2 ± 8.2	2.7 ± 5.6
FILAMENTOUS ALGAE	35.6 ± 31.7	57.9 ± 21.9	36.0 ± 30.4	48.1 ± 22.5	51.6 ± 28.6	66.1 ± 25	61.9 ± 26.6	75.6 ± 26	78.4 ± 20.9
ENC- RED- ALG	49.7 ± 34.6	16.0 ± 16.8	20.8 ± 22.5	19.7 ± 25.6	18.1 ± 24.9	6.7 ± 14.9	17.8 ± 21.7	5.5 ± 13.1	15.8 ± 17.8
HARD CORAL	3.2 ±11	4.8 ±11.2	10.2 ± 17	5.8 ±14.7	18.0 ± 26.0	2.6 ± 8.1	1.2 ± 6.9	3.5 ± 11	0.2 ± 0.9
SOFT CORAL	0.1 ± 0.6	10.5 ± 18	13.1 ± 26.5	0.4 ± 2.1	0.3 ± 1.6	0.1 ± 0.3	0.2 ± 0.9	0.6 ± 2.3	0.0 ± 0.0
BRYOZOAN	0.0 ± 0.0	0.4 ± 2.3	<0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.9	0.0 ± 0.0	0.1 ± 0.5
SPONGE	3.6 ± 5.5	3.0 ± 3.7	3.0 ± 3.6	6.6 ± 11.2	0.6 ± 1.2	0.3 ± 0.8	8.3 ± 15.8	3.6 ± 7.4	0.7 ± 1.0
ASCIDIAN	0.4 ± 1.2	<0.1 ± 0.2	<0.1 ± 0.2	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 1.5	0.4 ± 1.6	0.1 ± 0.5
ANEMONES	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 3.1	0.0 ± 0.0	0.0 ± 0.0
CORAL	3.3 ± 11.0	15.2 ± 22.9	23.3 ± 29.7	6.2 ± 14.7	18.3 ± 25.9	2.7 ± 8.1	1.4 ± 6.9	4.1 ± 11.3	0.2 ± 1.0
ALGAE	85.4 ± 14.9	74.0 ± 22.2	56.8 ± 30.3	67.8 ± 19.3	69.7 ± 23.9	72.8 ± 29.1	79.7 ± 20.1	81.2 ± 19.0	94.2 ± 14.4
Acroporidae									
Montipora sp	0.2 ± 0.0	0.4 ± 2.9	0.1 ± 0.9	0.2 ± 1.2	10.1 ± 24.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 3.0	<0.1 ± 0.1
Carophyllidae									
Coenocyathus brooki	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	<0.1 ± 0.1	0.0 ± 0.0
Pocilloporidae									
Pocillopora damicornis	0.1 ± 0.5	0.0 ± 0.0	0.9 ± 3.3	0.2 ± 1.4	0.0 ± 0.0	0.1 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Dendrophyllidae									
Dendrophyllia sp	<0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Tubastrea sp	0.2 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	<0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Turbinaria sp	0.9 ± 6.2	0.9 ± 6.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 1.5	0.0 ± 0.0	0.0 ± 0.0
Rhizopsammia sp	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	<0.1 ± 0.1
Faviidae									
Cyphastrea sp	0.0 ± 0.0	<0.1 ± 0.1	0.0 ± 0.0	<0.1 ± 0.1	0. 0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	<0.1 ± 0.1	0.0 ± 0.0
Goniastrea sp	0.0 ± 0.0	0.0 ± 0.0	<0.1 ± 0.2	0.1 ± 0.3	3.2 ± 7.8	2.1 ± 7.8	<0.1 ± 0.3	0.1 ± 0.4	0.0 ± 0.0
Leptastrea sp	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 6.4	0.0 ± 0.0	0.0 ± 0.0
Montastraea curta	1.7 ± 8.0	0.1 ± 0.3	3.1 ± 9.3	2.0 ± 9.2	3.4 ± 6.7	0.2 ± 1.6	0.0 ± 0.0	3.0 ± 10.8	<0.1 ± 0.4
Merulinidae									
Hydnophora pilosa	0.0 ± 0.0	3.1 ± 8.9	6.1 ± 14.3	3.2 ± 10.9	1.2 ± 5.6	0.1 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Poritidae									
Alveopora spongiosa	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.5	<0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Agariciidae									
Leptoseris sp	0.0 ± 0.0	0.0 ± 0.0	<0.1 ± 0.1	0.0 ± 0.0	<0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Pavona explanulata	0.0 ± 0.0	<0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Soft Coral	0.1 ± 0.6	10.8 ± 18.4	13.1 ± 26.5	0.4 ± 2.1	0.3 ± 1.6	0.1 ± 0.4	0.2 ± 0.9	0.6 ± 2.3	0.0 ± 0.0
Unidentified	<0.1 ± 0.1	<0.1 ± 0.1	<0.1 ± 0.2	0.1 ± 0.4	0.1 ± 0.4	0.1 ± 0.4	0.2 ± 0.8	0.1 ± 0.3	0.2 ± 0.8
Total no spp.	10	8	8	9	9	7	5	7	4
Shannon Weiner (H')	1.5	0.9	0.6	0.7	0.7	0.6	2.1	0.6	1.0
Margalef's (d)	7.5	2.6	2.2	4.4	2.8	6.1	11.2	4.2	0.2

Both hard and soft coral cover were significantly different among sites and depths within sites (Hard: site F = 9.7, $p < 0.01$, depth F = 4.1, $p < 0.01$; Soft: site F = 13.9, $p < 0.01$, depth F = 9.6, $p < 0.01$). Soft corals were less abundant than hard corals, with an average (± SD) of 2.9 ± 11.9 *versus* 5.2 ± 13.9 percent cover respectively, averaged over all depths at all sites. The highest total mean coral cover (i.e. both soft and hard) was recorded at the northern site of Rocks Southwest Meyer (23%; Table 2.1), whereas the lowest value (0.2%) was recorded at the southernmost site of Macauley Island, where four hard coral species and no soft coral species were present. Southeast Denham Bay had the greatest cover of hard corals (18%), and Rocks Southwest Meyer had the greatest soft coral cover (13%). Coral cover among sites and depths was highly variable (Fig. 2.3). Overall, mean coral cover (± SD) was lowest in the 12 - 15 m zone (< 8 % of cover), although the two sites around Parsons Rock had their highest recorded coral covers in this depth zone (PR = 5.3 ± 14.1%, OPR = 8.2 ± 12.5%). These deeper communities were dominated by *Leptastrea* sp. and *M. curta*.

Coral cover was less than macroalgal cover in all sites surveyed (DF = 130, $p<0.001$). Filamentous and encrusting macroalgae combined accounted for 57 – 94% of the benthic cover (Southwest Meyer and Macauley Is., respectively; Table 2.1). Filamentous macroalgal species observed included genera such as *Caulerpa, Enteromorpha, Codium* and *Corallina,* with encrusting species such as *Lithothamnion* sp., and *Apophloea* sp. also being common. Filamentous macroalgal cover declined with decreasing latitude, accounting for only 36% of the benthic cover west of Napier Island (29°S), compared to 94% at Macauley Island (30°S) though it was highly variable among neighbouring sites (e.g. 37 - 57% filamentous macroalgal cover at the three sites south of Meyer Island).

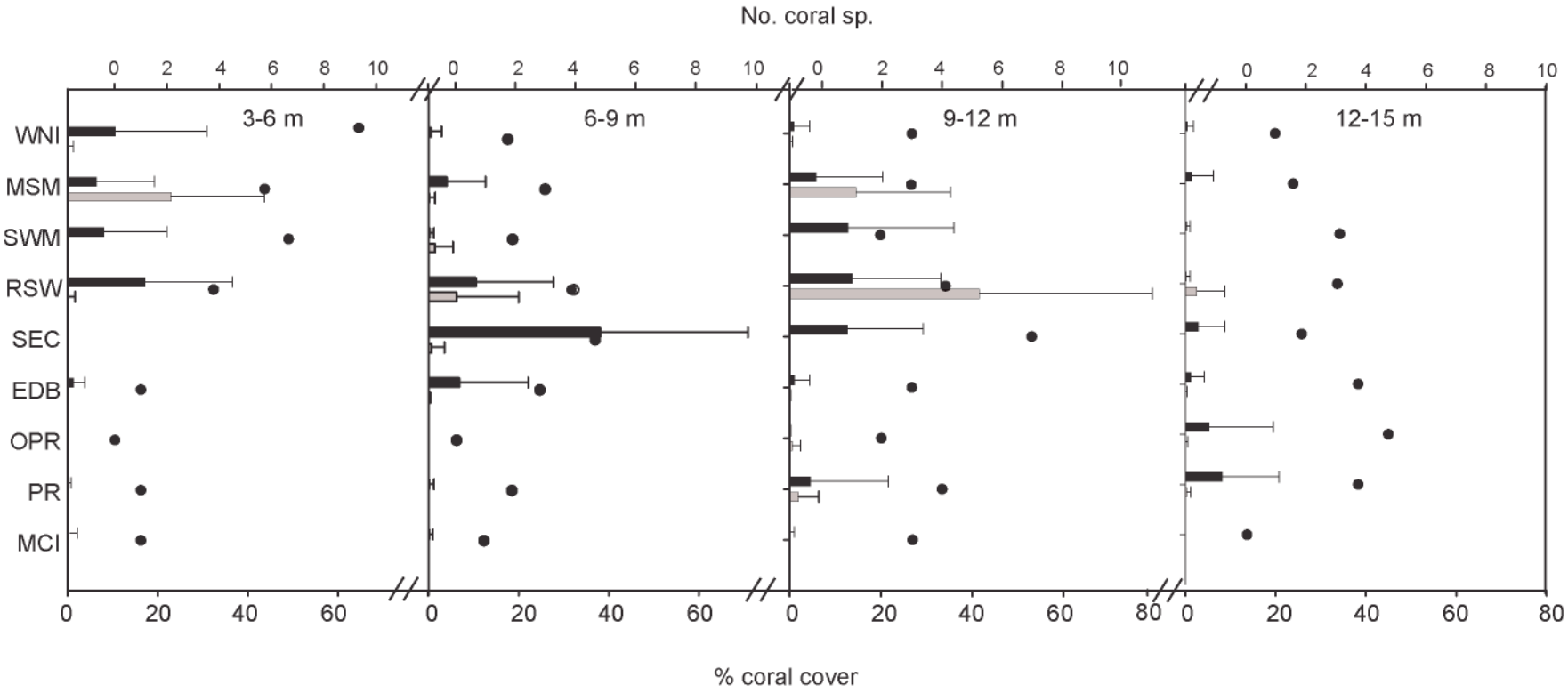


Figure 2.3 Mean percent coral cover ± SD (bottom axis) of hard (dark bars) and soft corals (light bars), and number of species (dots – top axis) present at each of the nine sites (left axis) in the four depth strata surveyed at the Kermadec Islands (for site abbreviations see Table 2.1). Sites ordered from north to south (top to bottom). EDB, East Denham Bay; SEC, South-east Denham Bay; OPR, Outer Parsons Rock; PR, Parsons Rock; WNI, West Napier Island; SWM, South-west Meyer; RSW, Rock South-west Mayer; MSM, Mid-south Meyer

Multivariate analysis of both benthic composition and coral community structure for the 9 sites (in 4 depth strata) around the Kermadec Islands revealed significant differences among sites and depths within sites (PERMANOVA: $p < 0.01$ for both tests). However, only 13 of 32 and 8 of 32 possible site comparisons had significantly different benthic compositions and coral community structures, respectively. Canonical ordination supports this finding, with a clear overlap of most sites, due to their dominance by macroalgae and coral species scarcity. The few outliers identified by CAP were sites with high abundances of hard or soft coral (e.g. RSW 9-12 m had 41% soft coral, Appendix 1).

The taxa that contributed most to the dissimilarity among the main grouping and the outliers were defined by SIMPER analysis. The site of Mid-South Meyer was significantly different to most other sites, due to its abundance of soft corals (19% mean cover) in addition to the hard coral *H. pilosa* (3% mean cover). The other outliers were characterised by their abundance of rare species (e.g. OPR 12-15 m had 4% *Leptastrea* sp.), or the relatively high abundance of one species (e.g. RSW 3-6 m had 14% *M. curta*). Two site/depth combinations were significantly different to other site/depth strata in both benthic (including coral) and coral species community structure: Rock Southwest Meyer 9-12 m, which was dominated by soft coral (42% cover), *H. pilosa* (13% cover) and filamentous macroalgae (23% cover); and Southeast Denham Bay 6-9 m with 38% hard coral cover (including 28% *Montipora* sp.). The southern site of Macauley Island was not significantly different in community structure to 5 of the other 8 sites (PERMANOVA, $p = 0.342$), despite its geographical isolation. The lack of clear groupings suggests a variable benthic community among sites and depths, rather than a clear latitudinal delineation.

2.3.2 Regional affinities

Cluster analysis (Fig. 2.4) revealed the existence of pronounced divisions among the tropical, sub-tropical and high latitude sites. The exclusion of azooxanthellate species from the analysis did not change the groupings, suggesting that such species contribute little to community structure similarities. Analyses using both untransformed and transformed data gave the same clusters, at comparable similarity levels. The SIMPROF grouped sites based on the abundance of each coral family into 7 clusters at $p < 0.05$, loosely defined as: A - Australasian high latitude, B - Japan, C - Central Pacific (though also including the Gulf of Aqaba), D - Multi-regional high latitude (including some eastern Australian sites), E - Houtman Abrolhos and American Samoa, F – GBR and Red Sea, G –Eastern Pacific, and Southwest Rocks (Australia) and Marquesas Islands (French Polynesia) were the two outliers.

The Kermadec Islands (29-31°S, 178°W) fell into the Australasian high latitude cluster with Cook Island (28°12'S, 153°34'E) and Julian Rocks (28°38S, 153°36'E), both in NSW, Australia. This cluster showed closest affinity with the cluster of high latitude sites of Japan (Iki Island 33°47'N, 129°43'E and Tsushima 34°25'N, 129°20'E), at $p = 0.055$. The two Australasian high latitude sites have similar coral cover to the Kermadec Islands (8%) with 8% and 5%, respectively, though their species counts are higher than at the Kermadec Islands (24 species) with 33 and 30 species, respectively. Notably, the other high latitude sites of Australia, including Lord Howe Island and the Solitary Islands, grouped with the South African and Persian Gulf Reefs, not the nearby Kermadec Islands or sites further south on the

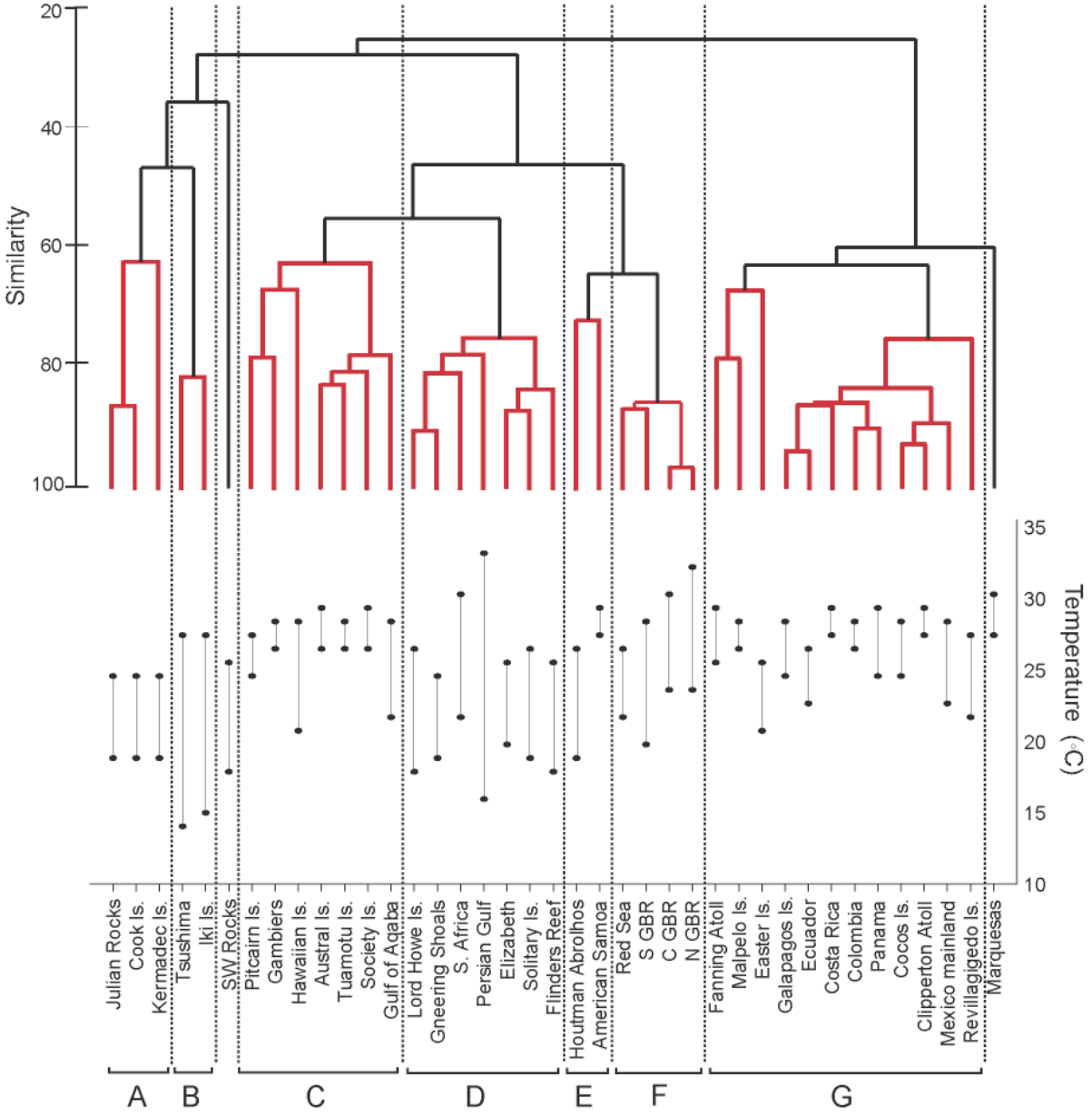


Figure 2.4 Cluster analysis of coral community structure patterns of the Kermadec Islands, locations in Australia, the Pacific and at marginal sites, with annual minimum and maximum temperatures. Temperatures of Hawaiian Islands, Red Sea and each specific GBR region are maximum and minimum over whole latitudinal range. GBR: Great Barrier Reef. Similarities calculated based on Bray-Curtis measure of square-root transformed coral species per family.

Table 2.2 Coral genera contributing to SIMPROF groupings (Fig. 2.4) of similarities in coral assemblages at Australasian and marginal sites, calculated with Bray-Curtis Similarity. Species are listed in order of % contribution to within site similarity. Letters in brackets under site refer to groupings in text. Numbers in brackets aside families indicates average number of species per group in site assemblage. GBR: Great Barrier Reef

Group	Australasian high latitude (A)	Japan (B)	Central Pacific (and Gulf of Aqaba) (C)	High latitude (D)	Houtman Abrolhos/Am. Samoa (E)	GBR/Red Sea (F)	Eastern Pacific (G)
Within group similarity	79%	80.70%	67.96%	77.70%	71.66%	87.60%	72.36%
Species contributing (mean number species)	Faviidae (7) Dendrophyllidae (6) Acroporidae (5) Siderstreidae (3)	Faviidae (17) Pectiniidae (4) Acroporidae (2) Poritidae (1)	Acroporidae (32) Pectiniidae (13) Agariciidae (9) Poritidae (7)	Acroporidae (31) Faviidae (22) Poritidae (8) Mussidae (5)	Acroporidae (83) Poritidae (20) Merulinidae (11) Siderstеidae (3)	Acroporidae (89) Faviidae (59) Poritidae (31) Fungiidae (27)	Agariciidae (6) Pocilloporidae (5) Sidersteidae (3) Poritidae (3)

Australian coast. Additionally, the most southern mainland site of Australia, Southwest Rocks (30.90°S 153.04°E) was separate to all other groups (similarity of 30%) due to the species-depauperate nature of the site; only 8 species were present. The coral community of the high latitude Australasian cluster was significantly different to all other tropical, subtropical and high latitude clusters ($p < 0.05$). Despite the surveys of all eastern Australian sites being conducted at 8 - 12 m, as opposed to the 0 – 40 m at the Kermadec Islands, two of the Australian sites still show closer affinities to the Kermadec Islands rather than other Australian sites.

SIMPER analysis revealed that the families Faviidae (25%), Dendrophyllidae (25%) and Acroporidae (18%) contributed most to the cluster similarities of the Kermadec Islands and two southern Australian sites (Table 2.2). The dissimilarity of the high latitude Australasian cluster to the Japanese sites was attributed to the abundance of species in the Faviidae at the latter (mean of 17 species compared to 7 species), and the high species counts in the Dendrophyllidae at the former (mean of 6 species compared to 1 species at the Japanese sites). The abundance of acroporids in the Central Pacific, Red Sea and GBR, and their absence at the Kermadec Islands, clearly explained the different coral community structures of the high latitude Australasian cluster *versus* the other clusters. Furthermore, the abundance of species in general, and specifically of acroporid and poritid species at the remaining high latitude sites (cluster D) caused the separation of this group from the Australasian high latitude sites. Whilst *Acropora* spp. dominate most sites in the Central and Western Pacific, including those at high latitudes, there is a lack of *Acropora* spp. in the far Eastern Pacific where species of the genera Agariciidae and Pocilloporidae dominate. Species counts overall are lowest in the Australasian high latitude sites and the Eastern/isolated Pacific sites.

A clear pattern exists between the clustering of sites, and their annual temperature ranges. The high latitude cluster, which includes some Australian sites, South Africa and the Persian Gulf (D on Fig. 2.4) are all subject to substantial annual temperature ranges (Persian Gulf 15 - 33°C, S. Africa 21 - 30°C), and these sites all host coral reefs. The cluster of the Kermadec Islands and the two southern Australian sites has annual average temperatures ranging from 18 - 24°C, and all sites host coral communities as opposed to reefs. Their temperature ranges are comparable with many of the other high latitude Australian sites, yet they exhibit a lack of affinity in coral community composition. These three sites, however, have the lowest annual maxima of 24°C. The Japanese sites, loosely clustering with the Kermadec Islands, are located at a higher latitude (34°N) and subject to average annual temperatures of 13 - 27°C. The Eastern Pacific sites (G on Fig. 2.4) are all characterised by low species diversity, ranging from 7 to 20 species, with high annual average temperature maxima (26.5 - 31°C).

2.4. DISCUSSION

2.4.1 Kermadec Islands

The coral community of the Kermadec Islands is dominated by subtropical species and features low coral diversity and cover, as might be expected at such a high latitude location. Seventeen coral species were identified from a known 24, with considerable differences in community structure between closely located sites (3 - 120 km). Although located at a high latitude (29 - 31°S), and subject to low annual SST (18 - 24°C annual range), the variability in coral diversity and cover at the

Kermadec Islands suggests that local-scale variations in the environmental regime are important for controlling the structure of this marginal community. On a regional scale, the Kermadecs were most similar to two rocky subtropical sites of south-eastern Australia (NSW); there was a lack of affinity with tropical sites, and a reduced affinity for other high latitude sites. A lack of congruence between depth of surveys and regional affinities suggests depth does not determine similarity in communities among sites in the present study. These different affinities are likely explained by local environmental factors such as wave energy, water quality and larval supply, rather than latitude and its associated influence on temperature.

The low coral diversity at the Kermadec Islands (24 spp.) is seemingly linked to the physiological tolerances of coral species to low temperatures. Many reef-building species cannot grow and survive outside the tropics (Harriott et al. 1994, Harriott et al. 1995) exemplified by the absence of some major reef-building taxa at the Kermadec Islands (e.g. staghorn *Acropora* and massive *Porites*), and at most subtropical Australian sites. However, coral communities with higher species diversity and cover have been observed in regions subject to lower annual SST minima than those seen at the Kermadec Islands. For example, along the coast of Japan, coral communities of up to 40 species and 20 - 30% cover have been recorded, usually subject to an annual average SST minimum of 17ºC (compared with 18ºC at the Kermadec Islands), and the most northerly of which are subject to a lowest annual SST of 13.3ºC (Yamano et al. 2001). Additionally, as observed in the present study, coral communities on the east coast of Australia separated by < 50 km, with similar annual temperature regimes have highly variable species diversity and cover. However, while low temperature goes some way towards explaining the low diversity and cover of corals at the Kermadec Islands, alone it cannot explain the lack

of similarities in coral community structure among sites from similarly high latitudes. Other, more local factors must play a role.

The hydrodynamic conditions, in terms of exposure to high wave energy, at the Kermadec Islands create a challenging environment for habitation. The absence of shallow sheltered rocky reefs and lagoons limit the substrate available for coral settlement. Locally, the southern site of Macauley Island is exposed to unrefracted ocean swell and storm surges (Brook 1999) and the community present reflects this challenging environment: filamentous macroalgae dominate with no soft corals present and <1% hard coral cover. The high wave energy experienced at the Kermadec Islands is also experienced at the rocky benthic communities of Julian Rocks and Cook Island (NSW, Australia), located 2 km and 600 m offshore respectively, with which it shows the closest affinities. Little is known of local hydrodynamic conditions at these 3 sites. Environments with high wave energy, such as these, favour compact growth forms of corals (Veron 1993b), with only those coral species that can withstand exposure to swell and storm surge able to establish there. Thus, the low abundance of branching species at the Kermadec Islands, Julian Rocks and Cook Island (e.g. acroporids, pocilloporids) may be due to the high degree of wave exposure. Comparatively, at Lord Howe Island, Australia, reef-building species are able to establish, but only in the lagoonal areas; they are uncommon on the seaward slopes where reef accretion capacity is very limited (Harriott et al. 1995). In addition to Lord Howe Island, the other high latitude sites considered here, all of which show greater species diversity than the Kermadec Islands, all feature sheltered areas, which potentially allow more fragile species to establish. Further, the decline in light penetration at these high latitude sites will prevent the development of corals at greater depths (Grigg 1982, Kleypas et al. 1999) where wave exposure

would be minimized thus allowing settlement. The lack of sheltered areas at the Kermadec Islands means that even if temperatures are sufficient to allow colonization by reef building species, hydrodynamic conditions may prevent their long-term establishment.

The high wave energy at the Kermadec Islands also favours the dominance of communities of calcareous algae, which can establish in wave-exposed conditions and outcompete corals for space and light (Hustan 1985). At high latitudes, competition between corals and algae plays a significant role in regulating the coral communities (Birkleland 1977, Birkleland & Randall 1981), because subtropical conditions (i.e. low SST) favour algae over coral. This is also linked to nutrient concentrations as, in general, inshore and higher latitude waters have greater nutrient concentrations than do offshore and lower latitude waters, which allows for the enhanced growth rates of macroalgae (Crossland 1983). The dominance by macroalgae, both filamentous and encrusting, at the Kermadec Islands, Julian Rocks and Cook Island (76%, 45%, and 67% respectively, compared to 25% on the GBR) suggests that algae are successfully out-competing corals for space at these sites, by overgrowth of the live coral tissue (Birkleland 1977). Additionally, any variation in nutrient availability among sites will affect community structure, as nutrients enhance growth and hence the competitive ability of macroalgae (Kojis & Quinn 1984, Hughes 1989, Nugues & Roberts 2003). Turbidity, a reflection of nutrient levels (Koenings & Edmundson 1991) is indeed known to be variable among sites at the Kermadec Islands (0 - 3 FTU, J. Gardner, unpublished data), however long-term measurements of turbidity and/or nutrients are not available. Further study is needed to assess the role that nutrient levels play in shaping the coral community at this location.

Larval supply to isolated sites is an additional factor that limits the species present. In a study of genetic connectivity between Lord Howe Island and the GBR (700 km apart), Ayre & Hughes (2004) showed that expanses of open-ocean between isolated reefs are far more effective barriers to dispersal than are similar distances within continuous reef systems. This highlights the potential for limited recruitment of coral larvae at the Kermadec Islands, given that they lie at a minimum distance of 750 km from any landmass, and are approximately 3000 km from the GBR. Indeed, at the Kermadec Islands, there is evidence of limited connectivity between even closely located sites (< 1 km), most likely associated with the hydrographic regime (Wood & Gardner 2007). The low diversity of the Kermadec Islands may also reflect their geological history, with no evidence of a land bridge at any time (Brothers & Searle 1970). Additionally, there appears to be a decline in coral recruitment with increasing latitude, linked to low water temperatures (Hughes et al. 2002, Nozawa et al. 2006), which at the Kermadec Islands likely compounds the problems associated with their remoteness. Finally, the mode of transmission of coral species will affect their likelihood of recruitment to the Kermadec Islands. Brooded larvae survive longer periods (greater than 1 month) in the water column (Richmond 1987, Harrison & Wallace 1990), which confers an adaptive advantage at isolated sites. Approximately half of the species present at the Kermadec Islands are brooding species, whilst the remaining species are broadcast spawners with a lower larval competency period (Babcock et al. 1985).

It is important to note that small, isolated, and often peripheral islands in the Pacific Ocean are usually subject to high faunal turnover rates, which are a major factor contributing to differences in community structure in time and space at these locations (Pauley 1989, Harriott et al. 1994, Harriott et al. 1995). Temporally, little is

known of the variability in community structure at the Kermadec Islands, as due to the Islands' isolation and inaccessibility, longer-term monitoring has not been possible. Previous short term studies have found large within-site variability (surveys 1991 - 1992 - Brook 1999; survey 2002 - Gardner et al. 2006), as well as variation between studies in depth-related patterns of coral distribution; however repeat sampling of the same sites has not been undertaken. Crucially, however, the present study identified the same coral species present at the Kermadec Islands as reported by Brook (1999), which suggests that although coral cover may be temporally variable, species presence is not. Hence, the analysis of regional species affinities remains valid.

2.4.2. Wider implications

In a time of global climate change and sea temperature warming, it is conceivable that 'marginal' coral communities may be more likely to survive than are 'optimal' communities, as they have an evolutionary history of persistence in stressful environments (Glynn 1996). On one hand, high latitude coral reefs or communities, at lower temperatures than their tropical counterparts, might be a refuge in the future as coral distributions change, and such sites become less marginal and more optimal (Riegl & Piller 2003). However, as stressed here, latitude should not be considered in isolation from other local environmental factors, which have the potential to exert marked influence on community structure. For example, whilst the Kermadec Islands may be somewhat protected from future sea surface warming due to their high latitude and ocean situation, their potential to act as a refuge for many taxa found at other high latitude locations is diminished, due to the extreme wave energy and their isolation. Further, the potential for species living at the Kermadecs to 're-seed' reefs

denuded of their coral fauna by global warming and other environmental stressors (Brown & Suharsono 1990, Glynn 1996) is limited, again by the isolation of this oceanic site and the limited capacity for coral larvae to be transported across such large distances.

The Kermadec Islands will be affected in the near future by the combined effects of sea temperature rise and ocean acidification., as high latitude sites are likely to be among the first affected on a global scale (Hoegh-Guldberg et al. 2007). The decline in accretion by the corals at the KI will reduce their competitive ability against macroalgae, and potentially reduce in coral cover. Conversely, the increased temperatures will aid in faster growth rates, increasing their competitive ability. A change in species composition is likely; with only those remaining that can cope with the new environmental conditions.

Future research to assess coral species-specific responses to environmental factors will contribute to a better understanding of the distribution and evolution of coral reefs through time, and what the future holds for both marginal and optimal coral reefs and communities. As the Kermadec Islands are uninhabited, they offer a valuable example of a marginal coral community that is free from anthropogenic impact, and ongoing monitoring of the changes in coral community composition at this site will be of great value in assessing changes linked to future environmental disturbances, such as global warming, at other high latitude sites.

Chapter 3

Local endemicity and high diversity characterise high latitude coral-*Symbiodinium* partnerships

ABSTRACT

Obligate symbiotic dinoflagellates (*Symbiodinium*) residing within the tissues of most reef invertebrates are important in determining the tolerance range of their host. Coral communities living at high latitudes experience wide fluctuations in environmental conditions and thus provide an ideal system to gain insights into the range within the symbiotic relationship can be sustained. Additionally, high latitude coral communities have the potential to act as refugia during periods of environmental change. Despite this, very little is known on the host and symbiont partnerships at high latitudes and thus *Symbiodinium* diversity and specificity of high latitude coral communities was explored using denaturing gradient gel electrophoresis (PCR-DGGE) analysis of the internal transcribed spacer regions (ITS1 and ITS2) of the ribosomal DNA at Lord Howe Island (31°S; Australia), and the Kermadec Islands (29°S; New Zealand). All but one hosts associated with clade C *Symbiodinium*, the exception being a soft coral (*Capnella* sp.) that contained *Symbiodinium* B1. Besides generalist *Symbiodinium* types C1 and C3, approximately 72% of the *Symbiodinium* identified were novel C-types, and zonation of symbionts in relation to environmental parameters such as depth and turbidity was evident in certain host species. The high latitude *Symbiodinium* communities showed little

overlap and relatively high diversity compared with communities sampled on the tropical Great Barrier Reef. Although host specificity was maintained in certain species, others shared symbionts and this potential reduction of fidelity at high latitude locations may be the result of locally challenging and highly variable environmental conditions. The combination of a highly diverse *Symbiodinium* community with a tolerance to a broad range of environmental conditions may, however, be beneficial if high latitude coral communities are to act as refugia in the future.

3.1 INTRODUCTION

Much of the success of coral reefs lies in the symbiotic relationship between reef invertebrates and dinoflagellates of the genus *Symbiodinium*. Current knowledge on the evolution, biogeography and ecology of *Symbiodinium* stems from our ability to, with the aid of molecular techniques, distinguish members of this highly diverse group of microorganisms. Thus far, 8 clades (A-H) of *Symbiodinium* have been identified of which six (A-D, F and G) form stable symbiotic associations with scleractinian corals (reviewed in Rowan 1998; Baker 2003; Coffroth and Santos 2005). Within each clade numerous sub-cladal *Symbiodinium* types have been identified (LaJeunesse et al. 2003, 2004a). Whilst the ecology of reef corals as well as some of their physiological traits have been correlated to the presence of different clades of *Symbiodinium* present within the host tissues (for example, clade D is indicated as thermally tolerant and clade C as thermally sensitive; Rowan and Knowlton 1995; Rowan et al. 1997; Baker 2001; Baker et al. 2004; Ralph et al. 2005; Berkelmans and Van Oppen 2006), increasing evidence indicates that functional

diversity of *Symbiodinium* may be better described at the sub-cladal level and distinct types exhibit host specificity, physiological differentiation as well as distinct ecological patterns (Iglesias-Prieto and Trench 1997; Warner and Fitt 1999; Tchernov et al. 2004; Sampayo et al. 2007, 2008, Frade et al. 2008a, 2008b).

Despite evidence that *Symbiodinium* plays an important role in driving tolerance ranges of the holobiont (host and symbiont) (Rowan and Knowlton 1995; Rowan et al. 1997; Iglesias-Prieto et al. 2004), little is known of the diversity of *Symbiodinium* in marginal environments where corals exist close to their survival thresholds (i.e., water temperature, light penetration and aragonite saturation; Kleypas et al. 1999). High latitude reefs represent marginal environments due to wide ranges in seasonal temperatures, low annual minimum temperatures and relatively low irradiance levels. In comparison to tropical reefs, high latitude coral communities generally sustain low species diversity, overall coral cover is low and existing corals experience reduced accretion rates (Crossland 1988; Veron 1995).

Living under highly variable or extreme conditions gives rise to unique coral communities that are adept to living under the specific environments governing higher latitude locations. Many common reef-building species do not occur outside the tropics (roughly 25°S and 25°N) and high latitude coral communities differ in species composition from their tropical counterparts (Veron and Done 1979; Kleypas et al. 1999; Harriott and Banks 2002) with subtropical species that are rare or absent on tropical reefs becoming more abundant with increasing latitude. For example, along the coast of eastern Australia certain species of the coral genera *Porites* or *Acropora* decrease in abundance with increasing latitude and are replaced by species such as *Acanthastrea lordhowensis*, *Hydnophora pilosa, and Favia speciosa* which are relatively uncommon, rare or absent in the tropics (Veron 1986; Van Woesik and

Done 1997). The absence of the dominant components of tropical reefs at high latitudes can result from limited dispersal (Harriott and Banks 2002) or a potential reduction in successful settlement. Although many factors are involved in successful recruitment and subsequent survival, the inherent physiological tolerance range of recruits and their resident symbionts are likely to be a major factor.

Although some data are available on the changes in community structure of reef corals as they extend into higher latitudes, similar information on the symbiont community is sparse. *Symbiodinium* of clades A, B, C and D have been found at high latitudes and thus distributional patterns appear inconsistent. A shift from clade C in tropical zones to clade A, B or D was recorded by studies on various invertebrate hosts from Japan, the northeastern Atlantic and Mediterranean Sea (*Anthopleura elegantissima* in LaJeunesse and Trench 2000; *Acropora longicyathus* in Loh et al. 2001; *Plesiastrea versipora* in Rodriguez-Lanetty et al. 2001; *Porites porites* in Savage et al. 2002). Interestingly, despite clade D *Symbiodinium* generally being indicated as thermally tolerant it was also found at high latitude reefs that experience low temperatures and limited light (Chen et al. 2003; Lien et al. 2007).

Members of clade C *Symbiodinium* largely dominate the tropical Indo-Pacific, and clear patterns of latitudinal zonation have been noted at the sub-cladal level (type) (Reimer et al. 2006; Macdonald et al. 2008). For example, distinct *Symbiodinium* types of clade C occur in *Stylophora pistillata* in relation to a latitudinal temperature gradient (Macdonald et al. 2008). Given that distinct *Symbiodinium* play an important role in driving the physiological tolerance range of their host (LaJeunesse et al. 2003; Iglesias-Prieto et al. 2004; Rowan 2004; Tchernov et al. 2004), understanding if and how symbiont communities associated with high

latitude reef corals are different from their tropical counterparts will provide clues as to how corals can survive under marginal or changing conditions.

Symbiodinium diversity was examined in the most commonly found corals from two high latitude reefs, Lord Howe Island (LHI, Australia) and the subtropical Kermadec Islands (New Zealand). LHI (31"33'S, 159"05' E, Fig. 1) represents the southernmost coral reef in the world and is situated over 1000 km from the southern Great Barrier Reef (GBR). Corals are believed to have reached LHI under the influence of the warm East Australian Current (EAC; Wolanski 1994), which maintains annual sea-surface temperatures of between 18 °C and 26 °C at LHI (Wicks, pers. obs.). The presence of the EAC provides conditions amenable to coral growth that are quite exceptional for a location of such high latitude. Regardless of its geographical isolation (Veron and Done 1979), latitude and small size (11 km by 2.5 km), and also thought to be a result from the connection with the EAC, LHI sustains high coral species diversity (83 species covering 33 genera) and coral cover comparable to tropical sites (Harriott et al. 1995).

The KI on the other hand, sustain only 24 scleractinian species that occur mainly in shallow waters less than 30 m deep (Brook 1999). The KI are 750 km off the northeast coast of New Zealand's North Island (Fig. 3.1), and approximately 3000 km from the GBR. Equidistant between temperate New Zealand and tropical Tonga, the KI are one of the few subtropical island groups in the western South Pacific Ocean, and form New Zealand's largest marine reserve (748,000 ha).

The two high latitude locations selected for surveys of *Symbiodinium* diversity are therefore fundamentally different. While LHI hosts a unique mix of tropical coral species at their southernmost limits as well as temperate coral species which are rare/absent on tropical reefs, the KI forms a non-reef building coral

community that hosts only temperate coral species. Nonetheless, given the geographical position and extreme conditions of both locations, we hypothesize that, similar to a reduction of coral species diversity with latitude (Kleypas et al. 1999), *Symbiodinium* diversity will be low and local endemicity will be high in comparison to the tropical GBR.

3.2 MATERIALS AND METHODS

3.2.1 Sampling and oceanographic parameters

In January 2007, fragments of 12 coral species were collected by SCUBA at six sites (Fig 3.1) around LHI (1 - 3 samples per species and location, Table 1). Collection sites were situated in the shallow lagoon (1 - 3 m) and on the fore reef (5 m and 15 m) on the eastern exposed side and the sheltered western side. At each of the locations it was ensured that collections were made of the three most common coral species, *Acropora cuneata, Goniastrea favulus* and *Pocillopora damicornis* in order to directly compare effects of the external habitat. Sampling at the KI was conducted at Raoul Island (29^{o} 14.75 S, 177^{o} 52.87 W, Table 3.1, n = 1 - 4) in November 2004. Of the 24 present species at this location, three of the most common hard corals and two soft corals were collected at 5 - 10 m (Table 3.1). All samples were collected more than 5 meters apart to avoid sampling of clone mates. Coral fragments were always removed from the top of colonies using a hammer and chisel, fixed in absolute ethanol and stored at -20°C until further processing. Seawater temperature, turbidity and salinity were recorded at each of the sites and at each depth using a CTD Unit (Richard Brancker Research Ltd XR 420). Collections were conducted on cloudless days of similar environmental conditions

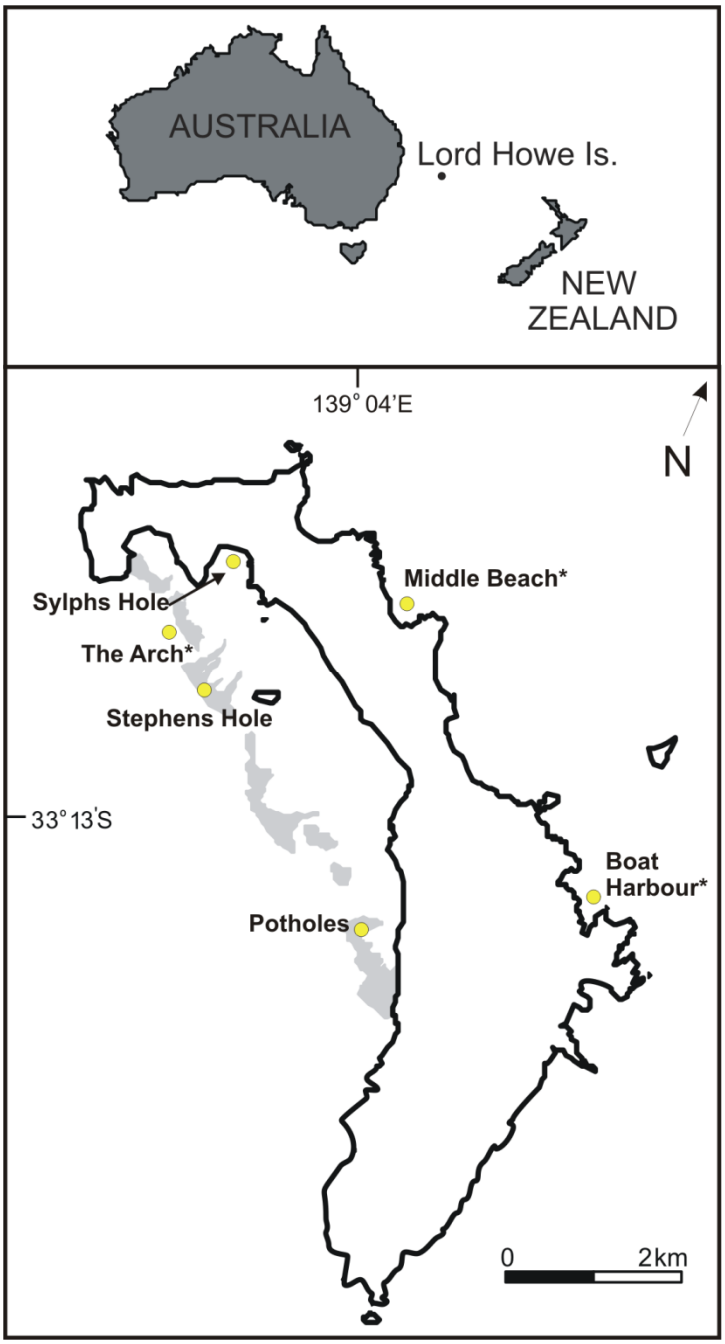


Figure 3.1 Location of (a) Lord Howe Island and the Kermadec Islands and (b) sampling sites around Lord Howe Island. Study sites are annotated by ●, asterisk marking sites sampled at 2 depths (5 m and 15 m). For species sampled at each site see Table 3.1.

3.2.2 DNA extraction and *Symbiodinium* identification

Symbiodinium cells were isolated prior to DNA analyses by removing the coral tissue from the skeleton using an airgun connected to a scuba cylinder. The tissue was homogenized using a tissue grinder in extraction buffer (1.2 M filtered seawater and 5 mM EDTA), the slurry was centrifuged at 1000 g, the buffer discarded and the remaining pellet was re-homogenised in 0.01% Triton X-100 extraction buffer, followed by a second centrifugation (1000 g) and homogenization step. DNA of the algal pellet was extracted using the DNeasy Plant Mini Kit following manufacturer's instructions (Qiagen).

Symbiodinium types were characterised using both the internal transcribed spacer 2 region (ITS2) and internal transcribed spacer 1 region (ITS1) of nuclear ribosomal DNA (cf. LaJeunesse 2001, 2002, LaJeunesse et al. 2008). The ITS2 region was PCR amplified using primers "ITS 2 clamp'' (CGCCCGCCGC GCCCCGCGCC CGTCCCGCCG CCCCCGCCC GGGATCCATA TGCTTAAGTT CAGCGGT-) and ''ITSintfor 2'' (GAATTGCAGA ACTCCGTG) (LaJeunesse and Trench 2000) under ''touchdown'' thermal cycle conditions specified in LaJeunesse (2002). The ITS1 region was amplified using primers "ITS1clamp" (CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCCGGGATCCGTT TCCGTAGGTGAACCTGC) and "ITS1intrev2' (TTCACGGAGTTCTGCAAT) (LaJeunesse et al. 2008). ITS2 amplified PCR products were subsequently run on a Biorad DCode Denaturing Gradient Gel Electrophoresis (DGGE) system, using a 30% to 65% gel during a 100V run for 14 h. PCR amplifications of the ITS1 region were run on a CBS Scientific DGGE system using an internal gradient of 50% to 90% denaturants and run at 115 V for 15 h. All DGGE gels were stained using 1X SYBR Green for 30 min and visualized on a UV transilluminator.

To identify distinct *Symbiodinium* type, prominent bands were excised from up to three representative samples of each characteristic ITS-DGGE fingerprint. Excised bands were eluted in 500 µl ddH20, kept at 4°C for 24 h and reamplified using GC-clamped primers (under conditions as above). Amplicons were re-run on the DGGE system to check band position and potential contamination with other bands during excision prior to sequencing. Clean bands running at the expected position in relation to the original sample were re-excised, eluted and reamplified with the non-GC clamped primer set (cf. LaJeunesse 2002; LaJeunesse et al. 2008). Amplifications were purified by ethanol precipitation and sequenced in both directions at the Australian Genome Research Facility (ABI BigDye Terminator v3.1; AB3730x1 sequencer). Chromatograms were checked using Seqman software (Lasergene) and aligned using Clustal-X (Thompson et al. 1997). The resulting consensus from each dominant or co-dominant band was identified by a BLAST search of previously recorded *Symbiodinium* types on GenBank (http://www.ncbi.nih.gov). Phylogenetic analyses of dominant rDNA ITS1 and ITS2 repeat sequences were performed in PAUP (version 4.0b10, Swofford 2000) under the maximum parsimony criterion treating gaps as a fifth character state and indels as a single evolutionary event with bootstrap values calculated based on 1,000 replicates.

3.2.3 Data analysis

Differences in measured environmental parameters (light, temperature, salinity and turbidity) among sites, depths and sampling location (lagoon, sheltered and exposed) were assessed using non-parametric Kruskal-Wallis and Dunn's posthoc tests (SPSS v16) because assumptions of normality and homogeneity of variances were not met.

To test the effects of environmental parameters on *Symbiodinium* distributions across sites in the three coral species sampled at all sites (*A. cuneata, G. favulus* and *P. damicornis)* multivariate analyses were conducted in PRIMER v6 with PERMANOVA add-on (PRIMER-E, Plymouth, Clarke & Gorley 2006), treating each coral colony as a replicate. The host was a significant factor in driving symbiont distributions (presence/absence data and Bray-Curtis similarity; ANOSIM, $p < 0.01$) and, with little overlap of symbionts among hosts, all subsequent analyses were conducted separately for each of the three host species.

BIOENV was used as an exploratory tool to correlate the similarity matrix derived for the symbiont distributions with an equivalent for the suite of environmental measurements taken at each site. Results are expressed as a Spearman's correlation coefficient (r), and indicate the proportion of variance in the community data explained by these environmental variables (see Clarke & Ainsworth 1993 for full details). Means of environmental parameters for each site (temperature, salinity, depth and turbidity) were (log+1) transformed, and normalized, and similarities calculated based on Euclidean distance. A distance-based redundancy analysis (dbRDA), which uses multiple regressions on any distance or similarity matrix, was performed to identify the proportion of total variation in the matrix (symbiont abundance) explained by predictor variables (environmental factors) (Legendre & Anderson 1999, McArdle & Anderson 2001).

Finally, community diversity of *Symbiodinium* at LHI and the KI was compared to published diversity surveys of the southern and central GBR (LaJeunesse et al. 2003, 2004a respectively). Due to differences in sampling intensity across studies, diversity estimates were generated as a normalization of species or

genera of host sampled (number of symbionts divided by the number of hosts sampled).

3.3 RESULTS

3.3.1 Symbiont community diversity and phylogenetic analysis

In total 12 and 5 coral species were sampled at Lord Howe Island (LHI) and the Kermadec Islands (KI) respectively (Table 3.1), with 80 samples collected in total. Across these different hosts at both locations, 18 *Symbiodinium* types were identified, of which only one was a member of *Symbiodinium* clade B (type B1) and occurred in the soft coral *Capnella.* The remaining 17 were members of clade C (Table 3.1, Fig. 3.2). In total 15 and 5 different *Symbiodinium* types were identified at LHI and the KI respectively, of which *Symbiodinium* types C1, C3, C1c, C22a, and B1 were previously been recorded on the tropical GBR (Table 3.1). Thus, the majority of identified *Symbiodinium* identified at these high latitude locations represented novel types, and were assigned a name following current ITS2 nomenclature for *Symbiodinium* (cf. LaJeunesse et al. 2003, 2004a) as types C1z, C1bb, C3w, C3gg, C3n-hh, C41, C100, C102, C104, C108, C111, C117, and C118. *Symbiodinium* community diversity at LHI was relatively high with 1.56 type/genera compared to 0.58 (23 types in 40 genera) and 0.43 type/genera (32 types in 74 genera) on the southern and central GBR respectively.

Phylogenetic analyses of both the ITS1 and ITS2 regions of the ribosomal DNA indicated that the ITS1 region is more conserved. For example, the ITS1 region amplified for DGGE showed no difference between *Symbiodinium* types C1 and C3.

Table 3.1 Sample list of host specimens and identified *Symbiodinium* type(s) from sites (and depths) around Lord Howe Island (LHI), Australia, and the Kermadec Islands (KI, NZ). Types hosted by same coral spp. or family on southern GBR (S GBR, LaJeunesse et al. 2003, Sampayo et al. 2007) and the central GBR (C GBR, LaJeunesse et al. 2004) noted († refers to type in closest coral spp.) Soft corals from Goulet et al. (2008). v: vertical transmission; h: horizontal transmission (Richmond + Hunter 1990).

Family/Species	Site	Depth (m)	n	ITS1	ITS2	S GBR	N GBR
Acroporidae							
Acropora cuneata (v)	Stephens Hole (LHI)	2-3 m	3	C1/C3	C3	C3†	C3k†, C1†, D2†, C3†, C3i†
	Potholes (LHI)	2-3 m	3	C1/C3	C3		
	The Arch (LHI)	5 m	3	C1/C3	C3		
	Boat Harbour (LHI)	5 m	2	C1/C3	C3		
		15 m	3	C1/C3	C3		
	Middle Beach (LHI)	5 m	2	C1/C3	C3		
		15 m	3	C1/C3	C3		
	Boat Harbour (LHI)	5 m	1	C100	C100		
	Middle Beach (LHI)	5 m	1	C100	C100		
	The Arch (LHI)	15 m	3	C102	C102		
	Sylphs Hole (LHI)	2-3 m	2	C1c	C1c		
	Sylphs Hole (LHI)	2-3 m	1	C1/C3	C3w		
A. abrotenoides (h)	Potholes (LHI)	2-3 m	3	C1/C3	C3	C3†	As above
A. glauca (h)	Stephens Hole (LHI)	2-3 m	2	C1/C3	C3	C3†	As above
			1	C1/C3	C3		
A. latistella (h)	Stephens Hole (LHI)	2-3 m	3	C1/C3	C3	C3	As above
Dendrophyllidae							
Turbinaria frondens	KI	5 m	4	C1/C3	C1	C22a†	C40, C3h, C1†
		15 m	1	C22a	C22a		
Faviidae							
Goniastrea favulus (h)	Potholes (LHI)	2-3 m	3	C1/C3	C3w	D1a, C3†, C1†	C1†, C3†, C3h†
	The Arch (LHI)	15 m	2	C1/C3	C3w		
	Boat Harbour (LHI)	15 m	2	C1/C3	C3w		
	Middle Beach (LHI)	5 m	2	C1/C3	C3w		
		15 m	2	C1/C3	C3w		
	Stephens Hole (LHI)	2-3 m	2	C1c	C1c		
	The Arch (LHI)	5 m	1	C1c	C1c		
		15 m	1	C1c	C1c		
	Boat Harbour (LHI)	15 m	1	C1c	C1c		
	Middle Beach (LHI)	15 m	1	C1c	C1c		
	Stephens Hole (LHI)	2-3 m	1	C1/C3	C108		
	Middle Beach (LHI)	5 m	1	C1/C3	C108		
	The Arch (LHI	5 m	2	C1/C3	C108		
Favites abdita (h)	The Arch (LHI)	5 m	3	C1/C3	C1	C3, C21†	C3, C3h
Montastrea curta (h)	Boat Harbour (LHI)	5 m	3	C1/C3	C1	C3	C3, D1a
	KI	5 m	3	C1/C3	C1		
Cyphastrea serailia (h)	Boat Harbour (LHI)	5 m	1	C102	C102	C1, C1i	C1, C3
			2	n.a.	C104		
Merulinidae							
Hydnophora pilosa	KI	5 m	2	C1/C3	C3w	C3†, D1a†	C3†, C3h†
		10 m	1	C1/C3	C3w		
		10 m	3	C1/C3	C1z		

Pocilloporidae							
Pocillopora damicornis (v/h)	Sylphs Hole (LHI)	2-3 m	3	n.a.	C41	C1c, C42,	C1j, C1c[†],
	The Arch (LHI)	15 m	1	n.a.	C41	C42a,	
	Boat Harbour (LHI)	15 m	2	n.a.	C41	C42b,	
	Middle Beach (LHI)	15 m	1	n.a.	C41	C33, C33a	
	Stephens Hole (LHI)	2-3 m	3	C100	C100		
	Potholes (LHI)	2-3 m	2	C100	C100		
	The Arch (LHI)	5 m	2	C100	C100		
		15 m	2	C100	C100		
	Boat Harbour(LHI)	5 m	3	C100	C100		
		15 m	1	C100	C100		
	Middle Beach (LHI)	5 m	3	C100	C100		
		15 m	2	C100	C100		
	Potholes (LHI)	2-3 m	1	n.a.	C118		
Stylophora pistillata (v)	Boat Harbour (LHI)	15 m	3	C1/C3	C1	C1, C78,	C8
				C1/C3	C3gg	C8a,	
						C35a, C79	
Seriatopora hystrix (v)	Boat Harbour (LHI)	15 m	3	C1/C3	C3n-hh	C3, C3nt	C3
Poritidae							
Porites cf. *heronensis (v/h)*	Boat Harbour (LHI)	5 m	2	C117	C117	C15[†],	C15[†], C60[†]
			1	C111	C111	C28[†]	
	Sylphs Hole (LHI)	2-3 m	3	C1/C3	C3		
Alcyoniidae							
Sinularia sp.	KI	5 m	3	C1/C3	C1z	C3j	C65, C1c,
		10 m	3	C1/C3	C1z		C1
Capnella sp.	KI	5 m	1	B1	B1		C, A

Some ITS2 types characterized by the presence of co-dominant repeats of the ribosomal array occurring in conjunction with the C1 or C3 sequence, such as ITS2 type C1z, C3gg, C3n-hh are 'collapsed' to the C1-C3 core for the ITS1 region (compare Fig 3.3a, b). Nonetheless, highly divergent sequences representing the dominant sequence within each symbiont type were consistent between the two DNA regions (Fig. 3.3a, b; for example C41, C102, C111, C117, C118) and overall tree topology was similar.

Finally, symbiont types identified in the present study were compared to those found on the tropical GBR. Phylogenetic analyses (not shown) indicated that symbionts from high latitudes did not share a common ancestry with symbionts found in corals that usually associating with highly specific lineages across the Indo-Pacific, such as *Porites* with the C15/ C17 lineages (LaJeunesse 2005). Examples of such symbionts are those in association with *Porites* cf. *heronensis* (C111, C117),

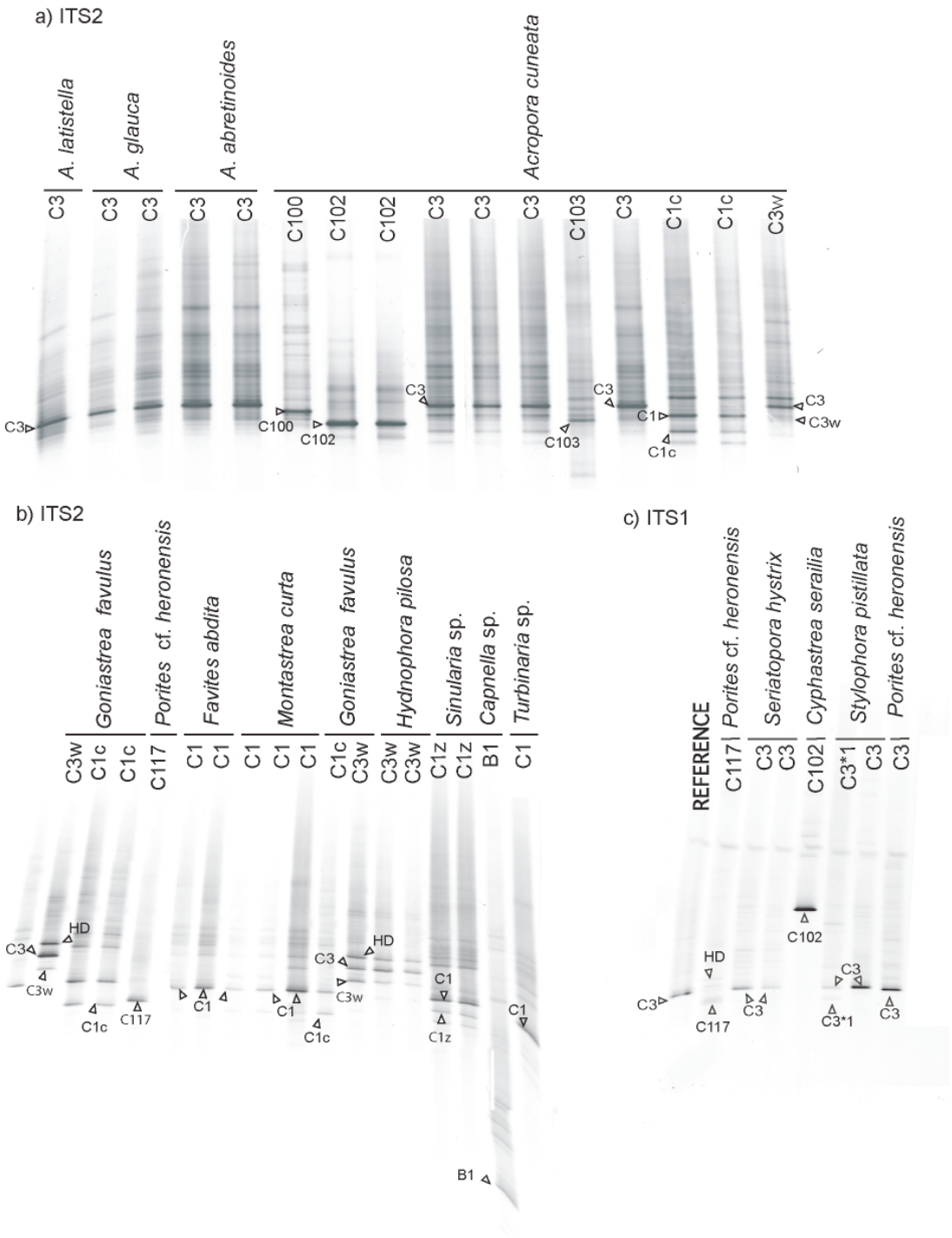
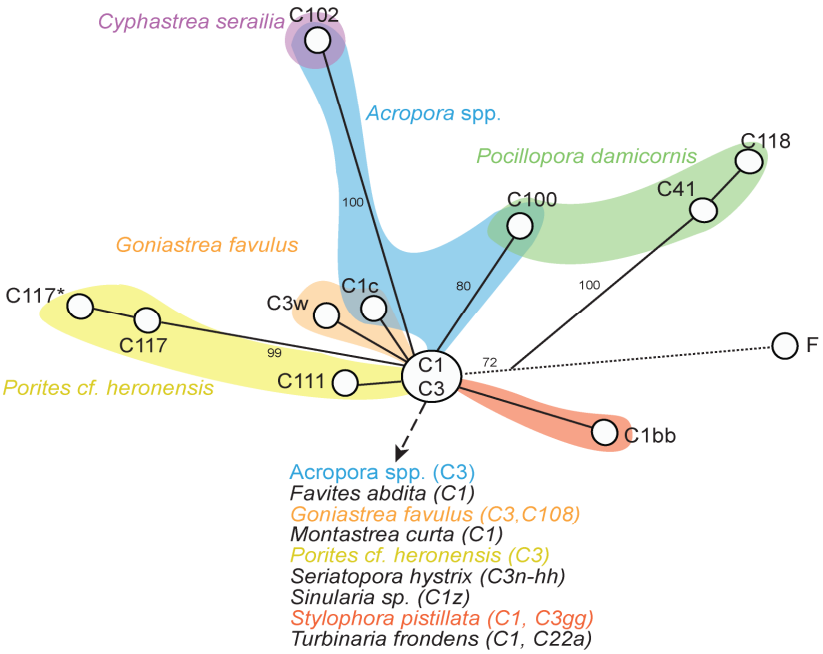


Figure 3.2 PCR-DGGE fingerprints of ITS2 (a, b) and ITS1 (c) *Symbiodinium* types identified in various coral species at Lord Howe Island (Australia) and the Kermadec Islands (New Zealand). Dominant and co-dominant bands in the ITS2-DGGE profile were excised (marked with arrows) to identify the resident *Symbiodinium* type (marked above each lane). For example, *Symbiodinium* type C1c represents a profile containing both the C1 and C1c sequence. HD: Heteroduplex.

(a) ITS1



(b) ITS2

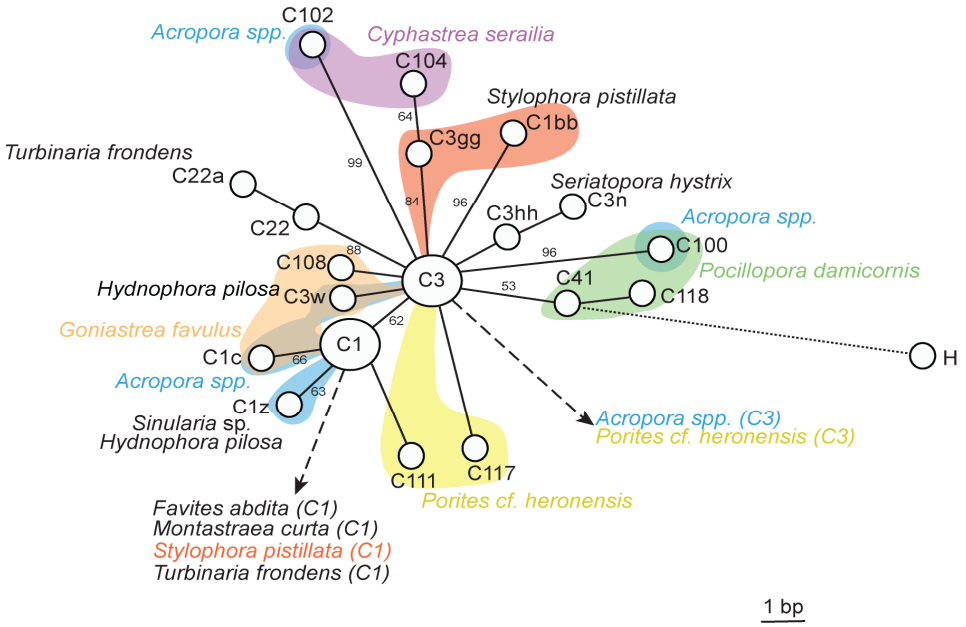


Figure 3.3 Most parsimonious un-rooted tree of the ITS rDNA of *Symbiodinium* isolated from various coral hosts repeats identified by (a) ITS1-DGGE and (b) ITS2-DGGE by direct sequencing of excised bands. Ancestral *Symbiodinium* types C1 and C3 are represented by *larger circles* compared to others but circle size is not representative of abundance. Branch length corresponds to the number of base changes between each sequence variant (number of changes shown next to each branch). Shaded areas of a single colour indicate that multiple symbionts were present; and colours indicate different hosts: *Acropora cuneata* (blue), *Cyphastrea serailia* (purple), *Goniastrea favulus* (orange). *Pocillopora damicornis* (green), *Porites* cf. *heronensis* (yellow).

Pocillopora damicornis (C41, C100, C118) and *Stylophora pistillata* (C1, C1bb, C3gg), all of which appear to have diverged from the C1 or C3 lineage as opposed to host specific radiations (LaJeunesse 2005). An exception is type C3n-hh found in *Seriatopora hystrix*, which is closely related to C3n-t commonly found on the southern GBR (Sampayo et al. 2007).

Host identity had a major influence on *Symbiodinium* distributions (ANOSIM, R = 0.404, $p < 0.001$) and generalist as well as host specialist symbionts were found (Table 3.1). The pandemic host generalist *Symbiodinium* type C3 associated with 6 species at LHI: *A. cuneata, Acropora abrotenoides, Acropora glauca, Acropora latistella*, *G. favulus,* and *P.* cf. *heronensis.* Type C1, also a generalist, was found in *Montastraea curta*, *Favites abdita* and *Stylophora pistillata* at LHI and *Turbinaria frondens.* A number of *Symbiodinium* types other than the pandemic host generalists C1 and C3, also associated with multiple hosts (Table 3.1). For example, C1c was found in *A. cuneata* and *G. favulus*, C1z in *H. pilosa* and *Sinularia* sp., C3w in *A. cuneata*, *G. favulus* and *H. pilosa*, and type C102 in *A. cuneata* and *C. serailia.* Although it appeared that symbionts were mainly shared by host species that have a horizontal mode of symbiont transmission it is interesting to note that vertical transmitters (but see discussion) *A. cuneata* and *P. damicornis* shared type C100 (n = 2 and n = 8 respectively; Table 3.1). Although symbionts appeared to be shared between hosts, such symbionts (except for C1 and C3) were usually dominant in one host and rare in the other. For example, C3w was present in only 4% of *A. cuneata* samples but was found in 52% of *G. favulus* samples. Of the total 18 *Symbiodinium* types, ten were exclusively found in a single host and were type B1 in *Capnella* sp., C22a in *T. frondens,* C41 and C118 in *P. damicornis,* C104 in *C. serailia*,

C108 in *G. favulus*, types C111 and C117 in *P.* cf. *heronensis*, C3n-hh in *S. hystrix* and C3gg as well as C1bb in *S. pistillata*

3.3.2 Environmental variability and symbiont diversity

At LHI, while turbidity was significantly higher at Sylphs Hole (2.4 ± 0.01 FTU, mean ± SD; H = 1464, DF = 2, $p < 0.01$), turbidity (1.3 - 1.7 ± 0.01 FTU, mean ± SD) was not significantly different between any of the other sites (Fig. 3.3b). Salinity (Fig 3a) was homogenous within the lagoon (31.1 ± 0.02, mean ± SD), excluding Sylphs Hole (31.0 ± 0.04, mean ± SD), and was significantly lower than the shallow and fore reef sites (31.2 ± 0.01 and 31.4 ± 0.02 respectively, mean ± SD; Kruskal Wallis, H = 7416, DF = 8, $p < 0.001$). Finally, at LHI the water temperature was significantly higher at the lagoon sites of Sylphs Hole, Potholes and Stephens Hole compared to the fore reef sites of The Arch, Middle Beach and Boat Harbour (Fig. 3c; Kruskal Wallis, H = 1943, DF = 9, $p < 0.001$). On the western fore reef (The Arch), mean temperature (± SD) was 0.1°C greater at 5 m (22.7°C ± 0.02) than 15 m (22.6°C ± 0.02; Fig. 4). At the eastern side (Boat Harbour) the temperature was 0.3°C lower at 10 m (22.5°C) than at 5 m (22.8°C), but temperature at Middle Beach was homogenous with depth (22.5°C ± 0.02). At the KI, mean water temperature (± SD) at time of sampling was 20.3 ± 0.01 at 5 m, decreasing by 0.2 ± 0.01 at 15 m. Mean turbidity (± SD) was 1.84 ± 0.01 FTU for all depths 5 – 15 m.

The influence of the above measured environmental variables on the *Symbiodinium* community was assessed using the three coral species, *A. cuneata, P. damicornis* and *G. favulus,* collected at all 6 sites around LHI. A total of five, three and three *Symbiodinium* types were found in *A. cuneata, P. damicornis* and *G. favulus* respectively (Table 1). Assessing the influence of environmental variables on symbiont distributions at LHI using

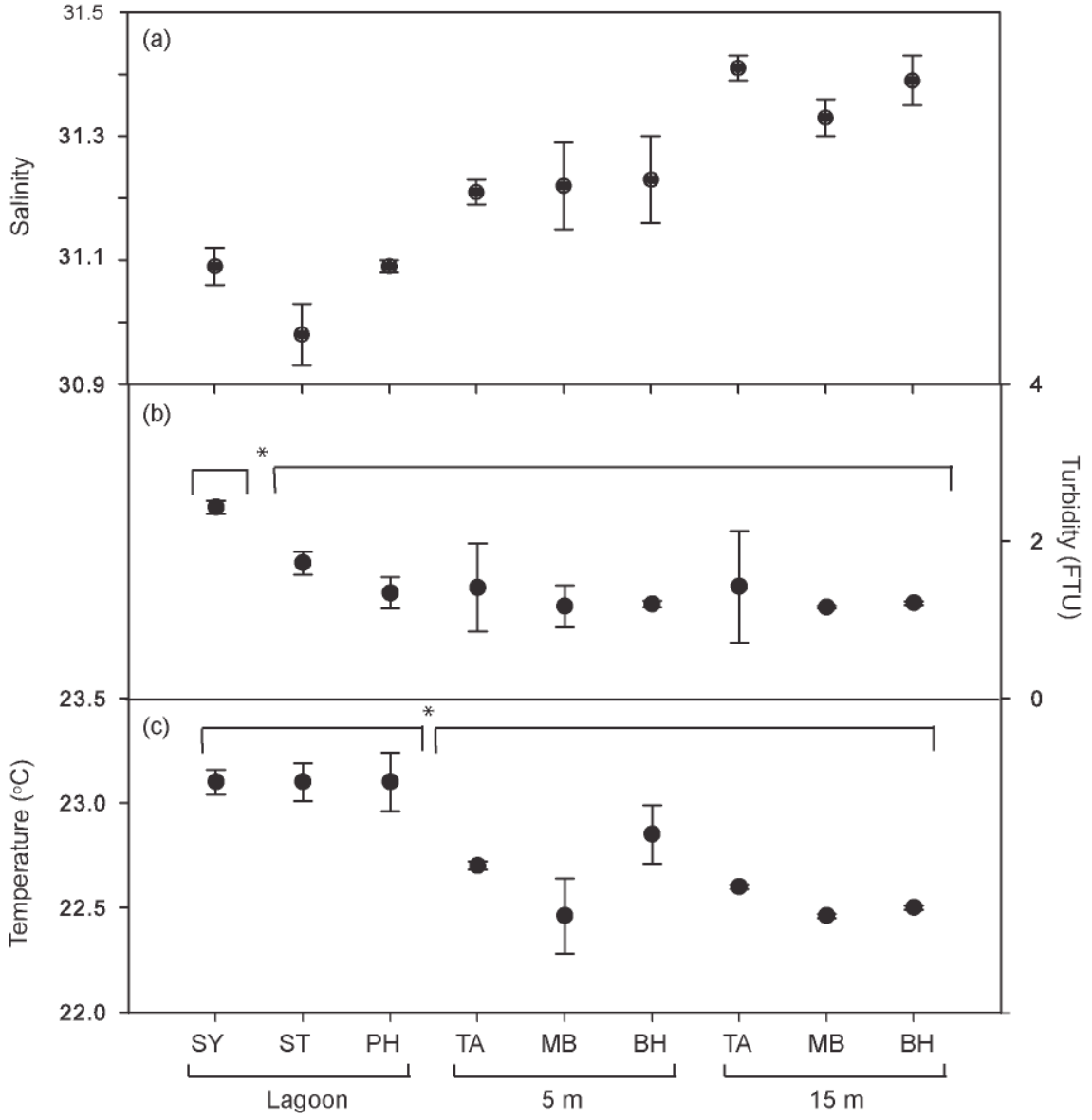


Figure 3.4 Mean (± SD) of environmental parameters measured at sites around Lord Howe Island at time of sampling: (a) salinity, (b) turbidity (FTU), (c) temperature (°C). SY: Sylphs Hole, ST: Stephens Hole, PH: Potholes, TA: The Arch, MB: Middle Beach, BH: Boat Harbour. Asterisk marks significant differences in environmental variable between groups of sites at $p < 0.05$.

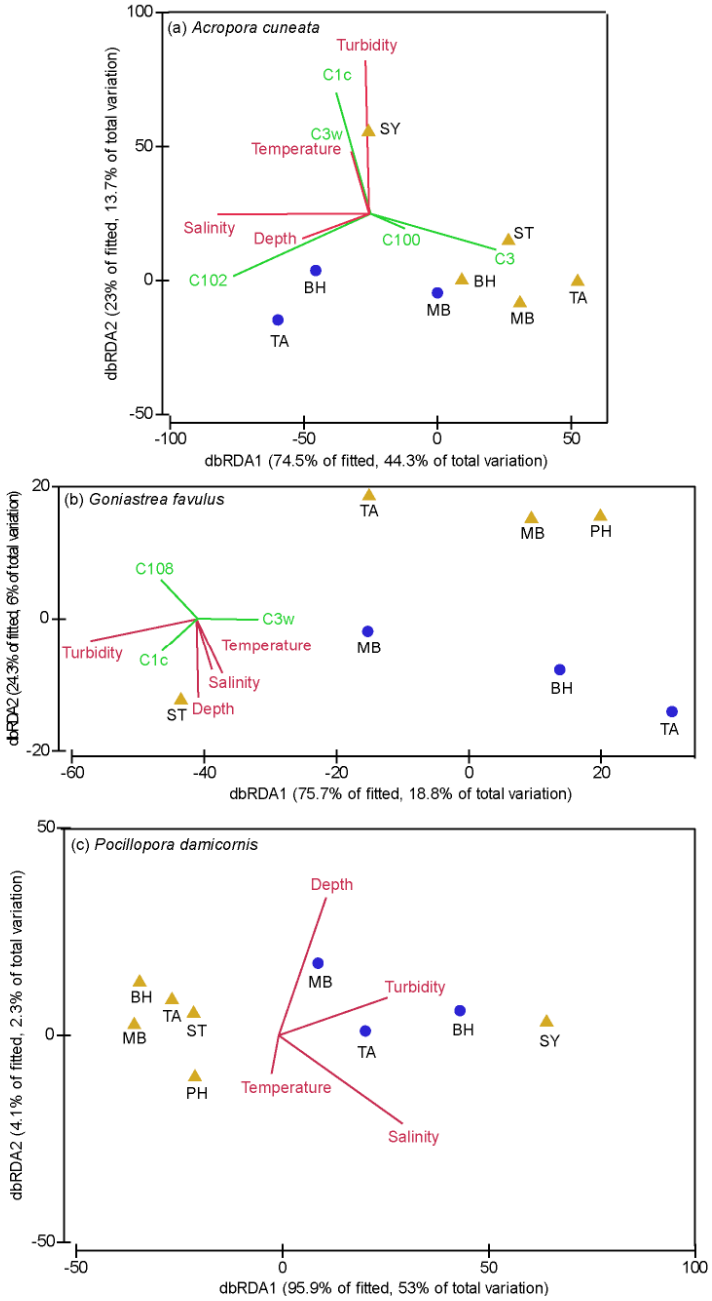


Figure 3.5 Distance based RDA ordination relating environmental variables to symbiont data, showing biplot projections for environmental data (in Figure 3.4) and symbiont types identified. Sites are labelled as abbreviations with symbols indicating depth (●deep ▲ shallow). Analysis was performed on Bray-Curtis dissimilarities of symbiont presence/absence data. '% of fitted' indicates the percentage of the variability in the original data explained by the axis and '% of total variation indicates the percentage of the variation in the fitted matrix (i.e. the fitted relationship between y and the explanatory variables) explained by the axis. Environmental variables (Ln+1) transformed and normalised. SY: Sylphs Hole, ST: Stephens Hole, PH: Potholes, TA: The Arch, MB: Middle Beach, BH: Boat Harbour.

multivariate analyses revealed complex patterns that were highly variable between host species (Fig. 4). Turbidity was the primary predictive environmental parameter for all host species and although the parameters of depth, salinity and temperature had varying effects they were correlated with each other indicating that the effects of each parameter on symbiont distributions could not be distinguished.

The predominant symbionts identified in *A. cuneata* (n = 27) were C3 and C102 (59% and 22% of samples) with C1c, C100 and C3w only occurring rarely (7, 7 and 4%). The main driving environmental variables explaining the observed distribution of symbionts in *A. cuneata* were turbidity and salinity (57% of the total variability; BIOENV R = 0.566, $p < 0.01$). The database redundancy analyses (dbRDA) depict the correlation of environmental variables to symbiont distributions in each of the three host species (Fig. 3.4), with the strongest effect of environmental predictors on symbiont distributions in *A. cuneata* (Fig. 3.4a). Turbidity is the main influential factor affecting symbiont distributions in *A. cuneata*, and is correlated with the presence of *Symbiodinium* C1c and C3w at the turbid site of Sylphs Hole. Depth dependence was observed for *Symbiodinium* type C100 and C102. C100 was found in colonies of *A. cuneata* from the western deep sites (>10 m) and C102 was only present in samples from the shallow, eastern exposed sites.

Of the sampled *P. damicornis* colonies (n = 24) 67% contained *Symbiodinium* type C100, 29% had C41 and a single sample had C118 (at the site Potholes, Fig. 3.1). Turbidity was the environmental parameters best explaining the observed symbiont distributions in *P. damicornis* (Fig. 3.4b; 34% of the variability; BIOENV R = 0.335, $p < 0.05$). The distribution of C41 and C100 are strongly correlated to both depth and turbidity, with C41 only found in colonies from Sylphs hole and at depths greater than 10 m, and C100 mainly present in colonies collected from shallow water or the lagoon.

Finally, 57% of *G. favulus* colonies (n = 21) associated with C3w, 33% with C1c and 9% with C108. In comparison to the other coral species, patterns of symbiont distributions of *G. favulus* were not significantly correlated to any of the measured environmental variables. Turbidity was the main factor identified, but explained the total variation in the symbiont community poorly (Fig 3.4c; 14%) and was not significant (BIOENV R = 0.139, $p = 0.17$). *Symbiodinium* C3w contrary to its presence in *A. cuneata*, where it was rare and only found at Sylphs Hole, was abundant and widely distributed in *G. favulus*. Host-specific C108 associated exclusively with colonies of *G. favulus* in the lagoon and shallow regions of the forereef (Table 3.1).

3.4 DISCUSSION

A high degree of diversity and endemicity of *Symbiodinium* was identified in corals at the high latitude locations of Lord Howe Island (LHI) and the Kermadec Islands (KI). Clade C types dominate the coral species at LHI, with local patterns of distribution of sub-cladal types driven by environmental parameters such as depth and turbidity, as well as being host-dependent. Furthermore, host specificity is significantly reduced compared to tropical reefs, such as the GBR (LaJeunesse et al. 2003, 2004a).

3.4.1 Biogeographic distribution patterns of *Symbiodinium*

The dominance of *Symbiodinium* types belonging to clade C at LHI and the KI was expected because many invertebrates, including corals, anemones and zoanthids, associate

with closely related clade C *Symbiodinium* in the Pacific (Baker and Rowan 1997; Loh et al. 2001; LaJeunesse et al. 2003,2004a, 2004b). At LHI and KI only B1 was identified in the soft coral *Capnella,* but all other *Symbiodinium* types identified were members of clade C (Table 1). Clade B *Symbiodinium* has been identified in the coral *Plesiastrea versipora* from high latitude regions, which harbours clade C in the tropics (Rodriguez-Lanetty et al. 2001). Studies of more temperate symbioses in the Atlantic region found a prevalence of both clades A and B *Symbiodinium;* clade A was present in *Anemonia* spp. in the Mediterranean and NE Atlantic (Savage et al. 2002), whilst *Anthopleura elegantissima* hosts clade B over a wide latitudinal range on the eastern North American coast (LaJeunesse and Trench 2000). High latitude corals on both sides of the Pacific, the Gulf of Cortez at 24°N (LaJeunesse et al. 2008), and Sesokojima, Japan at 27°N (Loh et al. 2001), however, also predominantly associated with clade C *Symbiodinium*. Furthermore, clade D symbionts, previously identified as 'heat tolerant' (Glynn et al. 2001; Baker et al. 2004), also occur in hosts at high latitudes (Lien et al. 2007), including *P. damicornis* (LaJeunesse et al. 2008), but was not found in the present study.

On a sub-cladal level, little is known of symbiont diversity at the high latitude limits of coral distribution. Most of the invertebrate hosts examined here at high latitudes associated with symbionts other than those found on the GBR (Table 1). For example, *F. abdita* and *M. curta* associate with C3 on the Southern GBR but are found with C1 at LHI, but both these symbionts are pandemic generalists. *H. pilosa* hosts *Symbiodinium* types C3w and C1z at the KI but other *Hydnophora* sp. associate with C3 throughout the whole range of the GBR (LaJeunesse et al. 2003). Only *Acropora* spp. hosting C3, and *Turbinaria* spp. with C22a in the present study are the same partnerships as on the southern GBR (Table 1; LaJeunesse et al. 2003).

Interestingly, whilst *Symbiodinium* C1c (and closely related symbionts C42a and C1b-c) is common to pocilloporid corals in locations ranging from Heron Island in the southern GBR to Lizard Island (north-central GBR; LaJeunesse et al. 2004b), Hawaii (LaJeunesse et al. 2004a), and the eastern Pacific (LaJeunesse et al. 2008), it is absent in Pocilloporid corals in the current study, where it hosts three novel symbiont types (C41, C100 and C118). These three symbionts are from a novel lineage and thus are likely to have developed separately from the widespread Pacific symbiont species. Interestingly, C1c is present in other hosts at LHI, namely *G. favulus* and *A. cuneata*, and these partnerships have not previously identified at other locations, in fact C1c was thought to be highly specific for *P. damicornis* in the western Pacific (Iglesias-Prieto et al. 2004). The absence of C1c with *P. damicornis* may suggest that that holobiont is not the optimal partnership for the variable environment at LHI.

3.4.2 Endemicity and diversity

The diversity of symbionts at high latitudes, and the endemicity observed in the present study may be driven by (1) host-dependent habitat specificity, (2) host-specificity and the reproduction strategy of the host, and (3) the biogeographical isolation of the sites.

Habitat specificity is observed on a local scale in the present study, and it is likely that the variable environment around LHI underlies the diversification of host-symbiont partnerships. For example, both *A. cuneata* and *P. damicornis* exhibit depth and turbidity-based patterns in symbiont diversity. *Symbiodinium* C100, which is prevalent in *P. damicornis* in all environments sampled, is also found on the shallow forereef when hosted by *A. cuneata*. Similarly, C1c and C3w are common to *G. favulus* across multiple locations whilst at the turbid site of Sylphs Hole these symbionts are found in association with *A.*

cuneata. The stratification of symbiont types linked to turbidity levels suggest that the symbiont types present at this site are specialized for the low light environment, but this pattern appears to be highly dependent on the host species in which the symbiont is found. Symbionts thus appear to have different environmental preferences according to the host they inhabit. Host-dependent thermal tolerance has been observed in symbionts associating with various *Acropora* spp. in the GBR*; A. tenuis* exhibited greater thermal tolerance whilst hosting C1 than D1, with the opposite responses observed in *A. millepora (*Abrego et al. 2008). The varying responses of host-symbiont partnerships was suggested to be due to (i) host factors, (ii) competitive ability of the symbionts in host cells, and (iii) the micro-environment of the host; possible explanations which require further exploration.

Interestingly, *P. damicornis* at the turbid site of Sylphs Hole was not found in association with any of the 'turbid' symbionts found in *A. cuneata* or *G. favulus* (C3w, C1c), but instead associated with *Symbiodinium* C41. The absence of C1c in *P. damicornis* suggests it is not a suitable symbiont under the current circumstances at LHI; these are a factor of the outside as well as the host species. C41 is likely to be shade adapted in combination with *P. damicornis* since it is also found at the deep sites.

These findings are consistent with light driven zonation patterns of *Symbiodinium* observed in many coral species. For example, the Caribbean coral *Montastraea annularis* is dominated by *Symbiodinium* clades A and B in shallow, well-illuminated sites, and by clade C in deep and poorly illuminated locations (Rowan and Knowlton 1995; Rowan et al. 1997). Depth based ecological stratification on a sub-cladal level has also been observed (LaJeunesse et al. 2003; Warner et al. 2006; Sampayo et al. 2007; Frade et al. 2008b), and for example in the coral genus *Madracis* functional differentiation between symbiont types is driven by light spectral niches (Frade et al. 2008a, 2008b). Whilst ecological zonation of

symbiont types with depth has been linked to the physiological adaptations of different symbionts to light regimes, depth variable parameters other than light intensity, such as temperature (Rowan 2004; Tchernov et al. 2004) or nutrient availability (Fitt and Cook 2001), may also play a role.

The second factor involved in driving observed host symbiont associations is linked to host specificity and this has been related to the various reproductive strategies employed by corals. Symbioses in corals that acquire their symbionts directly from their parent egg or brooded larvae (vertical transmission) are thought to be more specific for their symbionts due to the faithful transmission of symbiont type from parent to offspring (Yamamura 1996; Loh et al. 2001) compared to hosts that acquire their symbionts from the water column (horizontal transmission) (Loh et al. 2001; LaJeunesse et al. 2008; Stat et al. 2009). At LHI and the KI, 70% of the coral species sampled (with known transmission strategies) horizontally transmit their symbionts and thus have the opportunity to associate with a range of symbionts available from the water column. Most of the horizontal strategists either associate with the pandemic generalist types C3 or C1 but some contain locally endemic symbionts (i.e. *C. serailia* with C104, *G. favulus* with C108; Table 1).

Although host specificity in vertical strategists is usually high (Stat et al. 2009), 3 of the 4 known vertical transmitters in the present study harbour non-specific symbionts. Two of these hosts do additionally harbour host-specific symbionts (*P.* cf. *heronensis* with C111 and C117, and *S. pistillata* with C3gg). Interestingly, *A. cuneata*, a vertical strategist, harbours 5 non-specific symbiont types. Although one of these is a generalist (C3), the other types are all harboured by another host; C100 with *P. damicornis,* C102 with *C. serailia,* and C3w and C1c with *G. favulus*. The fact that symbionts are shared between vertical and horizontal transmitters may be driven by long-term isolation under challenging

environmental conditions, whereby perhaps normally host specific symbionts are acquired from the external environment rather than the generalist type. This scenario is rare, and has only been reported in *Fungia scutaria* with C1f in Hawaii (Weis et al. 2001; Rodriguez-Lanetty et al. 2004). Interestingly, at LHI, the horizontal strategist *G. favulus* harbours C108 in low frequency, whereas the two dominant symbionts (C3w and C1c) are present in multiple hosts. C108 may have evolved and be harboured by another vertical strategist not sampled in the present study. The ability of both horizontal and vertical strategists to associate with a range of symbiont types at these marginal locations may give them a greater flexibility to cope with the seasonally changing environmental conditions.

Where novel types are present, in many cases the host specific symbionts seem to have diverged independently from their Pacific radiations, which are widespread. For example *P. damicornis* harbours C118 and C41, which have the same lineage, separate to the C42a/C1c lineage harboured in other locations. The reproductive strategy of *P. damicornis* is unknown but can be suggested to be vertical due to the high specificity observed. Furthermore *Porites* spp. generally associate with symbionts of the C15 lineage in the Pacific (LaJeunesse et al. 2004b; Apprill and Gates 2007), but at LHI *P*. cf. *heronensis* harbours c117 and C111 from a separate lineage. The independence of divergences suggests not only that these locations are very isolated but also hints to the need for novel adaptations to challenging conditions.

The biogeographical isolation of the high latitude sites is likely to have contributed to the high levels of endemicity observed as well as the presence of host-specific locally divergent lineages of *Symbiodinium*. Although LHI is linked to the GBR through the flow of the EAC, coral populations are genetically isolated indicating that influx of recruits occurs infrequently (Ayre and Hughes 2004). The KI are even more isolated, 3300 km from

the GBR and 2200 km from LHI. Long distance dispersal of coral larvae from the GBR to both high latitude sites is rare, and low genetic diversity at LHI indicates that most populations are self-seeding, making them vulnerable to extinction (Ayre and Hughes 2004). In a study of *Symbiodinium* in a range of corals in the isolated Hawaiian Islands, high levels of endemicity were also observed (e.g., C75 hosted by *Porites* sp., LaJeunesse et al. 2004a). These findings are consistent with occurrence of biogeographical provinces across the Pacific (Udvardy 1975; Gaines and Lubchenco 1982), that have also been identified for *Symbiodinium* within the GBR (LaJeunesse et al. 2004a). Symbiont community composition differs between the northern, central and southern GBR, and based on our findings, the higher latitude reefs of LHI and KI would also represent a separate biogeographic province.

3.4.3 Ecological implications

The ecological patterns in symbiont distribution observed highlight the importance of sub-cladal identification. Despite the low numbers of coral colonies sampled in the present study high symbiont diversity was uncovered and increased sampling may be beneficial to uncover distinct patterns especially for corals hosting specialised symbionts (LaJeunesse et al. 2003, Sampayo et al. 2007).

The evolution of non-host specific partnerships between host and symbiont at high latitudes is no doubt linked to locally challenging environmental conditions. Whilst host specificity has previously been reported to be greatest in unstable and isolated environments (LaJeunesse et al. 2004a), the relaxation of host specificity at the high latitude isolated sites in the present study suggests the opposite. The presence of locally adapted symbionts likely indicates that, due to isolation and the hugely different

environment, symbionts have evolved that are more suitable to these locations. The flexibility of host-symbiont relationships is of great importance in light of evidence of stress-tolerant symbionts, and their role in increasing thermal thresholds of their hosts (Buddemeier and Fautin 1993; Rowan et al. 1997; Glynn et al. 2001; Berkelmans and Van Oppen 2006). Further, this study demonstrates the importance of sub-cladal designation, with patterns in host and environment specificity identified which would not have been revealed using cladal designation techniques. Questions ensue as to how these coral-symbiont partnerships will fare with warming oceans, how these novel symbionts vary in the physiological functionality, and whether certain host-symbiont partnerships will become prevalent in a future of climate change.

Chapter 4

Effect of sedimentation on the community structure and resilience of corals and their symbionts

ABSTRACT

Susceptibility to environmental stress varies between both coral species and their symbionts of the physiologically diverse genus *Symbiodinium,* and thus influences the distribution of the holobiont (host and symbiont). This study aimed to assess the influence of the stressor, sedimentation, on symbiont distribution, and its consequences for coral distribution and diversity across the reef system of Palmyra Atoll, USA. A low diversity of symbiont types was discovered at the sites, with only ten *Symbiodinium* types identified in 34 coral species from 9 genera: 8 clade C types and 2 clade D types. Multivariate analysis revealed that field-based estimates of sedimentation explained 79% of the spatial variation of coral species among sites, however this environmental variation has not affected the holobiont distributions. The low symbiont diversity and lack of correlation between potential environmental stressors suggests that the symbionts are robust enough to cope with the environmental regime. Further, the similarity in coral-*Symbiodinium* to other locations and the dominance of generalist symbiont types suggest a high degree of connectivity may exist with other reef systems in the central Pacific. The data highlight (1) the importance of host tolerance to environmental stressors, which should be considered simultaneously with symbiont sensitivity when considering the impact of environmental degradation on coral

communities, and (2) the low diversity of potentially stress-tolerant symbionts which may, however, be vulnerable to further environmental change.

4.1 INTRODUCTION

Coral reefs owe much of their success to the mutualistic endosymbiosis between corals and dinoflagellates of the genus *Symbiodinium*. The host relies on the dinoflagellates for survival, with evidence that the latter release substantial amounts of photosynthate to the coral, and aid the recycling and conservation of essential nutrients (Muscatine 1990, Wang & Douglas 1998). Within the genus *Symbiodinium*, high genetic and physiological diversity has been uncovered, with eight clades (A-H) and numerous sub-clades identified (Rowan & Powers 1991, Baker 2003, Coffroth & Santos 2005). The physiological diversity among symbiont types has ecological ramifications because the ability of corals to survive in a variety of habitats and environmental conditions has been attributed to their relationship with different *Symbiodinium* types (Iglesias-Prieto & Trench 1994, 1997a, Robison & Warner 2006, McCabe-Reynolds et al. 2008).

Few coral species can associate with a range of *Symbiodinium* types, specifically adapted to the local environment (Rowan & Knowlton 1995, Rowan et al. 1997, Ulstrup & van Oppen 2003, Mieog et al. 2007), with the host-symbiont combination driven by the specificity of a host for a specific symbiont type, or *vice versa* (Rowan et al. 1997, Baker 2003). Indeed, the physiological distinctions among symbiont types has wide-ranging significance, because they delineate host tolerance ranges and so influence host distribution on regional as well as wide latitudinal scales (Rowan & Knowlton 1995, Loh et al. 2001, Iglesias-Prieto et al. 2004, Rodriguez-Lanetty et al. 2004, Rowan 2004, Berkelmans & van

Oppen 2006). For example, irradiance-specific symbionts have been shown to explain the distinctive vertical distribution patterns of two dominant coral species using genetic and physiological analyses (Iglesias-Prieto et al. 2004).

Although some corals have the ability to harbour multiple symbiont types, many corals only host a single symbiont type (Baker 2003, Goulet 2006, 2007). Occasionally, mixed populations of symbionts occur not only within a single species, but also within a single colony, their distribution often correlating with irradiance levels (Rowan et al. 1997, Frade et al. 2008b). This simultaneous harbouring of different symbiont types, presumably with differing physiologies, has led to the suggestion that some coral species may adapt to a particular environment by 'shuffling' their symbionts; that is, shifting the dominant *Symbiodinium* type towards one that is well suited to the abiotic regime (Buddemeier & Fautin 1993, Fautin & Buddemeier 2004, Berkelmans & van Oppen 2006, Jones et al. 2008). Thus, the functional diversity of *Symbiodinium* types has implications for the host's ability to adapt/acclimate to environmental change, particularly if the host can shuffle its symbiont types, with the degree of host-symbiont specificity and flexibility also playing an important role.

On local and geographic scales, the structure and development of coral reefs is linked to a long list of environmental variables (Brown 1997ba), with those reefs occurring close to the thresholds for survival or in areas characterised by sub-optimal or widely fluctuating environmental conditions being termed as marginal (Kleypas et al. 1999). These variables include light, temperature, salinity, hydrodynamic factors and sedimentation (Achituv & Dubinsky 1990, Kleypas et al. 1999). Species-specific responses to temperature (Cook et al. 1990, Coles & Fadlallah 1991, reviewed in Baker et al. 2008), light (Done 1983, Cornell & Karlson 2000), salinity (Coles & Jokiel 1978) and turbidity (Rogers 1990,

McClanahan & Obura 1997, Fabricius 2005) have been well documented, and are reflected in the coral community established within an environment. For example, bleaching susceptibility varies among coral taxa (Hoegh-Guldberg & Salvat 1995, Marshall & Baird 2000), and thus following a bleaching event which results in selective mortality, communities will become dominated by 'hardy' coral species.

Sedimentation affects the distribution of coral species (Rogers 1990, Fabricius 2005), but little is known about how sediment regime affects the distribution of different *Symbiodinium* types specifically. Coral species exhibit differential responses to shading, abrasion and smothering which constitute the main mechanisms of sediment impact (Brown 1997ba). While some corals obtain nutritional benefits from ingestion of sediment particles (Anthony 1999) and have adapted to high sediment load habitats (Abdel-Salam et al. 1988, Stafford-Smith 1993, Riegl & Branch 1995), for most corals the direct impact of sedimentation on the host is clear; increased sedimentation leads to reduced irradiance, reduced species richness, and reduced coral cover, size and recruitment (Brown & Howard 1985, Rogers 1990, Brown 1997b, Fabricius 2005). With increasing sedimentation, coral growth rates decline, the corals lose their symbiotic dinoflagellates, and the coral tissue dies (Bak 1978, Rogers 1983, Peters & Pilson 1985). In addition to the role that sediment regimes play in the distribution and survival of corals, these species are only able to survive in the stressful environment if they host a dinoflagellate symbiont that is adapted to the conditions (Iglesias-Prieto et al. 2004). However, what little is known about this subject is limited to two studies. Toller et al. (2001) and Garren et al. (2006) identified cladal-level symbiont distribution patterns in response to sedimentation, but made no mention of host distributions. Thus, the aim of the current study was to assess the influence of sedimentation on symbiont distribution and its consequences for coral distribution and

diversity across a reef system. It was hypothesised that coral cover, coral diversity and symbiont diversity would all be lower in areas of high sedimentation than in areas of low sedimentation.

4.2 METHOD

4.2.1 Study site

The study was conducted between June and July 2007 at Palmyra Atoll (05°52'N, 162°06'W), an uninhabited atoll situated in the remote Central Pacific (Fig. 4.1), at the northwest end of the northern Line Islands. Palmyra has lacked an indigenous human population, but was extensively modified during WWII and abandoned shortly thereafter, with sparse human influence since (Collen et al. 2009). WWII construction disrupted the atoll's water-flow regime, leading to chronic sedimentation in some areas of the atoll, and catastrophic death of corals on the western side (Maragos et al. 2008a). In 2001, Palmyra became a National Wildlife Refuge, jointly managed by US Fish and Wildlife Service and The Nature Conservancy. The atoll is also part of the Pacific Remote Islands National Monument, established in 2009.

The atoll is 20 km long, made up of 50 islets and situated approximately 1,930 km south of the main Hawaiian Islands. Five sites were chosen from the backreef of the atoll, covering a range of sediment regimes (high, mid and low) at comparable depths (Fig. 4.1). The 6500 ha of shallow and deep reefs at Palmyra, and the marked spatial variability in sediment load, provide an ideal location for assessing the influence of sedimentation on coral and *Symbiodinium* distributions, and for examining the inter-relationship between these distributions.

4.2.2 Coral sampling and community surveys

At each study site, quadrats (1 m^2) were placed every 2 m along five 30-m transects at a constant depth of 2 - 3 m at high tide (n = 75 per site). Percentage cover of each coral species and other benthic categories (dead coral/rubble, sand, macroalgae, sponges) was estimated by eye *in situ*. In the case of *Montipora* spp., where species could not be accurately identified, corals were placed into 12 morphological groups (Appendix A4.1) as defined by Veron (2000). Coral species identifications were verified by a third party. Once the dominant and rare coral species at each site were ascertained, samples were taken for dinoflagellate symbiont identification. Samples (n = 3 per species) were taken of five coral species that were present at every site surveyed. Additionally, coral species found at only one or two sites were sampled. To avoid sampling the same clone, colonies more than 5 m apart were selected at each site. Fragments of 1 - 2 cm^3 were taken from the top of colonies using a hammer and chisel, fixed in absolute ethanol and stored at -20°C. Samples were transported in ethanol from Palmyra to New Zealand (NZ) for analysis.

4.2.2 Symbiont identification

At Victoria University of Wellington (NZ) *Symbiodinium* cells were isolated by removing the coral tissue from the skeleton. The tissue was rinsed, homogenised using a tissue grinder in extraction buffer (1.2 M filtered seawater and 5 mM EDTA), and the *Symbiodinium* DNA extracted using the DNeasy Plant Mini Kit following manufacturer's instructions (Qiagen). Briefly, the internal transcribed spacer 2 region (ITS 2) of nuclear ribosomal DNA was used to identify distinct *Symbiodinium* types (cf. LaJeunesse 2001, 2002). The ITS 2 region was PCR amplified using primers "ITS 2 clamp'' and ''ITSintfor

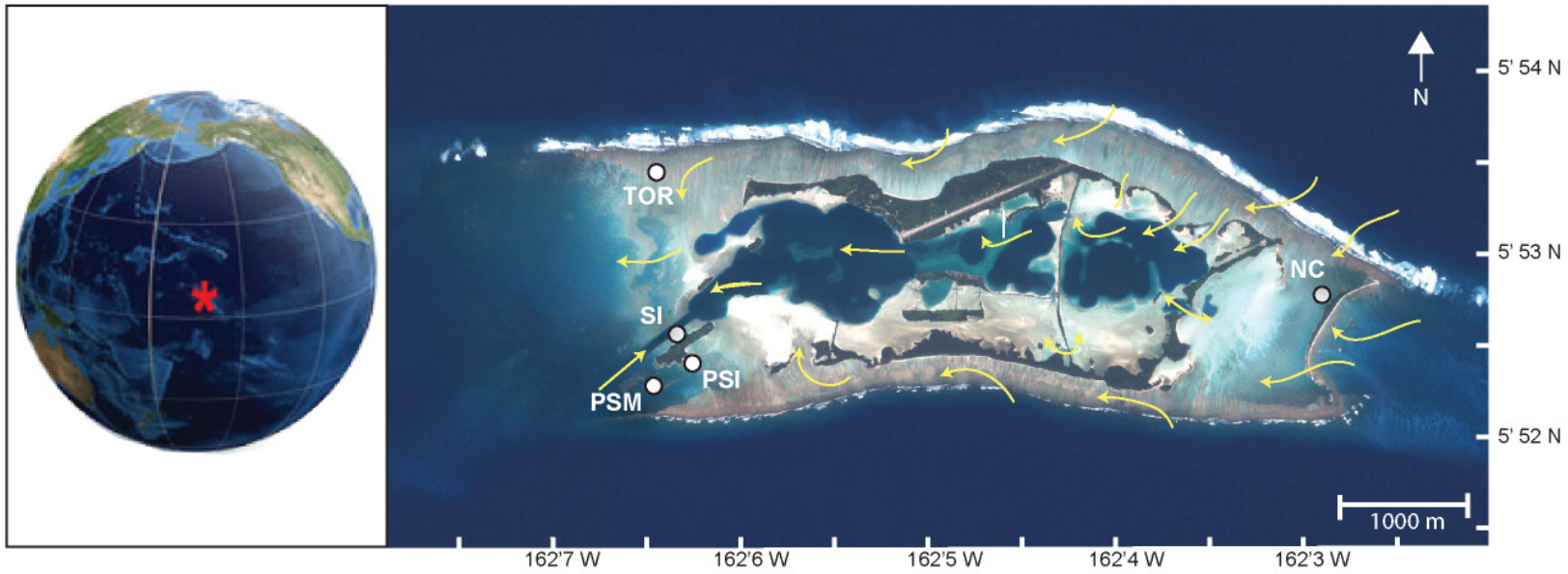


Figure 4.1 Location of Palmyra Atoll, USA and sampling sites. Arrows indicate current flow, sourced from Collen et al. (2009). TOR: Tortugonas, SI: Sand Island, PSI: Penguin Spit Inner, PSM: Penguin Spit Mid, NCG: North Coral Gardens

2'' (LaJeunesse & Trench 2000) under ''touchdown'' thermal cycle conditions specified in LaJeunesse (2002). ITS 2 amplified PCR products were subsequently run on a Biorad DCode Denaturing Gradient Gel Electrophoresis (DGGE) system, using a 30% to 65% gel during a 100V run for 14 h. with dominant (brightest) bands excised, amplified and sequenced. All DGGE gels were stained using 1X SYBR Green for 30 min and visualized on a UV transilluminator.

To identify distinct *Symbiodinium* type, prominent bands were excised from up to three representative samples of each characteristic profile. Excised bands from the ITS-DGGE fingerprint were eluted in 500 µl ddH20, kept at 4°C for 24 h and reamplified using GC-clamped primers (under conditions as above). Amplicons were re-run on the DGGE system to check band position and potential contamination with other bands during excision prior to sequencing. Clean bands running at the expected position in relation to the original sample were re-excised, eluted and reamplified with the non-GC clamped primer set (cf. LaJeunesse 2002; LaJeunesse et al. 2008). Amplifications were purified by ethanol precipitation and sequenced in both directions at the Australian Genome Research Facility (ABI BigDye Terminator v3.1; AB3730x1 sequencer). Chromatograms were checked using Seqman software (Lasergene) and aligned using Clustal-X (Thompson et al. 1997). Identical DNA sequences recovered from each dominant or co-dominant band were identified by a BLAST search on GenBank (http://www.ncbi.nih.gov). Phylogenetic analyses of dominant rDNA ITS1 and ITS2 repeat sequences were performed in PAUP (version 4.0b10, Swofford 2000) under the maximum parsimony criterion treating gaps as a fifth character state and indels as a single evolutionary event with bootstrap values calculated based on 1,000 replicates.

4.2.3 Environmental variables

At Palmyra Atoll, a range of environmental variables was recorded over a six-week period at the 5 sites to assess their influence on coral and symbiont distributions. Seawater temperature, fluorescence (as a proxy for chl-*a* concentration) and salinity were recorded once every minute for 7-day periods at 1-2 m depth at each of the sites using a CTD unit (Richard Brancker Research Ltd XR 420). Temperature and light loggers (HOBO, Onset Ltd) were deployed for a five-week period at three of the sites (Penguin Spit Middle, Tortugonas and North Coral Gardens), recording once every 30 min. Temperature measures from the CTD and HOBOs were combined for sites where both recordings were taken.

Sedimentation rates at 2 - 4 m depth at each of the sites were measured using sediment traps connected vertically to the seabed. Traps were 5.1 cm internal diameter PVC cylinders with a height of 100 cm. The mouth of each trap was located ~1.1 m above the seabed. Traps were deployed at least twice at each of the sites during the study period for varying periods of time (min 5 days; max 72 days). The trap contents were filtered onto 0.45-µm Whatman G/FC filter papers and dried at 60°C for 24 h to constant weight to determine total particulate matter (TPM – mg m^{-2} d^{-1}). To determine particulate organic matter (POM - mg m^{-2} d^{-1}) content the dry filter plus sediment was combusted at 500ºC for 24 h in a furnace to determine its ash-free dry weight (the weight loss on ignition equals the organic matter content). Percent organic matter content (PCOM) was determined as POM / TPM * 100. To extend the precision of the analyses, sedimentation data collected over a 3-year period (not just from the deployments in June and July 2007) were used in the analysis because preliminary testing revealed that no significant effect of time (date of deployment) was observed (ANOVA, $F = 2.34$, $p = 0.111$) on sedimentation rates (TPM, POM and

PCOM; ANOVA conducted on SPSS v16).

4.2.4 Statistical analyses

To determine the role that environmental variables play in controlling symbiont distribution, coral species distribution and their inter-correlation at Palmyra Atoll, a number of univariate and multivariate analyses were conducted. Indices used to assess diversity of corals per site were total number of species (S), Hill numbers N1 and N2 (to assess the influence of rare and dominant species on community diversity, respectively), and the modified Hill's ratio (N21′) (see Clarke & Gorley 2006 for a summary of each index). The modified Hill's ratio was used as a measure of equitability (spread among taxa) because it has an advantage over traditional measures of evenness by its non-dependency on the number of species (Rogers et al. 1999). All diversity indices were calculated using Primer v6.1.11 (Plymouth Marine Labs.) Percentage coral cover and environmental variables (temperature, salinity, chl-*a* and the 3 sediment measures of TPM, POM and PCOM) were compared among sites using one-way analysis of variance (ANOVA, with Tukeys posthoc test), testing the null hypothesis that there was no significant difference in aforementioned variables among the five sites. Where assumptions of normality and/or homogeneity of variance were not met, data were transformed using the square root transformation, or non-parametric Kruskal-Wallis and Dunns post-hoc tests used. Quadrats were used as the replicates for each site in the biotic analysis.

Multivariate analyses were conducted to examine differences in coral and holobiont community structure at each site, and the relationship of community structure with environmental variables, using Primer v6 with PERMANOVA v1.0.1 add-on. Analysis was conducted on the percent cover of all benthic groups (including all coral species), coral

species presence/absence and holobiont presence/absence. Presence of each holobiont type was restricted in all analyses to those present in five key coral genera: *Acropora*, *Astreopora, Favia, Montipora* and *Pocillopora,* with host colonies used as replicates, and analysis also conducted separately for each host species. Prior to the multivariate analysis, benthic percent cover data was subjected to a dispersion-weighted pre-treatment in which the abundances of the different species or groups were weighted differentially on the basis of their observed variability among replicate samples (1000 replications, Clarke et al. 2006a). This method compensates for the spatial clustering common to coral community studies (Clarke et al. 2006a). Similarities were calculated using the zero-adjusted Bray-Curtis similarity measure (Bray & Curtis 1957) to account for samples with denuded assemblages (see Clarke et al. 2006b). Non-adjusted Bray Curtis similarity was used for coral species and symbiont type presence/absence. Environmental variables were (log+1) transformed and normalised, with similarities then calculated using Euclidean distance.

Differences in benthic cover and coral species presence/absence among sites were assessed using the PERMANOVA routine with 999 permutations. PERMANOVA is a multi-factorial analysis of variance, unconstrained by the assumptions of normality and homogeneity of variance that limit the use of traditional MANOVA. It relies on comparing the observed value of a test statistic (pseudo *F*-ratio) against a recalculated test statistic generated from random reordering (permutation) of the data (Anderson 2001). Unconstrained multidimensional scaling (MDS) and constrained canonical analysis of principal components (CAP) were used to visualise the differences in benthic cover, coral species presence/absence, environmental parameters and holobiont presence/absence.

To assess the influence of environmental variables on distribution patterns, BIOENV was used as an exploratory tool to estimate correlations between the similarity

matrix derived for the benthic cover, coral species presence, and holobiont presence and the matrix of environmental measurements taken at each site. The null hypothesis tested was that there was no correlation between environmental variables and coral/holobiont distribution patterns. Where possible, mean, maximum and minimum values of each environmental measure were used in the analysis. Results are expressed as a Spearman's correlation coefficient (r), ranked in the order of which a single variable or combination of variables best explains the observed community patterns (Clarke & Ainsworth 1993). The results (correlation coefficient range of -1 to 1) indicate the proportion of variance in the community data explained by these environmental variables (see Clarke & Ainsworth 1993 for full details). The environmental variables were screened for multi-collinearity prior to running the BIOENV routine and only a representative of any highly correlated water quality variables (i.e., those with Spearman's ≥,0.8) was used. In a second step, only the environmental variables selected by BIOENV were included as explicative variables in the LINKTREE procedure (a non-parametric multivariate form of classification and regression trees; De'ath 2002). This non-parametric multivariate discrimination technique constructs a hierarchical tree through successive dichotomies of the set of observations. Each division is characterised by one or more variables, and ranges of their values, that appear to be responsible for discriminating each different assemblage grouping. LINKTREE is capable of demonstrating that an environmental variable is important for the internal assemblage structuring of one group of samples but not for another group (with similar value ranges).

4.3 RESULTS

4.3.1 Coral community structure

Sixty-seven coral species from 10 genera were identified from the 5 sites around Palmyra Atoll (Appendix A4.1 lists all coral species identified at Palmyra Atoll). Species diversity was greatest at Penguin Spit Middle and Tortugonas (both 41 spp.) and least at Sand Island (only 8 spp., Table 4.1). Sand Island was conspicuous in terms of its low density of coral species (2.64 ± 0.1 species per m^2, mean ± SE). N1, an index of the number of rare species at a site, was greatest at Penguin Spit sites (10.1 and 11.3), and lowest at Sand Island and Tortugonas (4.5 and 4.8 respectively). N2, an index of dominant species, was also greatest at Penguin Spit sites (6.6 and 6.8). Evenness (N21') of rare and dominant species was lowest at Tortugonas at 0.4 compared to the maximum of 0.7 at North Coral Gardens.

Mean coral cover (± SE) varied significantly among 3 of the 5 sites, from a minimum of 3.61 ± 0.33% at Sand Island, to a maximum of 66.73 ± 2.2% at North Coral Garden ($p<0.001$, NCG>PSM, PSI, TO> SI, Figure 4.2). The most abundant genus at the depauperate site of Sand Island was *Pocillopora*, with 5 species recorded (*P. eydouxi, P. damicornis, P. verrucosa, P. meandrina, P. brevicornis*). *Montipora* spp. dominated at North Coral Gardens, accounting for a mean (± SE) of 57 ± 2.5% of the benthic cover. *Montipora* spp. were also the most abundant corals in terms of percent cover at Penguin Spit middle (10.2 ± 1.2%). *Acropora* spp. had the greatest abundance of all coral genera at both Tortugonas and Penguin Spit Inner, accounting for 25 ± 3.4% and 20 ± 2.6% of the benthic cover, respectively. *Pocillopora* spp., *Montipora* spp. and Faviid spp. were the only genera present at all 5 sites surveyed. Species of the family Siderasteridae and soft corals were notably absent at North Coral Gardens, but present at the western sites (excluding

Sand Island, Table 4.1). No endemic coral species were identified.

4.3.2 Symbiont identification

Ten *Symbiodinium* types were identified in 34 coral species (172 samples) from 9 genera: 8 clade C types and 2 clade D types (Table 4.2, Figure 4.3). All *Acropora* spp. hosted type C3, as did both *Favia* spp. *Astreopora gracilis* hosted C1 solely, with C1 also present in *Pavona* spp., *Gardinoseris planulata*, *Leptastrea purpurea, Psammacora nierstrazi* and *Fungia paumotensis*. Two novel *Symbiodinium* clade C types were identified (yet to be named), $C1_{xx}$ in *F. paumotensis* (present with C1) and $C3_{xx}$ in *Merulina ampliata*; $C1_{xx}$ was 2 basepairs divergent from ancestral C1, with $C3_{xx}$ 2 basepairs divergent from ancestral C1 (Fig. 4.3). ITS-DGGE fingerprints and sequences of novel types, yet to be named, were consistent and reproducible.

Porites lobata hosted *Symbiodinium* C15 and C15b, and C15 was also present in a range of *Montipor*a spp. C1c and C42a were hosted by *Pocillopora* spp., with C1c also harboured by *Pavona duerdeni.* Finally, C1b was present in *L. purpurea* with C1. Of the D types identified, D1 and D1a were found in *Montipora* spp. and *Echinophyllia* sp., while D1a alone was present in *Turbinaria reniformis.*

There was no pattern of symbiont diversity among sites. The key genera/families identified (*Acropora, Astreopora, Favia, Montipora, Pavona* and *Pocillopora*) all associated with similar symbionts across the atoll (Table 4.2). *Acropora, Astreopora* and *Favia* consistently hosted one type across all sites samples at the atoll. Similarly, *Montipora* hosted C15 and D1a at all sites. Minor variation in symbiont distributions were seen in *Pavona* and *Pocillopora* spp. *Pavona* hosted C1 and C3 at all sites sampled (3

Table 4.1 Coral community measures and environmental parameters recorded at 5 backreef sites around Palmyra Atoll, USA. Benthic categories and coral genera shown as percent cover ± SE. Mean ± SE of environmental variables shown. Temperatures recorded by HOBO over a 5-week period or CTD over a 7-day period in June/July 2007. CTD also recorded chlorophyll fluorescence (µg l^{-1}) and salinity. Sedimentation, measured using sediment traps, given as total particulate matter (TPM), particulate organic matter (POM) and percent organic matter (PCOM).

	Sand Island	Penguin Spit Inner	Penguin Spit Mid	Tortugonas	North Coral Gardens
Dead/Rubble	76.4 ± 1.6	62.2 ± 3.1	80.7 ± 1.7	69.1 ± 3.3	33.3 ± 2.2
Sand	19.9 ± 1.7	13.8 ± 2.7	1.04 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
Macroalgae	< 1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Sponge	< 1 ± 0.1	< 1± 0.1	< 1± 0.1	< 1± 0.1	< 1± 0.1
Total coral cover	3.6 ± 0.3	23.9 ± 2.4	18.5 ± 1.5	30.9 ± 3.3	66.7 ± 2.2
Fungiidae	0.0 ± 0.0	0.2 ± 0.1	0.8 ± 0.1	0.2 ± 0.1	< 0.1 ± 0.0
Pocilloporidae	3.4 ± 0.3	1.4 ± 0.3	1.1 ± 0.2	0.7 ± 0.2	1.2 ± 0.3
Poritidae	0.1 ± 0.1	0.0 ± 0.0	< 0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.2
Siderasteridae	0.0 ± 0.0	< 0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
Acropora spp.	0.0 ± 0.0	9.9 ± 2.6	2.8 ± 1.1	25.1 ± 3.4	0.6 ± 0.4
Astreopora sp.	0.0 ± 0.0	3.7 ± 0.6	0.3 ± 0.2	0.1 ± 0.1	3.1 ± 0.7
Montipora spp.	0.3 ± 0.1	3.3 ± 0.6	10.2 ± 1.2	3.0 ± 0.6	57.4 ± 2.5
Dendrophyllidae	0.0 ± 0.0	< 0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Faviidae	0.1 ± 0.1	2.4 ± 0.6	1.0 ± 0.4	0.6 ± 0.2	1.1 ± 0.4
Merulinidae	0.0 ± 0.0	0.4 ± 0.3	0.1 ± 0.1	0.1 ± 0.0	2.4 ± 1.1
Agariciidae	0.0 ± 0.0	1.0 ± 0.3	0.7 ± 0.2	1.1 ± 0.3	0.7 ± 0.4
Mussidae	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	< 0.1 ± 0.0
Soft corals	0.0 ± 0.0	1.4 ± 0.4	1.5 ± 0.4	0.1 ± 0.0	0.0 ± 0.0
Diversity Indices					
Coral					
Total no. species	8	39	41	41	34
N1	4.5	10.1	11.3	4.8	6.7
N2	2.9	6.6	6.8	2.3	5.0
N21’	0.5	0.6	0.6	0.4	0.7
***Symbiodinium* (in key coral species)**					
Total no. types	3	6	5	6	6
Environmental					
Mean temperature (°C)	28.7 ± 0.8	28.3 ± 0.3	28.2 ± 0.3	28.2 ± 0.3	28.4 ± 0.5
Max. temperature (°C)	31.1	29.5	31.3	31.1	34.8
Mean Chlorophyll (µg/l)	3.4 ± 3	0.71 ± 0.5	0.4 ± 0.2	0.29 ± 0.5	0.86 ± 2.1
Mean TPM (g m^{-2} d^{-1})	52.5 ± 55	9.3 ± 12.3	11.5 ± 21.6	7.9 ± 20	1.4 ± 1.3
Max. TPM (g m^{-2} d^{-1})	129.8	43.8	75	74.4	3.81
Mean POM (mg m^{-2} d^{-1})	1670 ± 2077	78 ± 46	64 ± 46	156 ± 433	23 ± 18
Max. POM (mg m^{-2} d^{-1})	4730	201	131	1596	53
Mean PCOM (%)	37.3 ± 34	10.9 ± 6	13.8 ± 0.6	12.6 ± 6	30.1 ± 11
Mean Salinity (PSU)	34.1 ± 0.3	34.7 ± 0.1	34.6 ± 0.1	34.7 ± 0.1	34.5 ± 0.3

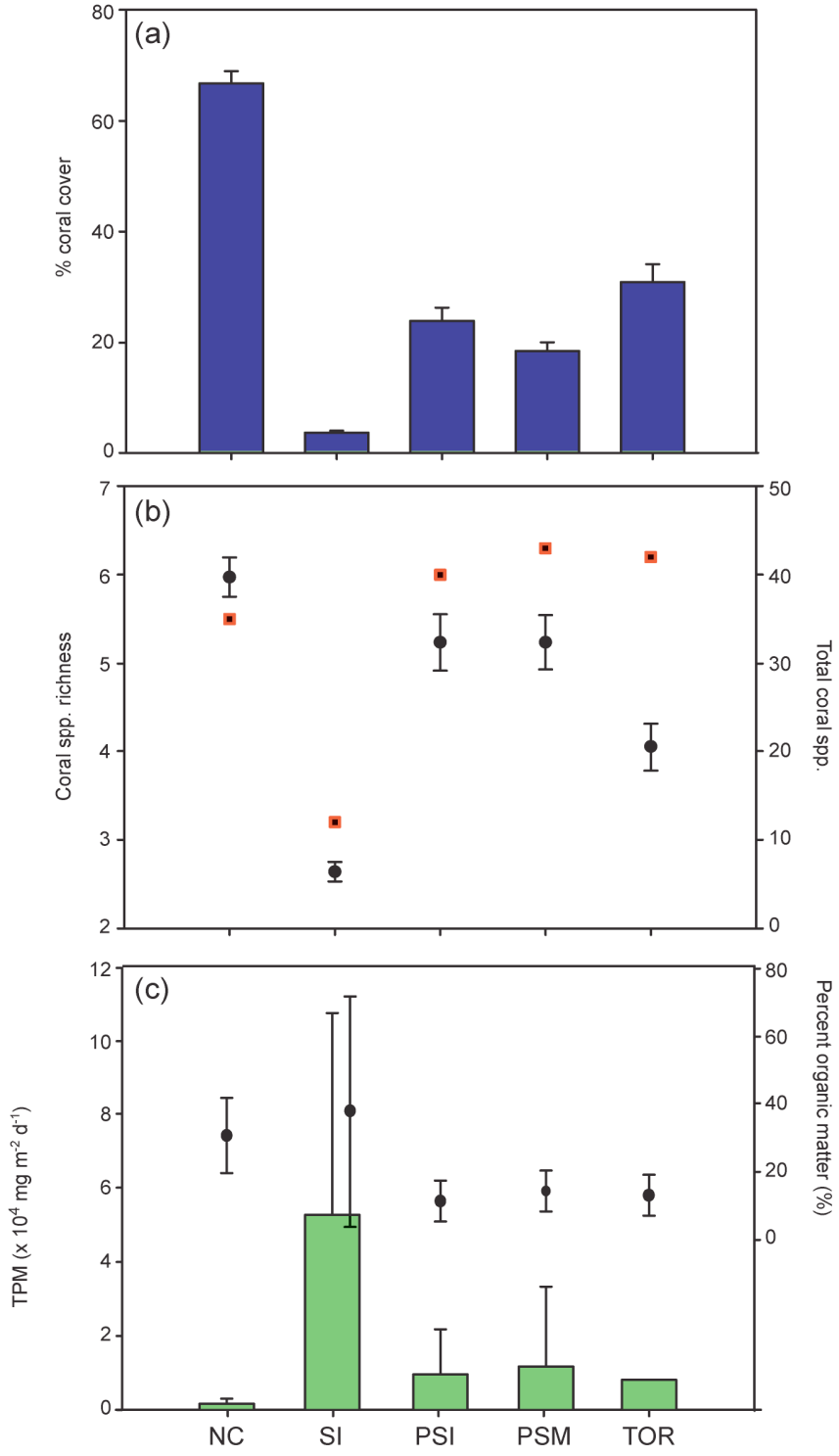


Figure 4.2 Variation in (a) coral % cover (mean ± SE) (b) no. of coral spp. per m^2 (mean ± SE , ●, left axis) and per site (right axis ■), and (c) sedimentation measures (mean ± SD) of total particulate matter (■, left axis) and percent organic matter (● right axis, both mg m^{-2} d^{-1}) between five sites at Palmyra Atoll, USA. NC: North Coral Gardens, SI: Sand Island, PSI: Penguin Spit Inner, PSM: Penguin Spit Mid, TOR: Tortugonas.

western sites) but C1c was also present at Penguin Spit Middle. Similarly, *Pocillopora* spp. associate with C1c and C42a at North Coral Gardens, Penguin Spit Inner and Tortugonas, but C1c alone is harboured by *Pocillopora* spp. at Penguin Spit Middle and Sand Island.

4.3.3 Environmental variables

Temperatures differed significantly among sites (Table 4.2, $p < 0.001$) however on an ecological scale the variability was low, with means ± SD ranging from 28.2 ± 0.03°C to 28.7 ± 0.8°C. The maximum temperature recorded over the 5-week period, 34.8°C, was at North Coral Gardens, with the minimum temperature of 25.4°C recorded at Penguin Spit Middle. Salinity (PSU) ranged from 34.1 ± 0.3 to 34.7 ± 0.1, and was significantly different among 4 of the 5 sites ($p<0.001$, Table 4.2). Chlorophyll-*a* concentration (measured as fluorescence: FIC), was significantly different among all sites, with the largest mean value (± SD) recorded at Sand Island (3.4 ± 3 μg l^{-1}). All other sites recorded a mean FIC less than 1 μg l^{-1}, with the minimum of 0.29 ± 0.5 μg l^{-1} measured at Tortugonas.

Levels of sedimentation were measured as total particulate matter (TPM), particulate organic matter (POM) and percent organic matter (PCOM) (Table 4.1, Fig. 4.2). TPM was significantly greater at Sand Island than North Coral Gardens ($p < 0.05$), but was not significantly different among any other site combinations. Sand Island exhibited the highest mean (± SD) TPM of 52.5 ± 55 g m^{-2} d^{-1}, with TPM at North Coral Gardens being the lowest at 1.4 ± 1.3 g m^{-2} d^{-1}. Mean TPM at the three other sites ranged from 7.9 – 11.5 g m^{-2} d^{-1}, although all measures were highly variable (Table 4.1). Mean POM (± SD) ranged from 23 ± 18 mg m^{-2} d^{-1} (North Coral Gardens) to 1670 ± 2077 mg m^{-2} d^{-1} (Sand Island), again a highly variable measure. Significant greater POM was recorded at Sand

Table 4.2 *Symbiodinium* types identified in coral species from 5 backreef sites at Palmyra Atoll, USA, using ITS2-DGGE. NC: North Coral Gardens, SI: Sand Island, PSI: Penguin Spit Inner, PSM: Penguin Spit Mid, TOR: Tortugonas.

Host species/genus	NC	PSI	PSM	SI	Tort
Acroporidae					
A. acuminata		C3			C3
A. cerealis		C3			
A. cytherea			C3		
A. danai		C3			
A. elseyi		C3			
A. formosa					C3
A. glauca			C3		
A. nasuta					C3
A. valida	C3				C3
A. verweyi					C3
Astreopora gracilis	C1	C1	C1		C1
Montipora Gp 2	D1/a				
Montipora Gp 3	C15, D1/a	D1/a	C15	C15, D1/a	
Montipora Gp 4	C15	D1/a	D1/a		D1/a
Montipora Gp 6	C15	C15	C15		
Montipora Gp 7	C15				
Montipora Gp 8	C15	C15	C15		C15
Pectiniidae					
Echinophyllia sp.			D1/a		
Faviidae					
Favia matthai		C3			
Favia stelligera	C3	C3	C3		C3
Leptastrea purpurea				C1/b	
Fungiidae					
Fungia paumotensis			C1 + $C1_{xx}$		
Agariciidae					
Gardinoseris planulata		C1			
Pavona duerdeni		C1 + C3	C1/c, C3		C1 + C3
Pavona varians		C1	C1		
Siderastreidae					
Psammacora nierstrazi			C1		
Merulinidae					
Merulina ampliata	$C3_{xx}$				
Pocilloporidae					
Pocillopora damicornis	C1c	C1c + C42a		C1c	
Pocillopora eydouxi	C1c + C42a				C1c
Pocillopora meandrina	C1c	C1c	C1c	C1c	
Pocillopora verrucosa		C1c			C1c + C42a
Poritidae					
Porites lobata	C15/b			C15/b	
Dendrophyllidae					
Turbinaria reniformis		D1a			

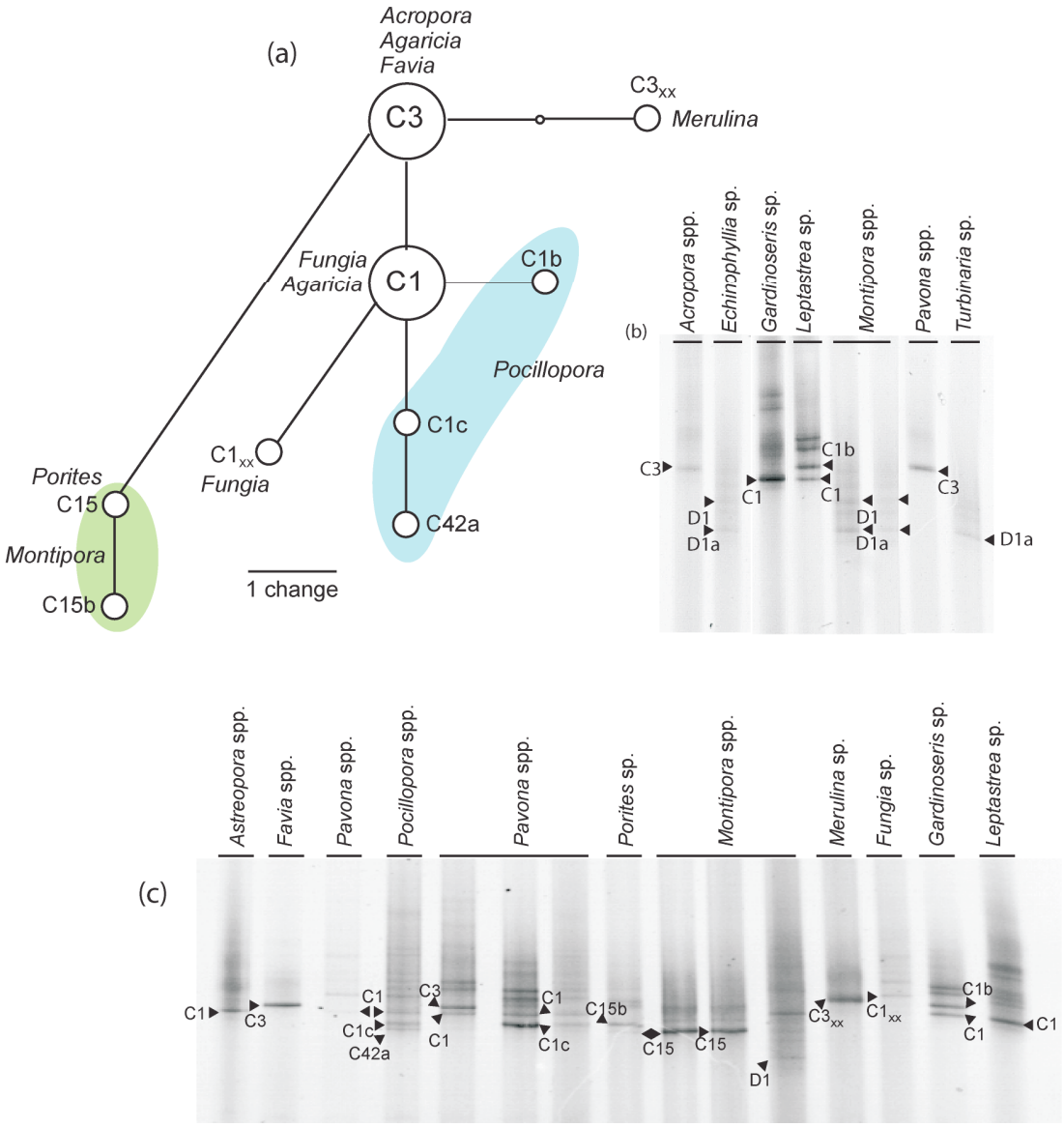


Figure 4.3. *Symbiodinium* types identified in corals at Palmyra Atoll, USA, based on ITS2 DGGE. (a) Unrooted phylogenetic sequence reconstruction showing the position of ITS2 types in relation to ancestral types C1 and C3, using maximum parsimony. Connections 90% connection support, and internodal branch lengths represent distance in terms of basepairs. Host genera from which symbionts identified noted. (b) and (c) ITS-DGGE gels showing symbiont profiles found in various host genera. Identified sequences are shown next to bands. $C3_{xx}$ and $C1_{xx}$ are novel types, yet to be named.

Table 4.3 Summary of ANOVA/Kruskal-Wallis test of differences in environmental variables between sites at Palmyra Atoll, USA. F tests statistic refers to ANOVA test, H refers to Kruskal-Wallis with Dunns post-hoc test.

Factor	DF	Test statistic	*p*	Sites
Temperature	4	F = 1364	<0.001	SI>NC>PSM, TOR>PSI
Salinity	4	F = 1350	<0.001	SI>NC>PSM, TOR>PSI
Chl-*a*	4	F = 4947	<0.001	SI>NC>PSI>PSM>TOR
TPM	4	H = 17.6	<0.001	SI>PSM>PSI, TOR>NC
POM	4	H = 13.7	<0.001	SI>TOR>PSI,PSM,NC
PCOM	4	H = 11.9	<0.05	NCG, SI>PSI
Coral cover	4	F = 117	<0.001	NCG>PSM, PSI, TO> SI

Island than at all other sites ($p < 0.05$), with a maximum value of 4.7 g m^{-2} d^{-1} recorded at the site. PCOM also varied significantly among sites, with significantly greater values recorded at North Coral Gardens and Sand Island, than Penguin Spit Inner ($p < 0.05$). Maximum PCOM recorded was at Sand Island (89%).

4.3.4 Multivariate analyses of corals, symbionts and environmental variables

Multivariate analysis revealed significant differences in coral species presence and benthic cover among all sites (PERMANOVA, F = 63.77, $p < 0.001$, and F = 42.81, $p < 0.001$, respectively), yet no difference in holobiont distribution among all sites was observed (PERMOANOVA, F = 31.23, $p = 0.456$). All sites were grouped separately in terms of their biotic community, with North Coral Gardens a clear outlier when assessing similarities in benthic cover among sites. Differences in coral species presence separated the sites of Sand Island and Tortugonas from the three other sites (Fig. 4.4). Both

constrained and unconstrained ordinations (CAP and MDS) revealed comparable patterns of similarities of benthic cover and coral species presence among sites (Appendix 4.2).

Significant correlations (Spearman's ≥ 0.08) were recorded among maximum and mean temperature, minimum TPM and POM, chl-*a* concentration and salinity, PCOM mean and maximum, mean and maximum TPM, and mean and maximum POM. These pairs of variables were treated as single variables in following analyses.

BIO-ENV analysis showed that the combination of environmental variables that best explained the spatial variation of coral species presence were the sedimentation measures of maximum and mean particulate organic matter (POM, $\rho^w = 0.814$). These variables explained 81% of the variation in coral species presence among sites ($R = 0.81$, $p < 0.01$). In terms of benthic cover the variables minimum total particulate matter (TPM) and maximum TPM explained 80% of the spatial variation ($R = 0.8$, $p < 0.01$). TPM minimum was the best single environmental predictor, explaining 79% of the variability in benthic cover. Other important parameters explaining the spatial variation in both benthic cover and coral species presence, when considered alone, are percent organic matter ($\rho = 0.19$), chl-*a* ($\rho = 0.27$) and salinity ($\rho = 0.19$), but the latter two showed significant correlations with each other. BIOENV analysis on symbiont distribution revealed a lack of correlation between the presence of symbionts and environmental variables ($\rho = 0.002$, $p = 0.85$).

LINKTREE analysis showed that when linking the coral species presence and optimum environmental variables together, all sites grouped separately, with sediment measures primarily responsible for discriminating between coral species assemblages (Fig. 4.5). The first split in the assemblage data is between Sand Island and Tortugonas, and the

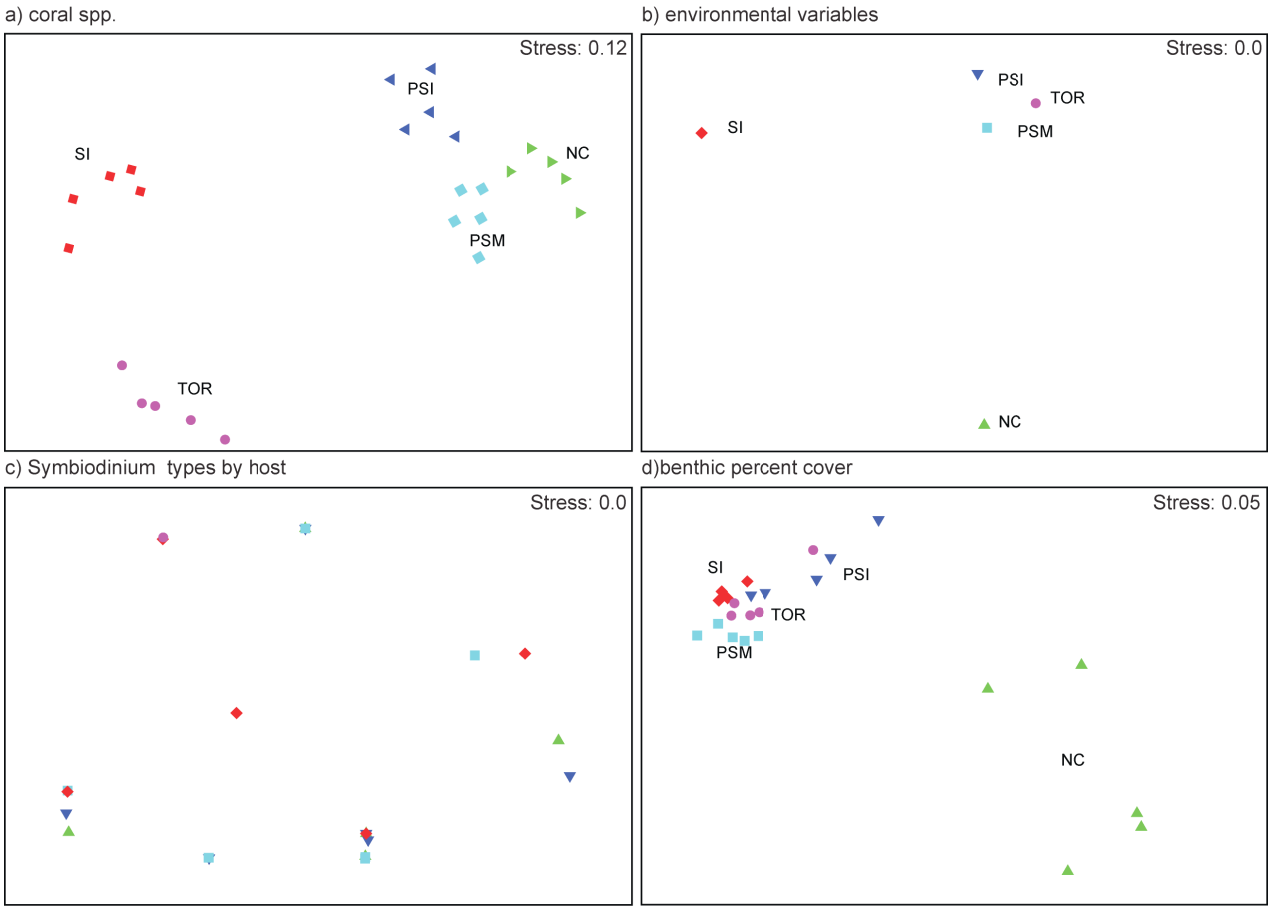


Figure 4.4 MDS plots showing dissimilarities in the (a) coral species presence, (b) environmental variables, (c) *Symbiodinium* types by host, and (d) benthic percent cover at five sites at Palmyra Atoll, USA. MDS similarities for (a), (c), and (d) based on Bray Curtis similarity of presence/absence data (a,c) and dispersion weighted data (d). Similarities for environmental data (b) based on Euclidean distance. Site codes shown near symbols for clarity where possible. NC: North Coral Gardens, PSI: Penguin Spit Inner, PSM: Penguin Spit Middle, SI: Sand Island, T: Tortugonas.

three other sites (ANOSIM R = 0.83, Linktree B % = 96%). The former two sites had the greatest mean and maximum POM (> 1596 mg m^{-2} d^{-1} and > 156 mg m^{-2} d^{-1} respectively). The differences in coral species assemblages between Tortugonas and Sand Island were correlated with higher maximum PCOM, and mean TPM/POM at Sand Island (27%, 7918 mg m^{-2} d^{-1} and 156 mg m^{-2} d^{-1} respectively). The sites of Penguin Spit Mid and North Coral Gardens were aggregated together. These two sites were separated from Penguin Spit Inner (ANOSIM R = 0.61, LINKTREE B % = 43) because the minimum TPM (2448 mg m^{-2} d^{-1}) and maximum POM (201 mg m^{-2} d^{-1}) were higher at the latter site. The final division between North Coral Gardens and Penguin Spit Mid was attributed to greater minimum POM (15.5 mg m^{-2} d^{-1}) and PCOM (3.4%), and lower max TPM (3815 mg m^{-2} d^{-1}) at the former (ANOSIM R = 0.88, LINKTREE B % = 32). Linktree analysis on benthic cover produced similar results to those described for coral species cover (see Appendix A4.2).

4.4 DISCUSSION

The variation in environmental parameters, particularly sedimentation, around Palmyra Atoll has led to diversification of coral communities; however this environmental variation has not affected the symbiont communities. *Symbiodinium* diversity was low, with many coral species harbouring generalist symbionts. The potential cause of this low diversity and the diversification of coral and symbiont distributions at Palmyra will be discussed.

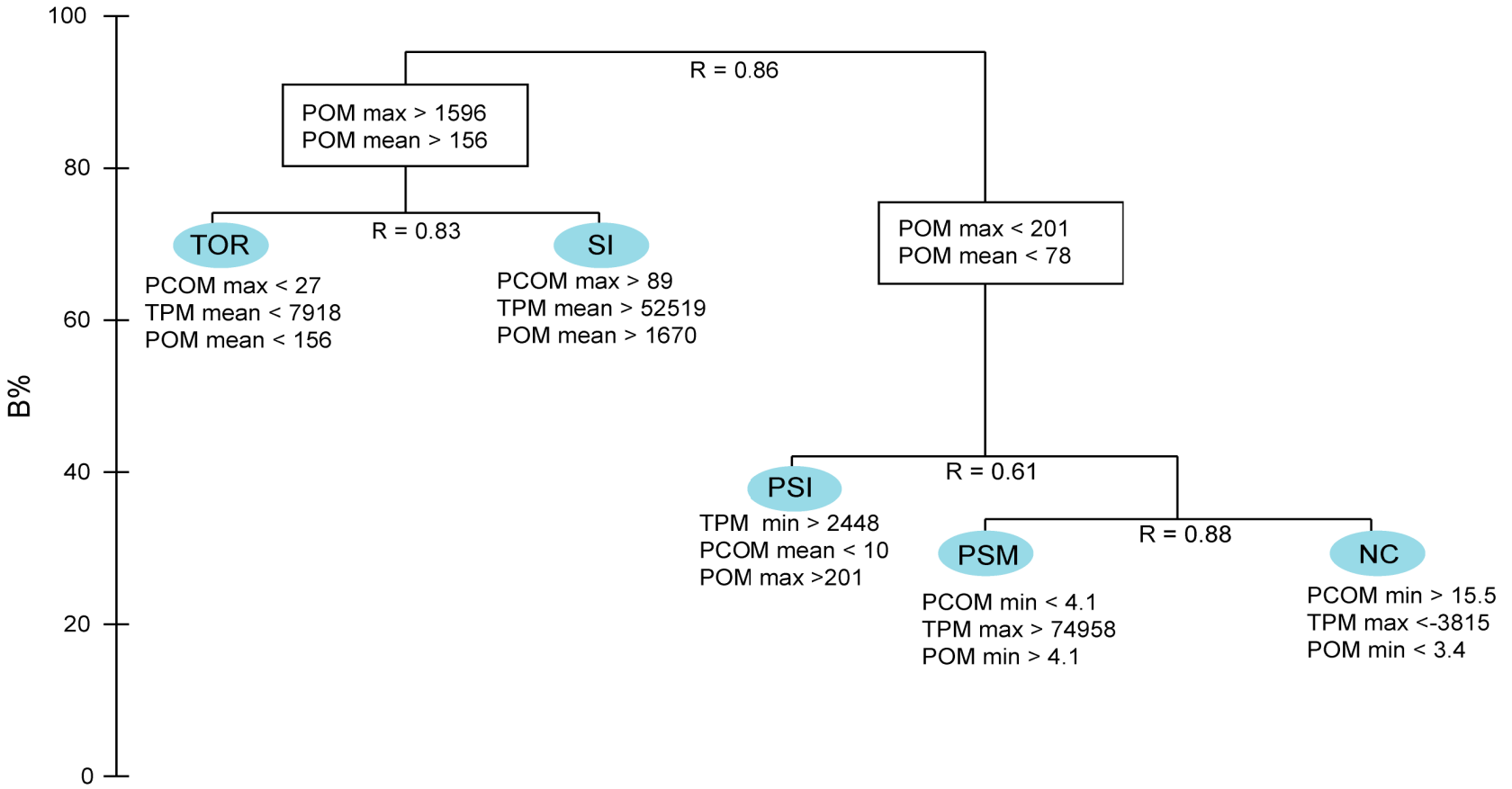


Figure 4.5. LINKTREE classification and regression tree analysis (with Simprof test of 1000 permutations) showing major splits in coral species presence between sites in link-tree dendrogram by most significant environmental variable(s). B% is the absolute measure of group differences. For each split the ANOSIM test statistic R is shown. NC: North Coral Gardens, PSI: Penguin Spit Inner, PSM: Penguin Spit Mid, SI: Sand Island, T: Tortugonas. POM IN %, TPM/POM in mg m^{-2} d^{-1}

4.4.1 *Symbiodinium* at Palmyra Atoll

Diversity of *Symbiodinium* types at Palmyra Atoll was low, with only 10 types identified in 34 coral species (from 9 genera), compared to 16 in 24 hosts (in 12 genera) in Hawaii (LaJeunesse et al. 2004b), and 20 types in 37 hosts (in 8 genera) in the Caribbean (LaJeunesse 2002) Of the 10 *Symbiodinium* types identified, only two were novel types ($C1_{xx}$ and $C3_{xx}$), with the generalist ancestral types C1 and C3 being dominant. Indeed, Clade C types overall were dominant at Palmyra, and represent the most frequent types found in scleractinian corals within the Pacific (Baker & Rowan 1997, LaJeunesse et al. 2004a). At a sub-cladal level, many of the host-symbiont specificities correlate with those observed in the Hawaiian Islands and the Great Barrier Reef (GBR, LaJeunesse et al. 2003, LaJeunesse et al. 2004a). For example, *Acropora* spp. at Palmyra only harbour type C3 *Symbiodinium*, as do *Acropora* spp. on the GBR (van Oppen et al. 2001, LaJeunesse et al. 2004a, although see Chapter 3). Both *Pocillopora* spp. and *Porites* sp. sampled at Palmyra host the same symbionts as their counterparts in Hawaii (C1c and C15, respectively; LaJeunesse et al. 2004b). At Palmyra, only 3 coral genera were seen to associate with more than one symbiont type: *Pavona, Pocillopora* and *Montipora*. The low diversity of *Symbiodinium* types found in scleractinian corals at Palmyra Atoll may reflect (1) the atoll's high degree of connectivity to other reef systems, (2) the lack of knowledge of symbionts in the Central Pacific, or (3) may be a result of previous environmental perturbation, leading to the dominance of a few stress-tolerant symbionts.

The similarity among the host-symbiont partnerships at Palmyra Atoll, the Hawaiian Islands and the GBR is surprising, given the location of the former in the Central Pacific. Situated 1930 km south of Hawaii, the high coral biodiversity (176 species) may be

due to the location of the atoll in the Inter Tropical Convergence Zone, with the eastward flowing North Pacific Equatorial Countercurrent and the Pacific Equatorial current (Hamann et al. 2004), bringing larvae from multiple directions (Maragos et al. 2008b). The nearest reef, Kingman Reef, is 76 km north of Palmyra and hosts 162 coral species. The proximity of Kingman Reef, the rest of the Line Islands, and the Phoenix Islands could enable them to act as stepping stones in the transfer of coral larvae, as has been seen within a 1700 km length of the GBR (Ayre & Hughes 2004). In addition to the high coral species diversity, the low diversity of symbiont types at Palmyra may be a factor of the high connectivity leading to a lack of co-evolution. Usually, geographic distinctness leads to genetic drift and the evolution of endemic/rare symbiont types, as observed at many remote locations (e.g. Gulf of California, LaJeunesse et al. 2008; Lord Howe Island, Chapter 3). Although some novel host-specific *Symbiodinium* types were identified ($C3_{xx}$ in *M. ampliata*, and $C1_{xx}$ in *F. paumotensis*), the abundance of generalist symbionts and of similar host-symbiont partnerships to Hawaii suggests that co-evolution is rare at Palmyra Atoll, perhaps due to the connectivity with other reef systems. *F. paumotensis* in Hawaii hosted C3h, whereas *M. ampliata* hosted C3 and C1, all types closely related to the novel ones identified at Palmyra.

The presence of the generalists C1 and C3, which have been found in a wide range of hosts and environments, and clade D types being harboured by *Echinophyllia* sp., *Montipora* spp. and *Turbinaria* sp., which all have the ability to associate with C types too (LaJeunesse 2005), suggests that they may have experienced stressful environmental conditions. Clade D *Symbiodinium* (particularly D1a) have been shown to be resistant to high sedimentation rates and elevated temperatures (Toller et al. 2001b), remaining in host tissue when other symbiont types are depleted (Baker 2001, Jones 2008). By hosting these

potentially stress-tolerant symbionts, the corals may have survived the stressful conditions during the modification of the atoll, however hosting stress-tolerant symbiont may be at a cost to the host, in terms of their photosynthetic efficiency and growth (Rowan 2004, Little et al. 2004). Further testing of physiological tolerances of the host-symbiont partnerships at the atoll is needed, to determine whether a recent bleaching event is causing the low symbiont diversity observed.

4.4.2 Environmental variability

The effect of environmental variables such as temperature, light, wave exposure, salinity and sedimentation on coral communities is well known (Wells 1957, Connell 1978, Johannes et al. 1983, Smith & Buddemeier 1992, Kleypas et al. 1999). Differences in coral community structure were best explained at Palmyra by the different sediment regimes around the atoll. Parameters such as total particulate matter showed a strong inverse relationship with coral cover, consistent with findings from other locations such as French Polynesia and Singapore (Adjeroud et al. 2000, Dikou & van Woesik 2006). In a study of which specific aspect of sedimentation most affects coral communities, Dikou and van Woesik (2006) found that reduced light availability has the strongest negative effect. Measures of light were not taken in the present study, however it was clear that light levels were markedly lower at Sand Island, particularly during periods of strong tidal activity (L. Wicks, pers. obs), and this correlates with the lowest coral species diversity and percentage cover at the site. The measured levels of sedimentation were within normal ranges of coral reefs, as defined by Rogers (1990), of 10,000 mg cm^{-2} day^{-1} or less, although Sand Island did experience some periods of greater sediment load. Heavy levels of sedimentation have been associated with depressed rates of photosynthesis and enhanced respiration and mucus

production in corals (Riegl & Branch 1995, Yentsch et al. 2002), in addition to a decline in growth rates, a loss of symbiotic algae and eventual tissue death (Bak 1978, Rogers 1983, Peters & Pilson 1985). Sedimentation also negatively affects rates of survival and settlement of coral larvae (Babcock & Davies 1991, Gilmour 1999), with low levels of coral cover, fewer coral species and lower general health of corals all associated with high sediment levels (Fabricius 2005).

The impact of sedimentation was further seen in the distribution of particular coral species, in addition to a decline in species diversity with increasing sediment load. *Porites* spp. are considered poor sediment rejectors (Stafford-Smith & Ormond 1992): at Palmyra this is reflected in their highest abundance being at North Coral Gardens which was the site with lowest sedimentation. *Porites* spp. were, however, present as small isolated colonies at Sand Island and Penguin Spit, and this may be linked to their ability to tolerate sediment deposition through heavy mucus secretion, trapping and subsequent sloughing (Dikou & van Woesik 2006). Solitary corals of the family Fungiidae possess morphological and physiological adaptations to highly turbid environments, such as broad septal edges and polyp inflation to allow sediment to slide off (Hubbard & Pocock 1972, Schuhmacher 1977). Accordingly, *Fungia* spp. at Palmyra Atoll are more abundant on the western, more turbid side of the atoll. Many genera, such as *Echinophyllia, Turbinaria* and *Hydnophora* (L. Wicks, pers. obs.) are common in deep or turbid waters and are only found to the western side of the atoll. *Pocillopora* spp. have been cited as being characteristic of turbid waters in the Indo-Pacific (Rogers 1990): at Palmyra this is reflected in their dominance of the coral fauna at the highly turbid site Sand Island. *Acropora* spp. are poor sediment rejectors (Bak & Elgershuizen 1976) and intolerant of low light (Roy & Smith 1971, Sheppard 1980, Bouchon 1981): at Palmyra their dominance at Tortugonas may be linked

to their ability to escape sedimentation due to their branching morphology, although their presence suggests that turbidity levels are low enough to allow sufficient light for photosynthesis.

The environmental variables influencing the diversity of coral communities at Palmyra have not affected the symbiont communities. Some coral species may harbour multiple physiologically-distinct symbiont types, sometimes simultaneously, with their presence or dominance being in response to the external environment (Rowan et al. 1997, van Oppen et al. 2001, Venn et al. 2008b). For example, *Symbiodinium* clades A, C and D were found evenly distributed in *Montastrea* spp. in areas of high sedimentation in Panama, with clade B dominant in the clearer waters of nearby Belize (Garren et al. 2006). At Palmyra, however, coral species tend to host single *Symbiodinium* types, commonly the generalist types C1 and C3 (LaJeunesse et al. 2003) which have the ability to associate with a range of hosts in various environments. The community structures of *Symbiodinium* types characterized from Palmyra's host assemblage is similar to those observed in Caribbean and western Pacific reefs where a greater percentage of host taxa share a common symbiont type (LaJeunesse 2002; LaJeunesse et al. 2003), attributed to increased extinction rates and the increased faunal turnover of scleractinian corals. Exceptions to this at Palmyra included *Montipora* spp., which hosted both C15 and 'stress tolerant' D1/a (Baker 2001, Thornhill et al. 2006a). However, both symbionts were present at all sites, excluding the turbid Sand Island at which *Montipora* spp. only hosted C15. It may be that both C15 and D1a are equally tolerant to potential stressors; indeed, *Montipora digitata* hosting C15 on the GBR was found to be less sensitive to thermal stress than were other *Montipora*-symbiont partnerships (LaJeunesse et al. 2003). This reduced host-specificity uncoupled with environmental conditions was observed in *Pocillopora* spp. at Palmyra, which had the

ability to host C1c, either alone or with C42a, and *Pavona* spp. could host C3, C1 or C1/c. The homogeneity of coral-*Symbiodinium* partnerships across the atoll indicates that the partnerships are able to tolerate the wide range of environmental conditions.

The susceptibility of the host, rather than the symbiont, to sedimentation appears to drive coral distribution patterns at Palmyra Atoll. Host factors have been demonstrated to be critical to stress susceptibility (Fitt et al. 2009), however the nature of this susceptibility is less well recognised. For example, symbiont type C3 has been observed to be stress-susceptible in *Acropora* spp. but stress-tolerant in *Favia* and *Goniastrea* spp. (LaJeunesse et al. 2003). Differences in the capacity of host species to use mechanisms to combat sedimentation might therefore determine the host distributions in response to stressful environments. Much remains to be learnt of the physiological variability between symbionts and hosts, and the flexibility of the relationship between the two in response to changes in the environment.

4.4.3 Future plans for Palmyra Atoll

Although at present sedimentation levels at Palmyra are within normal levels of corals reefs, the proposed restoration of the atoll will change this, and may negatively impact the coral communities present. The man-made causeways are breaking down, with sediment being redistributed by physical processes (Collen et al. 2009), potentially affecting corals across the atoll. Restoration would involve the creation and widening of breaks in the causeways to re-establish flow to pre-WWII conditions, leading to short-term increases in sediment load, but long-term reduction in turbidity and water temperature in the shallow reef areas (Maragos et al. 2008a).The current host-symbiont partnerships tolerate a range of sediment regimes across the atoll, and thus may be able to withstand low levels of

environmental perturbation. In the event of restoration going forward, host-symbiont communities should be monitored.

4.4.4 Ecological implications

Sedimentation is just one of many large-scale disturbances that can exert considerable impact on coral communities. Coral reefs face a rapid change in their environment from increasing sea surface temperature (SST), and questions exist as to how (if) they will adapt and whether their rates of adaptation will be rapid enough to match those of the changing environment. Palmyra is located to the north of equatorial upwelling, and so year round experiences temperatures greater than 28ºC (Maragos et al. 2008b) comparable to temperatures on other Pacific reefs where bleaching commonly occurs (Oliver & Palumbi 2009). In the shallow lagoon regions of the atoll where corals flourish, water temperature will increase beyond this 28ºC on a diurnal basis. Further, El Niño conditions can increase annual SST at Palmyra, which in the past has led to bleaching events and the catastrophic death of staghorn and tabular corals on the southwest side of the atoll (Maragos et al. 2008a).

The lack of correlation between symbiont community and environmental attributes at Palmyra suggest that the symbionts are robust enough to cope with the environmental regime, which may be less severe than expected. However, if environmental conditions (i.e. increased SST, heavy sedimentation) do reach a level where stress-induced bleaching occurs, the low diversity of symbionts may put these corals at a greater risk of extinction, than reefs hosting diverse symbiont assemblages (LaJeunesse et al. 2003). The presence of ‘stress-tolerant’ clade D symbionts may allow resistance to bleaching by some coral species

(Glynn et al. 2001, Baker et al. 2004, Rowan 2004, Berkelmans & Van Oppen 2006), but only the coral species able to host tolerant symbiont types will have the capacity to survive.

Despite the relatively high water temperatures experienced at Palmyra, and the high sedimentation regimes at some areas of the atoll, diverse coral communities still exist, and recovery from bleaching has been reported (Maragos et al. 2008b). Reefs experiencing no direct anthropogenic impact such as Palmyra have been reported to display a greater capacity than reefs experiencing anthropogenic impacts to survive or recover from bleaching episodes, due to the protection from added stressors of overfishing and pollution (Sandin et al. 2008). Further, the high degree of connectivity among Palmyra and other reef systems does mean that in the case of regional or global scale disturbances, a significant influx of larvae from multiple sources will help the community recover.

Chapter 5

Photophysiology of *Symbiodinium* in response to environmental stress at a high latitude, seasonally variable site

ABSTRACT

Biogeographic variability in the partnerships of corals and their dinoflagellate symbionts (*Symbiodinium* spp.) is linked, in part, to the physiological difference between the symbionts. *Pocillopora damicornis* hosts genetically distinct and novel types of symbiont at the high latitude site of Lord Howe Island (LHI), yet why these novel types exist at LHI is unknown. In this study, I examined whether one of the novel holobiont partnership at LHI is physiologically adapted for this high latitude site and so help explain the success of this marginal coral reef. High and low thermal bleaching thresholds of the coral-symbiont partnerships were measured as 14°C and 30°C. Photochemical sensitivity to temperature (15°C and 29°C) and light treatments (100% and 40% of sunlight), measured as effective quantum yield and fast induction curves, were also determined over a 72 h period. A lesser impact on the photochemical processes of LHI symbionts was recorded in response to a 3°C temperature decrease from annual minima, than a 3°C temperature increase from annual maxima. Water temperatures at LHI annually range from 18 - 26°C, but corals still did not bleach when temperature was reduced to 15°C for 72 h, with minimal bleaching in the 14°C treatment. In contrast, after 72 h, a 94% decline in photochemical efficiency were recorded in the 29°C treatment ($\Delta F/F_m' < 0.05$), compared to 37% loss in the control.

Response to warm and cold temperature stress were both enhanced by high light levels. This study suggests that the types of *Symbiodinium* at LHI may be specialised for cooler and more variable temperatures, so contributing to the success of corals at this marginal location.

5.1 INTRODUCTION

The success of coral reefs results from the symbiotic relationship between scleractinian corals and dinoflagellates of the genus *Symbiodinium.* The coral relies on its dinoflagellate symbionts for survival, as they release substantial amounts of photosynthate to the host, and aid the recycling and conservation of nitrogen (Muscatine 1990, Wang & Douglas 1998). In return, the host provides carbon dioxide for photosynthesis and nutrients (e.g. nitrogen and phosphorus) required for synthesis of cellular constituents, including the photosynthetic apparatus.

The photosynthetic efficiency of the dinoflagellates is a function of both their environment and the diversity within the genus *Symbiodinium* (Iglesias-Prieto & Trench 1997b, Rowan 2004, Tchernov et al. 2004, Berkelmans & Van Oppen 2006, Abrego et al. 2008). The genus is highly diverse, with 8 clades (A-H) and numerous sub-clades identified (reviewed in Rowan 1998, Baker 2003, Coffroth & Santos 2005). As a reflection of the high genetic diversity within the genus *Symbiodinium*, considerable physiological diversity exists at both cladal and sub-cladal levels, including in terms of tolerance to irradiance and/or temperature (Iglesias-Prieto & Trench 1997b, Iglesias-Prieto et al. 2004, Robison & Warner 2006, McCabe-Reynolds et al. 2008, Takahashi et al. 2009). This physiological diversity is also evident in the distribution of these symbiotic dinoflagellates, at both global

and local scales, and in bleaching patterns of host corals (Baker et al. 2004, Berkelmans & Van Oppen 2006, Jones et al. 2008).

Despite evidence that these symbiotic dinoflagellates play an important role in determining the environmental tolerance of the holobiont (host and symbiont), and potentially its survival in response to environmental stress, little is known of the physiology of *Symbiodinium* types that live on so-called 'marginal' coral reefs. Marginal coral reefs are those living near or beyond the normal limits of reef distributions, close to the environmental thresholds for coral survival (Kleypas et al. 1999). Marginal reefs frequently occur at high latitudes i.e. above 25°S and 25°N, with well known examples being those of the Persian Gulf, Bermuda, Japan and Lord Howe Island (LHI), Australia. High latitude or temperate reefs are generally subject to wide ranges in seasonal temperature (highest in Arabian Gulf; 18 - 36°C, Riegl 2003), for example temperatures at LHI annually range from 18-26°C (L Wicks, personal observation) compared to the central Great Barrier Reef's (GBR) annual range of 23 - 30°C (Ulstrup et al. 2006). They also experience low annual minimum temperatures (lowest 13.3°C, Japan, Veron 2000) and relatively low irradiance levels (Kleypas et al. 1999).

From the limited information available, it has been shown that the specific partnerships between corals and their dinoflagellate symbionts at high latitude reef sites differ from their tropical counterparts (see Chapter 3, LaJeunesse & Trench 2000, Loh et al. 2001, Rodriguez-Lanetty et al. 2001, Savage et al. 2002, Loh et al. 2006). In a recent study at the high latitude site of LHI, many corals contain different types of clade C *Symbiodinium* to those found in the same coral species on the GBR (Chapter 3). LHI (31"33'S, 159"05' E, Fig. 5.1) is situated over 1000 km from the southern GBR, and is home to the southernmost coral reef in the world. Despite the high latitude conditions and

isolation of the island, both symbiont and coral diversity are high (Chapter 3, Harriott et al. 1995), with flexible relationships identified in many coral species. Of particular note, *Pocillopora damicornis* hosts three novel C types (C41, C100, C118) at LHI, but is known only to associate with types C1, closely related types to C1, and D1 elsewhere in the Indo-Pacific (Ulstrup et al. 2006, Sampayo et al. 2007). These novel types at LHI are environmentally specialised, with one type only found in deeper and turbid waters, yet why these novel types exist at LHI, particularly in terms of their photo-physiological attributes, is unknown.

To assess physiological variability between *Symbiodinium* types, photosynthetic responses to thermal and irradiance stressors can be determined. Symbionts exposed to stress can undergo photosynthetic damage when temperature (either high or low) hinders photosynthetic CO_2 assimilation by the symbiont, so causing light capture to exceed the rate of light utilization (Jones et al. 1998, Saxby et al. 2003, Smith et al. 2005). In response to increased temperature, excess energy leads to the production of reactive oxygen species (ROS), which can disrupt cellular components and induce physiological malfunction, and lead to expulsion of the symbiont from the host (Warner & Fitt 1999, Tchernov et al. 2004, Weis 2008). Excessive irradiance or thermal stress can lead to permanent damage, including dysfunction of Photosystem II (PSII) and degradation of the associated D1 protein, energetic uncoupling in the thylakoid membranes, and impairment of the Calvin-Benson Cycle (Jones et al. 1998, Warner & Fitt 1999, Tchernov et al. 2004, Hill et al. 2006). Few studies have examined the impact of cold temperature stress on PSII; however, on the southern GBR, where temperatures annually average 17 - 25°C, significant loss of photochemical efficiency was recorded after 12 h exposure to 12°C (Saxby et al. 2003) and bleaching at 13.3°C was observed *in situ* (Hoegh-Guldberg et al. 2005).

To enable symbionts to cope with such stressors, several photo-protective pathways exist in *Symbiodinium* cells, to dissipate the excess excitation energy (Brown et al. 1999, Jones & Hoegh-Guldberg 2001, Winters et al. 2003). These include down-regulation of PSII reaction centres and alternative electron transport pathways, such as the xanthophyll cycle (Brown et al. 1999, Jones & Hoegh-Guldberg 2001, Winters et al. 2003, Warner & Berry-Lowe 2006, McCabe-Reynolds et al. 2008). Down-regulation also occurs on a diurnal basis, where photosynthetic capacity declines following exposure to midday high light (Hill & Ralph 2005). The efficiency of photo-protective mechanisms is thought to vary between *Symbiodinium* types, although the underlying mechanism is poorly understood (Takahashi et al. 2009).

This study aimed to determine if the novel holobionts at LHI are photo-physiologically adapted for this high latitude site and so help explain the success of this marginal coral reef. The study focused on responses to temperature and light intensity, with the underlying hypothesis being that these high latitude *Symbiodinium* types are able to withstand a greater range of temperatures than are their tropical counterparts. Elucidation of the relationship between *Symbiodinium* photo-physiology and biogeography will further our understanding of the mechanisms involved in local adaptation to thermal and solar conditions, and the persistence of coral reefs at marginal sites. It is hypothesised that these high latitude *Symbiodinium* types would be able to withstand a greater range of temperatures, than their tropical counterparts on the GBR. By elucidating the relationship between *Symbiodinium* physiology and biogeography, this study aimed to further our understanding of the mechanisms involved in local adaptation to thermal and solar conditions, and the persistence of coral reefs at marginal sites.

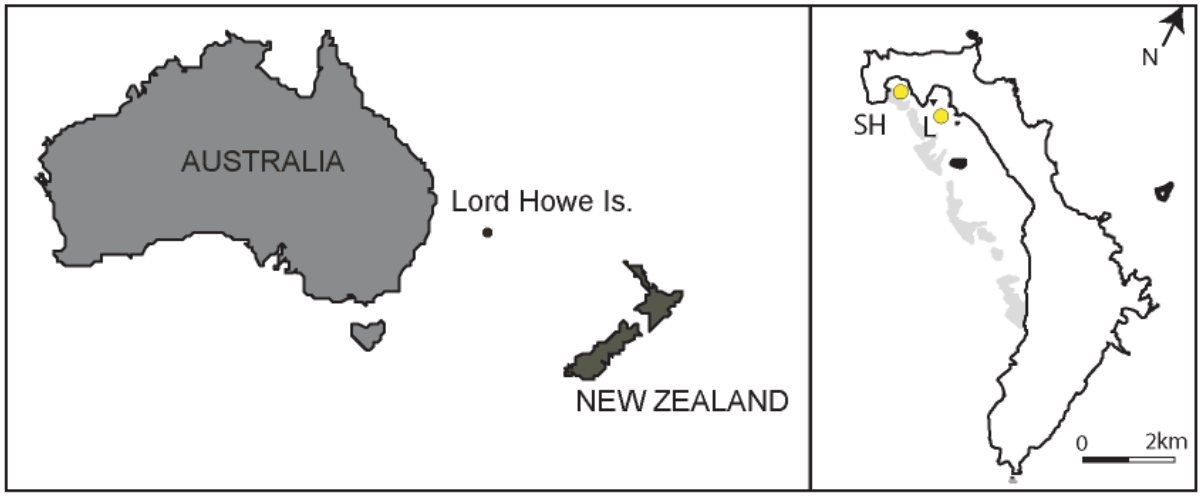


Figure 5.1. Lord Howe Island, Australia, with location of sample collection. SH: Sylphs Hole, L: Lagoon**.**

5.2 MATERIALS AND METHODS

5.2.1 Sampling

In September 2008, colonies of the scleractinian coral *P. damicornis* were collected from 2 - 3 m deep (during low tide) at two lagoon sites at LHI (31°33′S 159°05′E): the highly turbid site of Sylphs Hole and off Lagoon Beach in the central lagoon (Fig. 5.1). Colonies were collected from two sites to increase the chance of sampling multiple symbiont types. A previous study found that *Symbiodinium* C41 predominated in *P. damicornis* colonies at Sylphs Hole, whilst C100 was dominant at other lagoon sites (Chapter 3). Water temperatures at time of collection ranged between 19°C and 22°C. Colonies were collected from the central lagoon and maintained in indoor tanks at ambient lagoon temperature (18 - 19ºC) for 2 days.

5.2.2 Experimental procedure

All experiments were set up in direct sunlight close to the shore at LHI, with 60-L tanks angled to all receive the same duration and intensity of sunlight, and fresh seawater used throughout. Portable air pumps aerated and circulated the water, and 50% of the water was changed every 2 hours to control for solar heating effects; this ensured that the water temperature did not deviate by more than ±1° C from the experimental temperature.

To determine upper and lower bleaching thresholds of *P. damicornis* at LHI, corals were exposed to 5 different temperatures (14°C, 15°C, 20°C, 29°C and 30°C) for a 62-hour period, beginning at 6 am (dawn) (n = 6 per treatment). All temperature treatments were run simultaneously, with one tank per treatment due to the logistical limitations of working at this remote site. Temperatures in the tanks were maintained using a chiller (Aqua Medic HL-260CA) for the 14°C and 15°C treatments, and 2 heating elements (Jager 150 W, EHEIM) for the 29°C and 30°C treatments. In the control treatment (20°C), temperatures were maintained by regular water changes with lagoon water. Temperatures were recorded each minute throughout the experiment using HOBO pendant loggers (Onset). Measurements of effective quantum yield ($\Delta F/F_m'$) (see *Physiological measurements*) were taken using a submersible Pulse Amplitude Modulated fluorometer (Diving-PAM, Walz) every two hours between 6 am and 8 pm for the 62-h period. Sunrise was at ~ 6 am, with sunset at ~6 pm. The intensity of photosynthetically active radiation (PAR) was logged every minute by a submersible light meter (Odyssey). Bleaching thresholds were defined as the temperature at which >50% of nubbins bleached (visible bleaching and $\Delta F/F_m' < 0.1$).

Once the upper and lower thermal thresholds had been established (14°C and 30°C), 4 new colonies per site were divided into six nubbins and exposed to six different temperature/light combinations for 72 h: 15°C, 20°C (control) and 29°C, each at both high

irradiance (full light at 30 cm depth, peak irradiance ~ 1900 μmol quanta m^{-2} s^{-1}) and low irradiance (60% neutral density filter, peak irradiance ~ 1000 μmol quanta m^{-2} s^{-1}). These low and high temperatures were chosen as they were ~1°C clear of the determined upper and lower bleaching thresholds. Coral nubbins were placed in the tanks at dawn on day 1 at ambient lagoon temperature (18 - 19°C) and the temperature ramped upwards or downwards to reach the required temperature over 6 hours. It should be noted that the rate of ramping of the temperatures was relatively rapid due to experimental limitations, and thus the experiments act as a proxy for determining temperature stress reactions.

Fast induction curves (see *Physiological measurements*) were taken every 3 hours starting from dawn (5.30 am) until after dark (9 pm), on each of the three days of the experiment. Where samples appeared dead, as indicated by chlorophyll fluorescence signals and tissue loss, they were removed and preserved for genetic analysis of the dinoflagellate symbionts. In these cases (< 5% of samples), physiological measures could not be taken.

5.2.3 Physiological measurements

Photosynthetic health of symbionts was measured with chlorophyll fluorescence techniques, namely the Diving-PAM and PEA (Plant Efficiency Analyser). Diving-PAM (settings: measuring intensity = 8, saturation intensity > 4500 μmol photons m^{-2} s^{-1}, saturation width = 0.8, gain = 12) takes measures of the effective quantum yield of PSII (Φ_{PSII}) in a light-acclimated state. Dark-acclimated maximum quantum yield (F_v/F_m) was recorded at dawn and after dark. In corals, a decline in Φ_{PSII} precedes coral bleaching (Jones & Hoegh-Guldberg 2001, Lesser & Gorbunov 2001, Warner et al. 2002), and Φ_{PSII} varies both diurnally and seasonally (Jones and Hoegh-Guldberg 2001, Hill and Ralph 2005, Ulstrup et al. 2008). Evidence of down-regulation can be found from diel

experiments where F_v/F_m recovers by the evening or following morning; photodamage is implied when this recovery takes longer to occur (several days). To better characterize the physiological performance of the symbionts, we calculated the maximum excitation pressure over PSII (Q_m) as described in Iglesias-Prieto et al. (2004):

$$Q_m = 1-[(\Phi_{PSII})/(F_v/F_m)]$$

where Φ_{PSII} is the effective quantum yield of fluorescence recorded at noon, and F_v/F_m is the maximum quantum yield after dark.

The PEA measures polyphasic fluorescence-induction kinetics, to obtain detailed information about the photochemical state of PSII, as well as the plastoquinone (PQ) pool (Govindjee 1995, Strasser et al. 1995, Hill et al. 2004a). Fast induction curves (FIC) have been employed to assess the impact of bleaching conditions on symbiont photosynthesis in terms of the condition of PSII and the redox state of the primary (Q_A) and secondary (Q_B) electron acceptors (Hill et al. 2004a, Hill & Ralph 2006), as well as changes in these mechanisms both diurnally and seasonally (Hill & Ralph 2005). Prior to each PEA measurement, dark adaptation was conducted for 5 min to allow substantial re-oxidation of the primary electron acceptor (Q_A, Schreiber 2004). The measuring head of the PEA was attached to a dark adaptation chamber (Hill et al. 2004b) before each measurement, and a pre-amplifier (x 10) was used. Illumination was provided by an array of six red LEDs (peak wavelength 650 nm) focused on an area of 4-mm diameter, resulting in a maximum excitation irradiance of 3200 μmol photons m^{-2} s^{-1}. For the first 2 ms of saturation the fluorescence signal was measured every 10 μs, followed by 1 ms sampling up to 1 s, and then 100 ms sampling up to 5 s. All curves were normalized to the fluorescence yield at 0.05 ms (F_o) to eliminate any changes linked to a possible reduction in chlorophyll concentration (or symbiont density) due to stress (Iglesias-Prieto 1995, Jones et al. 2000).

When plotted on a $\log_{10}$ time scale, the fast induction curves followed the O-J-I-P steps of the 'Kautsky' curve. The 'O' step (F_o, the origin) occurred at 0.05 ms, the J step (intermediate) at 1 ms, the I step (second intermediate) at 100 ms, and the P step (F_m, maximum) at 2 s (Hill et al. 2004a).

Both prior to and following the treatments, coral tissue was removed from each nubbin with an airbrush. The tissue slurry was diluted in 5 ml of filtered seawater (FSW; 1 μm), and centrifuged at 6,000 × *g* for 5 min. The remaining algal pellet was resuspended in 5 ml FSW and homogenized by vigorous shaking; 0.5 ml was removed for symbiont cell counts, and 0.5 ml preserved at -20°C in darkness for determination of chlorophyll concentrations. The remaining sample was centrifuged for 5 min at 8,000 × *g*, the supernatant discarded, and the pellet preserved in 20% DMSO buffer and stored at −20°C for symbiont identification. Symbiont densities were estimated by using a haemocytometer (Neubauer) with 10 replicates of each sample, and expressed per unit surface area of the coral, obtained by the wax weight method (Stimson & Kinzie 1991). Chlorophyll extractions were undertaken in darkness, using 100% acetone at 4°C overnight, and concentrations determined with a spectrophotometer as described by Jeffrey & Humphrey (1975). Chlorophyll concentrations were determined per cell and per cm^2 of coral surface area.

5.2.4 Genetic data

The preservation buffer was removed using a 3-fold DNAB washing step and the DNA extracted using a DNeasy plant kit (Qiagen) according to the manufacturer's instructions. The ITS2 region of the ribosomal DNA was amplified with the primers ITSintfor and ITS2clamp (LaJeunesse & Trench 2000). Amplified ITS2 fragments were separated by

using 30–60% DGGE gels (DCode system, Bio-Rad) during a 14-h run at 100V, after which gels were stained with SYBR green (Invitrogen). Symbiont profiles were compared with those previously identified in *P. damicornis* from Lord Howe Island (Chapter 3).

5.2.5 Data analysis

Repeated measures ANOVA (rmANOVA) was used to determine if the fluorescence parameters (F_v/F_m, Φ_{PSII}, Q_m, and O, J, I and P steps) differed significantly ($\alpha = 0.05$) between temperature and light treatments in the 3 day period (19 time points). rmANOVA also tested for significant differences in Φ_{PSII} between temperature treatments in the bleaching threshold test. Due to the similarity of photo-physiological responses between the two collection sites, inter-site comparisons are not considered further here, except in relation to symbiont density and chlorophyll concentrations where differences existed. One-way ANOVA assessed significant differences in chl-*a* concentration and symbiont density between treatments, and pre- *versus* post-treatment. Assumptions of normality and of homogeneity of variances were tested using Kolmogorov-Smirvov's and Levene's tests, respectively. If these assumptions were not met, transformations were performed (Arcsine for F_v/F_m, Φ_{PSII}, and Q_m; square root for chl-*a* concentration, symbiont density, and O, J, I and P). Where rmANOVA determined a significant difference, Box's test of equality of covariance matrices was used to test the homogeneity of variances between treatments. The assumption of sphericity was tested, and where assumptions were not met, the Greenhouse–Geisser adjustment was performed. Tukey's multiple comparisons were used to identify significant differences between individual temperature treatments. Each physiological parameter was analysed separately, with temperature and light as between-subject factors.

Time was analysed as a within-subject factor, as photochemical measures were repeated on the same colony. All analyses were performed using SPSS software v16.0.0.

5.3 RESULTS

5.3.1 Symbiont identification

All coral colonies (n = 10) collected from the lagoon harboured *Symbiodinium* type C100, while three of four colonies collected from the more turbid site of Sylphs Hole also harboured this type; the fourth colony from Sylphs Hole contained type C41.

5.3.2 Bleaching thresholds

In the bleaching threshold experiment, all *P. damicornis* samples contained C100. The effective quantum yield of PSII (Φ_{PSII}) declined from 6 am to 12 pm in all temperature treatments, with significantly higher Φ_{PSII} recorded for the control nubbins than the nubbins subject to high (29 and 30°C) or low (14 and 15°C) temperatures ($p < 0.001$ Fig. 5.2). Furthermore, the 29°C and 15°C treatments exhibited significantly higher Φ_{PSII} than did the 30°C and 14°C treatments, throughout the experiment ($p < 0.001$ Fig. 5.2).

Bleaching was only observed in the 14°C (50% of nubbins), 29°C (17% of nubbins) and 30°C treatments. Indeed, after 6 am on day 2 (i.e. 24 h after the start of the treatments), all nubbins (n = 6) exposed to 30°C had completely bleached or died. Mean F_v/F_m (± SE) of nubbins exposed to 29°C was 0.2 ± 0.01 at 72 h, less than half the F_v/F_m of the control (0.44 ± 0.03). Three of the six nubbins exposed to 14°C had $F_v/F_m < 0.1$ at 24 and 48 h, with F_v/F_m of the three remaining nubbins being ~ 0.25 at these same time points. Considerable

changes in Φ_{PSII} occurred over the first 24 h of the experiment. At 24 h, mean Φ_{PSII} (± SE) in the control treatment was 0.46 (± 0.02), which was significantly higher than in the 29°C and 15°C treatments ($\Delta F/F_m$' = 0.31 ± 0.04 and 0.22 ± 0.03 respectively), and four-times the mean Φ_{PSII} (± SE) in the 14°C treatment (0.12 ± 0.02; all nubbins at 30°C were dead) at this time-point.

5.3.3 Photochemical efficiency

Once the bleaching thresholds of 14 and 30°C were established, an experiment on the responses of photochemical efficiency to extreme temperature and light treatments was performed. Maximum (F_v/F_m) and effective quantum yield (Φ_{PSII}), and fast induction curves (FICs) were measured every 3 hours from dawn to dusk in nubbins exposed to three temperature treatments (29°C, 20°C, 15°C), each at two light levels (100% and 40% full irradiance, referred to as high and low light) over 72 h. Significant differences in each of the parameters measured between temperature and light treatments are displayed in Table 5.1.

The well-known inverse relationship of photosynthetic quantum yield with irradiance (Brown et al. 1999, Hoegh-Guldberg & Jones 1999, Hill & Ralph 2005) was observed for all *P. damicornis* nubbins in all treatments (Fig. 5.3). Maximum F_v/F_m was recorded at 6 am, with Φ_{PSII} then decreasing diurnally from 9 am, reaching a minimum at ~3 pm following the 12 pm peak in natural sunlight intensity. In the afternoon, $\Delta F/F_m$' recovered as natural sunlight intensity decreased, with F_v/F_m reaching a maximum

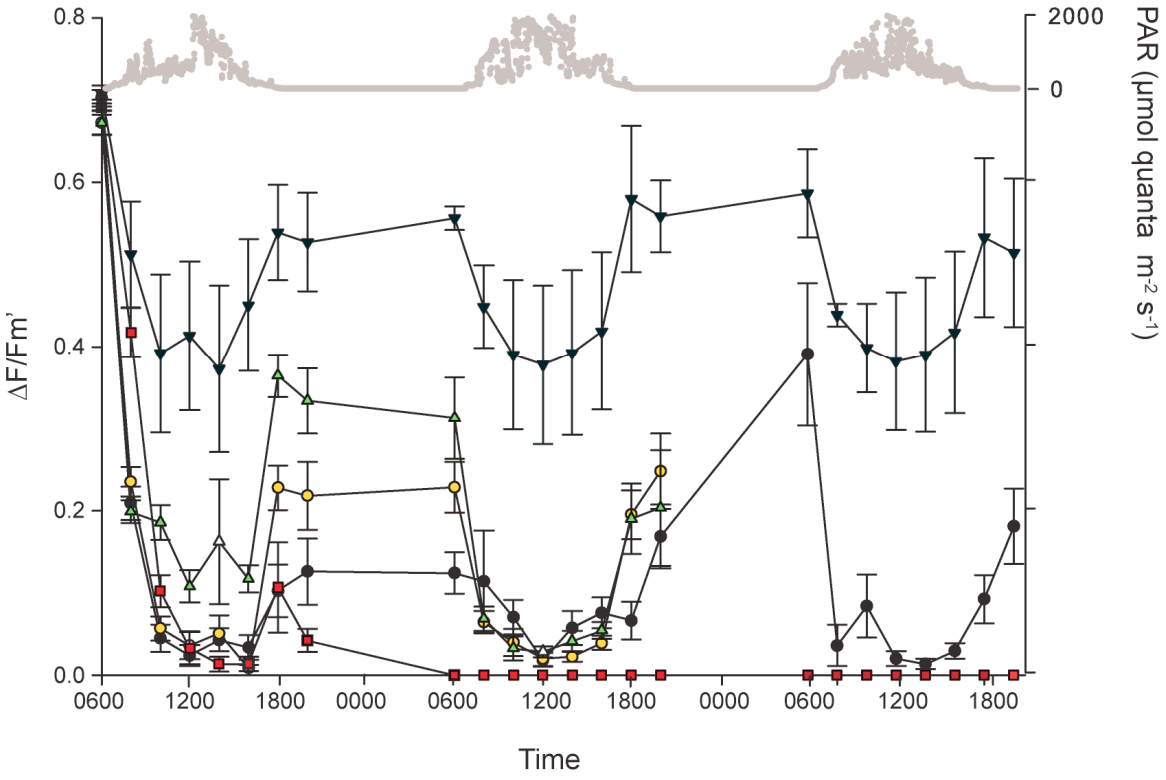


Figure 5.2 Φ_{PSII} in *Pocillopora damicornis* nubbins over 62 hour treatment period at 5 temperature treatments: ●14°C ● 15°C ▼20°C ▲ 29°C ■30°C. Photosynthetically active radiation (µmol quanta m-2 s-1) on right axis (●).

at ~9 pm. Overnight, F_v/F_m did not increase or decline significantly (ANOVA, $p < 0.01$). Significant differences in Φ_{PSII} were recorded between both light and temperature treatments, in addition to the additive effect of both factors (rmANOVA, $p < 0.001$ Refer to Table). Φ_{PSII} was significantly higher over the 72 h period in low light than in high light for all treatments (rmANOVA, $p < 0.001$ Table 5.1). After 72 h at low light, $\Delta F/F_m$' maintained 88%, 63% and 45% of initial readings at 20°C, 15°C and 29°C, respectively,

compared to 63%, 14% and 9% in the high light treatment. Under both low and high irradiance, coral nubbins in the 20°C temperature treatment maintained the highest ΔF/Fm' over the 72 h period, which was significantly greater than in the 29°C and 15°C treatments (rmANOVA, $p < 0.001$ Table 5.1). Φ_{PSII} varied among temperature treatments over time at both light levels; the 20°C/low light treatment (0.58 ± 0.03), was greatest, closely followed by 20°C/high light and 15°C/low light (0.43 ± 0.03 and 0.42 ± 0.04 respectively). The lowest mean Φ_{PSII} overall was recorded in the 29°C/high light treatment, which remained at < 0.05 for the final 21 h of the experiment.

PSII excitation pressure (Q_m) was significantly higher in nubbins exposed to high light than low light on days 1 and 3 of the experiment (rmANOVA, $p < 0.001$), however this varied among temperature treatments (Fig. 5.4, Table 4.1). Assessed alone, neither temperature nor light significantly affected Q_m over time ($p = 0.722$ and $p = 0.784$, respectively). In high light, nubbins exposed to 29°C had significantly higher Q_m than corals in the control or 15°C treatments (rmANOVA, $p < 0.01$). In contrast, in low light, no significant difference in Q_m was observed among temperature treatments (rmANOVA, $p = 0.342$). In high light, Q_m either declined or was stable from day 1 to 2, depending upon treatment. The greatest decline in mean Q_m (± SE) from day 1 to 2 in high light was in the 20°C treatment (0.83 ± 0.02 to 0.61 ± 0.11). Q_m in the 29°C and 15°C/low light treatments remained stable over the 3 days (range 0.8 - 0.88). For the low light treatment, mean Q_m rose from day 1 to 2 for all temperature treatments (from 0.52 ± 0.02 to 0.66 ± 0.11 at 29°C, 0.32 ± 0.04 to 0.74 ± 0.02 at 20°C, and 0.62 ± 0.05 to 0.85 ± 0.03 at 15°C), with the day 2 Q_m being sustained to day 3 in the 15°C treatment (0.84 ± 0.02). In the 20°C treatment, however, mean Q_m declined on day 3 to 0.61 (± 0.06), while in the 29°C

Table 5.1 *p* values of the rmANOVA analyses which tested for changes in the maximum (F_v/F_m) and effective ($\Delta F/F_m'$) quantum yields, O, J, I and P steps and PSII excitation pressure (Q_m) over the 72 h experiment for three temperature treatments (29°C, 20°C and 15°C) at two light levels (high and low) of *Pocillopora damicornis* from Lord Howe Island.

	$\Delta F/F_m'$	F_v/F_m	O	J	I	P	Q_m
Within subjects							
Time	<0.001*	<0.001*	<0.001*	<0.01	<0.001	<0.001	<0.001
Time x Temp	<0.001*	<0.01*	<0.001*	0.09	0.058	0.062	0.722
Time x light	<0.001*	<0.001*	<0.01*	<0.05	<0.05	<0.05	<0.001
Time x temp x light	0.339	0.458	0.292	0.384	0.391	0.924	0.171
Between subjects							
Temp	<0.001*	<0.001*	0.229	<0.01*	<0.001*	<0.001*	<0.01*
Light	<0.001*	<0.001*	<0.05*	<0.001*	<0.001*	<0.01*	<0.001*
Temp x light	0.517	0.938	0.735	0.326	0.51	0.561	0.157
	15, 19 > 28	19>28>15	~	15, 19 > 28	15, 19 > 28	15, 19 > 28	15, 19 > 28

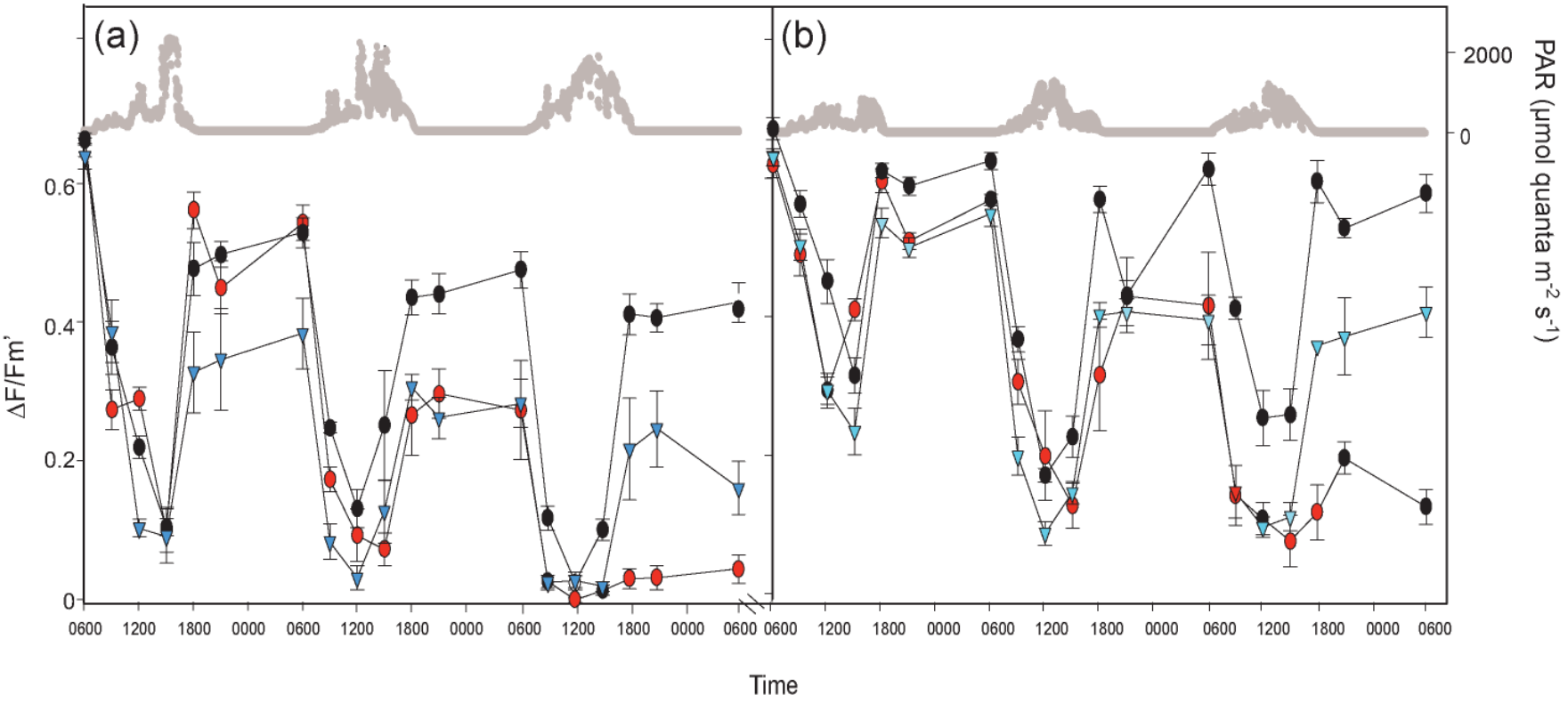


Figure 5.3 Φ_{PSII} in *Pocillopora damicornis* nubbins over 72 hour thermal treatment period at high light (a) and low light (b). Treatment temperatures were 29°C (●), 20°C (●) and 15°C (▼). For all tests, averages (±SE) shown (n = 4). Photosynthetically active radiation (PAR, µmol quanta m^{-2} s^{-1}) on right axis (●).

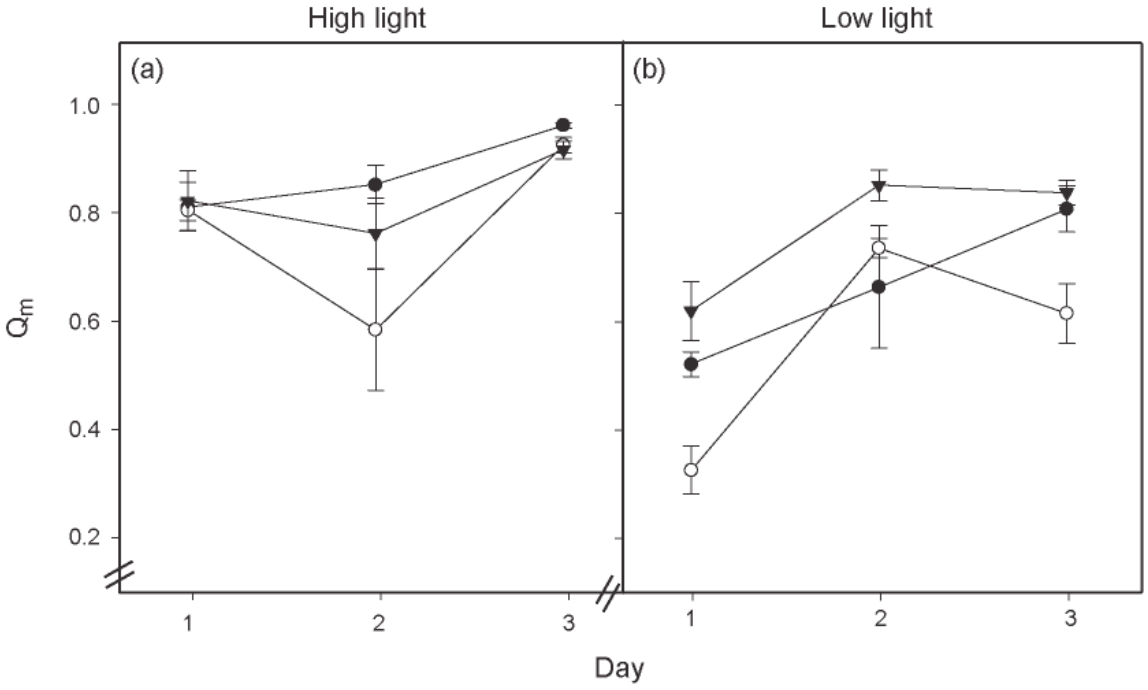


Figure 5.4 PSII excitation pressure (Q_m) in *Pocillopora damicornis* nubbins over 3 day thermal treatment period at high light (a) and low light (b). Treatment temperatures were 29°C (●), 20°C (○) and 15°C (▾). For all tests, averages (±SE) shown (n = 8).

treatment it increased further to 0.81 (± 0.04). However, no significant difference in Q_m was observed among temperature treatments in low light (rmANOVA, $p = 0.342$). In high light, nubbins exposed to 29°C had significantly higher Q_m than corals in the control or 15°C treatments (rmANOVA, $p < 0.01$).

5.3.4 Fast induction kinetics

Figure 5.5 a–f shows the fast induction curves (FICs) of *P. damicornis* for a series of thermal treatments at two light levels (100% and 40% of natural daylight, high and low light respectively). For each treatment, a selection of FICs normalized to F_o is shown from the 72-h period (T = 0, 9, 24, 33, 48, 72 h). At each timepoint in all temperature treatments,

the FIC amplitude of nubbins subjected to high light was lower than that of nubbins subjected to low light. In the low light treatment at all temperatures, the P step remained high at 24 h (i.e. dawn on day 2), despite falling at 9 h (3 pm on day 1). At >24 h in low light, the P step declined in the 29ºC and 15ºC, however in the 20ºC treatment it remained high. In the high light treatment, the P step declined over time in all temperature treatments. Least variability among FICs over time was recorded in the 20ºC and 29ºC/low light treatments, with greatest variability in the 29ºC/high light treatment.

The mean amplitudes of the O, J, I and P steps over the 72-h period are shown in Figures 5.6a-f. In all treatments, the amplitude of steps varied diurnally, declining from a maximum at 6 am to a minimum at 3 pm (following maximum PAR at 12 pm) followed by an increase in the afternoon. For all steps, the amplitudes were significantly lower at high light than low light, in all temperature treatments over the 72 h (rmANOVA $p < 0.01$, Table 5.1). Magnitude of the J, I and P steps was significantly higher in the 20°C treatment than in the 29°C or 15°C treatments (rmANOVA, $p < 0.05$), with O significantly variable among temperature treatments over time. No significant synergistic effect of temperature and light was observed for any of the steps (rmANOVA, $p > 0.3$, Table 5.1).

Diurnal changes in the O, J, I and P steps varied among temperature treatments at both light levels. In the 20°C/low and high light treatments, each step had increased in amplitude by 6 pm, and returned to pre-dawn measurements by 9 pm. Similarly, in the 15ºC/low and high light treatments, amplitudes of each step at dusk were comparable to pre-dawn measures, after the decline associated with the high natural irradiance from 12 - 3 pm. However, at 29ºC in both light levels, amplitudes of each peak did not return to their

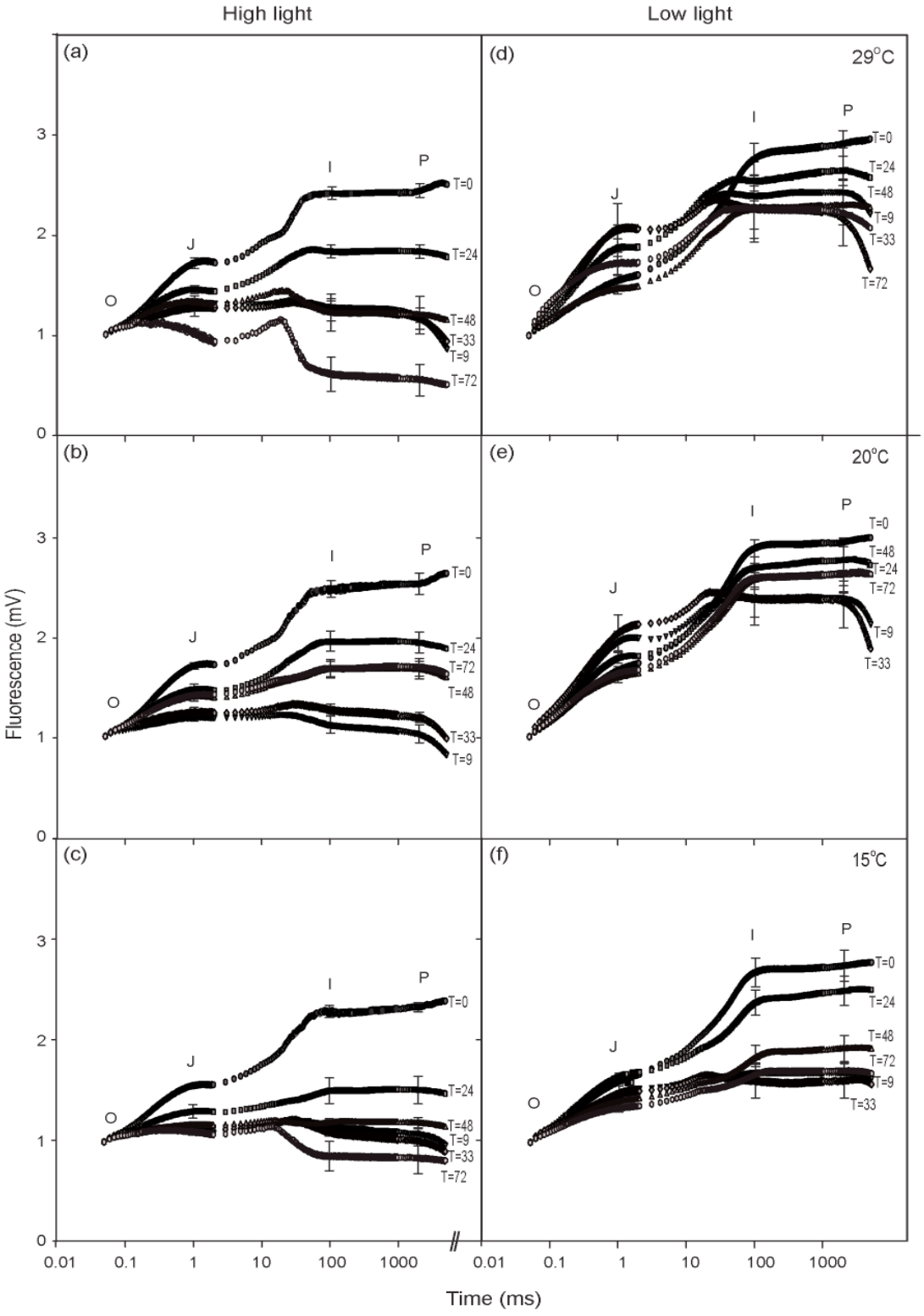


Figure 5.5 Fast kinetic induction curves (FICs) of thermal treatments of *Pocillopora damicornis* at 7 timepoints over a 72-hour period at high light (a - c) and low light (d - f). Treatment temperatures were 29°C (a, d), 20°C (b, e) and 15°C (c, f). Curves are normalised to F_0 and are plotted on a log10 time scale. Average curves are shown (n = 6 - 8), with SE given at the J, I and P steps (labelled).

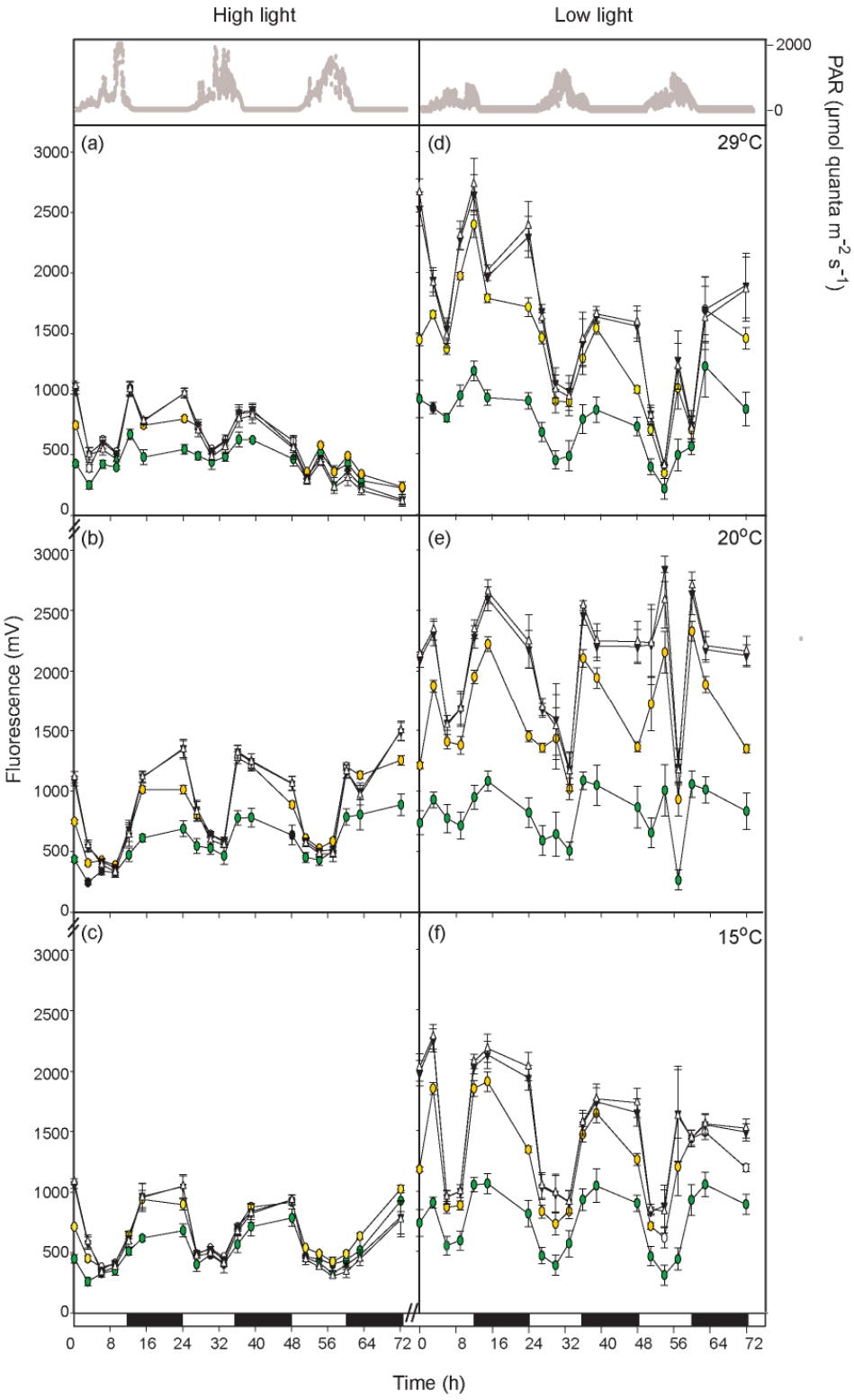


Figure 5.6 Amplitude of the O, J, I and P steps (●●▼△respectively) along fast induction curves (FICs) in *P. damicornis* over a 72-hour period at high light (a-c) and low light (d - f). Treatment temperatures were 29°C (a, d), 20°C (b, e) and 15°C (c, f). Average values (n = 7) from all nubbins and SE shown. Photosynthetically active radiation (PAR, µmol quanta m^{-2} s^{-1}) on right axis (●).

pre-dawn maxima, and were significantly lower at 72 h than at 0 h.

At 72 h, differences among the magnitude of each of the steps in response to light levels and temperatures were apparent, with values significantly lower in nubbins subject to high light than low light (one way ANOVA, $p < 0.05$). In low light, the O step was not significantly different from initial readings at 72 h in any temperature treatment. However, over the 72 h in high light, the O step had increased significantly in the 20ºC and 15ºC treatments (by 101 % and 100 %, respectively), but declined in the 29ºC treatment by 55 % (rmANOVA, $p < 0.001$). Over 72 h at low light, percent change in J (normalized to F_o) in the 29ºC treatment was not significantly different to that of J in the 15ºC treatment (1% increase in both treatments), however in high light, percent change in the 29ºC treatment was significantly greater than in the 20ºC and 15ºC treatments (67% decline *vs.* 67% and 42% increase, respectively; $p < 0.001$). For the I and P steps, magnitude and variability were greater in low light than in high light, but similar responses to temperature existed for both light treatments over the course of the experiment. In the 29ºC treatment at both light levels, I and P declined over 72 h (low light: 26% and 3% declines, respectively; high light: 86% and 88% declines, respectively). At 15ºC however, the decline was significantly less in both I and P than the 29ºC ($p < 0.01$; low light: 25% decline for both steps; high light: 24 and 28% declines, respectively). However, in the 20ºC treatment, I and P actually increased after 72 h in high light (by 37% and 34%, respectively), but declined in the low light (by 2% and 1%, respectively).

5.3.5 Symbiont density and chlorophyll concentrations

P. damicornis colonies from the lagoon had significantly higher mean (± SE) densities of symbionts than those from Sylphs Hole (8.08 ± 0.97 x 10^6 cm^{-2} *vs.* 4.87 ± 0.66 x 10^6 cells cm^{-2}, respectively; Table 5.2, $p < 0.05$). Change in symbiont density over the 72 h was variable among treatments (Fig. 5.7), with symbiont loss significantly greater in the 29°C treatment than in the 20°C and 15°C treatments (Table 5.2, ANOVA, $p < 0.001$). No significant effect of light or collection site on percent change in symbiont density was recorded (one way ANOVA, $p = 0.274$ and $p = 0.774$, respectively).

Chlorophyll concentration per symbiont cell at the start of the experiment did not differ significantly with site (ANOVA, $p = 0.342$, Table 5.3), however did vary per cm^2 coral (ANOVA, $p<0.01$). Following treatment, chlorophyll concentration per symbiont cell did not differ significantly with temperature (15°C, 20°C and 29°C) or collection site, but was highly variable among nubbins ($p = 0.225$, $p = 0.984$, respectively, Fig. 5.8). However, light did significantly affect chl-*a* loss (one way ANOVA, $p < 0.01$); in the low light treatment, all cells gained chl-*a* per cell (means 12-29% over 72 h for three temperature treatments); however in high light most cells lost chl-*a* (mean of 11-23% loss).

Chlorophyll-*a* cm^{-2} declined in all nubbins under all treatments. Neither light level nor collection site had a significant effect on the loss of chl-*a* cm^{-2} ($p = 0.226$ and $p = 0.758$, respectively). However, decline in chl-*a* cm^{-2} was significantly greater in the 29°C treatment than the 15°C and 20°C treatments at both light levels (mean ± SE: low light 85 ± 6% vs. 43 ± 13% and 23 ± 21% respectively, high light: 83 ± 7% vs. 65 ± 9 % and 33 ± 17%, ANOVA $p < 0.05$, Table 5.3).

Table 5.2 Symbiont density (per cm^2 x 10^{-6}), and chl-*a* concentration (per symbiont cell and per cm^2) in *Pocillopora damicornis* from Lord Howe Island. Measurements were taken pre-experiment and at 72 h, at each temperature/light treatment (29°C, 20°C and 15°C, high and low light). Mean ± SE (n = 8).

Site	**Light level**	**Treatment**	Zoox. density (cm^{-2} x 10^6)	Chl *a* $cell^{-1}$ (pg)	Chl *a* (μg cm^{-2})
Lagoon		pre-treatment	8.08 ± 0.97	3.99 ± 0.83	16.34 ± 3.64
	High	28	0.45 ± 0.26	4.88 ± 1.56	0.89 ± 0.38
		19	7.39 ± 0.63	3.52 ± 0.2	12.89 ± 0.86
		15	2.25 ± 0.52	7.33 ± 3.46	4.37 ± 10.4
	Low	28	1.04 ± 0.4	3.31 ± 0.39	1.82 ± 0.71
		19	4.37 ± 0.19	5.82 ± 0.85	12.52 ± 1.41
		15	2.95 ± 0.26	6.81 ± 0.31	10.03 ± 1.04
Sylphs		pre-treatment	4.87 ± 0.66	6.46 ± 0.82	16.33 ± 2.81
	High	28	0.99 ± 0.39	2.06 ± 0.21	1.46 ± 0.05
		19	2.36 ± 0.52	4.67 ± 0.56	5.67 ± 1.52
		15	3.97 ± 0.62	2.56 ± 0.37	5.28 ± 1.45
	Low	28	0.56 ± 0.22	5.91 ± 0.95	1.45 ± 0.85
		19	2.05 ± 0.19	7.96 ± 2.55	5.51 ± 0.8
		15	2.61 ± 0.21	5.92 ± 1.13	7.51 ± 1.23

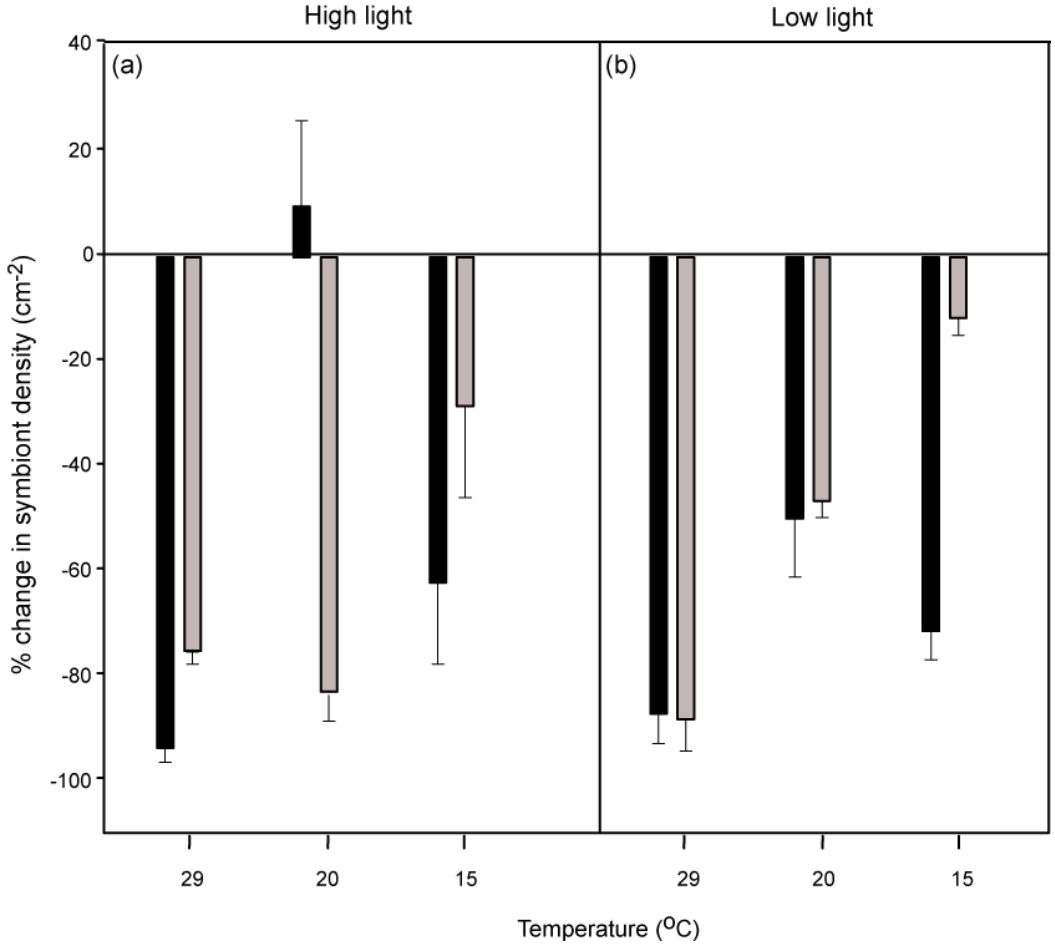


Figure 5.7. Relative change (%) of symbiont density (x10^6 cm^{-2}) following 72 h exposure to temperature treatments (29°C, 20°C and 15°C) in *P. damicornis* nubbins at a) high light and b) low light, from the lagoon (black bars), and from Sylphs Hole (grey bars)). Mean ± SE (n = 4).

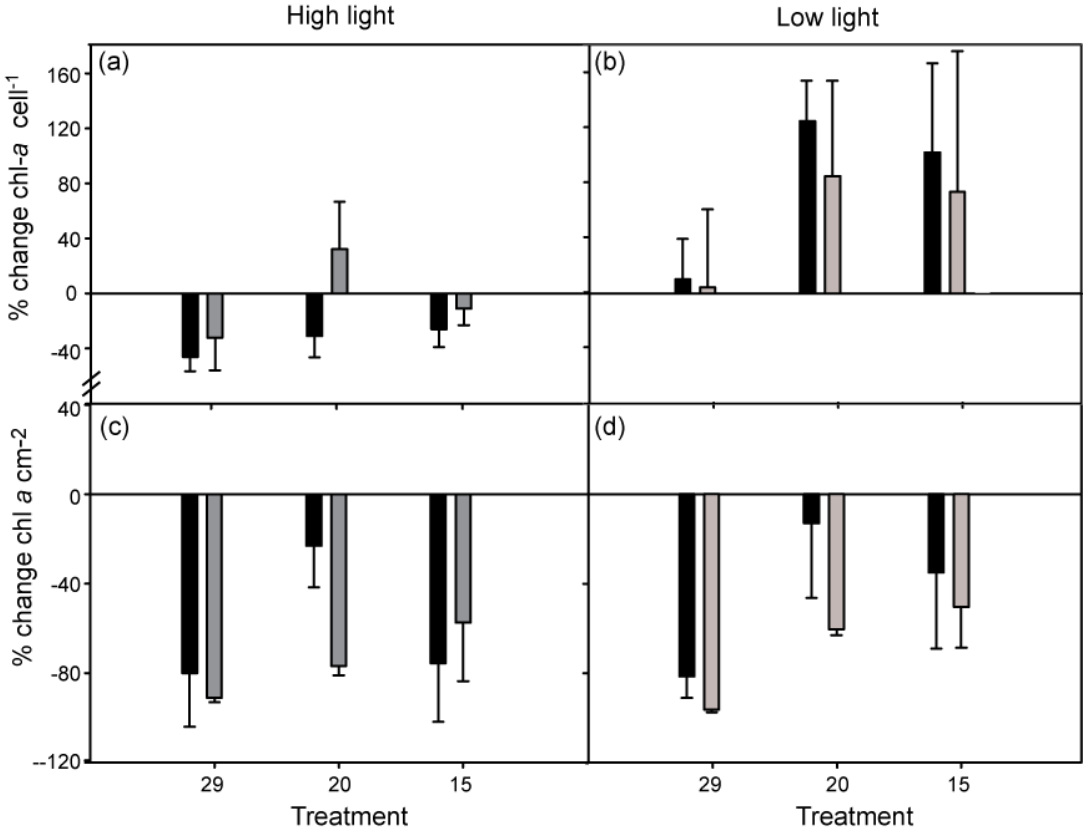


Figure 5.8 Relative change (%) in chl-*a* of *Pocillopora damicornis* nubbins after 72 h of exposure to elevated or lowered temperatures (29°C, 20°C and 15°C) at (a, c) high light and (b, d) low light. (a, b) Chlorophyll concentration per symbiont (pg cell^{-1}). (c, d) Chlorophyll concentration per unit surface area (pg cm^{-2}). Nubbins from the lagoon shown as (black bars, and nubbins from Sylphs shown as grey bars. Mean ± SE (n = 4).

Table 5.3 *p* values of one-way ANOVA which tested for changes in symbiont density, and chlorophyll *a* concentration in *Pocillopora damicornis* nubbins over a 72 h experiment for three temperature treatments (29°C, 20°C and 15°C) at two light levels (high and low), from two locations.

	Percent change		
Factor	Symbiont density (cm^{-2} x 10^6)	Chl *a* zx^{-1} (pg)	Chl *a* ($\mu g\ cm^{-2}$)
Temp	<0.001	0.225	<0.01
Light	0.274	<0.05	0.335
Site	0.774	0.984	0.124
Temp x light	0.419	0.759	0.718
Temp x site	<0.01	0.909	0.186
Light x site	0.211	0.295	0.223
Temp x light x site	0.225	0.674	0.602
	29 > 15, 20		29 > 15, 20

5.4 DISCUSSION

The results of this study demonstrate a greater tolerance of the dinoflagellate symbionts of *P. damicornis* at LHI to a 3°C temperature decrease than a 3°C temperature increase from the annual temperature range (18 - 26°C). Both temperature extremes were 1°C clear of their thermal thresholds. An additive effect of light and cold temperature stress was demonstrated, with high light and high temperature having the greatest impact on PSII photochemical efficiency, maximum excitation pressure, symbiont density and chl-*a* concentration per cell. The results suggest that the presence of novel types of clade C *Symbiodinium* at LHI may be due to photo-physiological differences between the LHI symbionts and those found at lower latitudes, such as clade C1 in *P. damicornis* on the GBR. Essentially, this study suggests that the types of *Symbiodinium* at LHI may be specialised for cool and variable temperatures, so contributing to the success of corals at this marginal location

5.4.1 Photo-physiology of Lord Howe Island *Symbiodinium*

P. damicornis at LHI has been observed to host three novel symbiont types, C41, C100 and C118, with the local scale distribution of these three types relating to environmental conditions (Chapter 3). In terms of the physiological parameters of *P. damicornis* from LHI, symbiont density was 6 times that of *P. damicornis* from the southern GBR (23.3° S, 151.5° E, Jones & Hoegh-Guldberg 2001, Ulstrup et al. 2006, 2008), when both were sampled during the winter-spring. The high density of symbionts in *P. damicornis* colonies at LHI is probably linked to decreased irradiance at this high-latitude site, which is similarly known to cause increased symbiont density when linked with depth (Falkowski & Dubinsky 1981, Stimson 1997). Further, the high density corroborates the findings of Ulstrup et al. (2006) of an increase in symbiont density with increasing latitude (and thus decreasing irradiance) across the range of the GBR (14.4°S – 23.3°S).

On a local scale, symbiont density was nearly 50% higher in corals sampled from the lagoon, compared to corals from Sylphs Hole. The latter site is characterised by high turbidity and low irradiance (2.4 FTU at Sylphs Hole compared to 1.7 FTU in the lagoon, Chapter 3), and thus the lower density at Sylphs Hole is unexpected, as reduced irradiance usually equates to increased symbiont density (Falkowski & Dubinsky 1981, Stimson 1997). However, the low symbiont density in *P. damicornis* at Sylphs Hole may be attributable to the increase in nutrients at this site, whereby the corals may be making greater use of the heterotrophic carbon from particulate matter (Grotolli et al. 2006), and thus have less need for a high density of symbionts. A freshwater spring has been reported at Sylphs Hole (LHI MPA Manager, *pers. comm.*), but further study is needed to confirm these observations of high nutrient loads. Interestingly, chl-*a* concentration per cell was

greater in Sylphs Hole than the lagoon, which may compensate for the lower symbiont density, allowing photosynthetic output to remain high despite low cell densities (Falkowski & Owens 1980, Kinzie et al. 1984).

5.4.2 Photochemical responses to low temperature

Symbiotic dinoflagellates in corals at LHI are seasonally exposed to extremes of temperature, with annual averages ranging from 18 - 26°C (Chapter 3). Cold temperature stress in the present study caused a decline in effective photosynthetic yield, particularly evident in the first 32 h of the experiment. It is well documented that decreased temperatures intensify photoinhibition in higher plants (Smilie et al. 1988, Aro et al. 1990, Greer 1990) due to a reduction in the rate at which the quenching of PSII occurs (Krause 1992). However, few studies have documented the effect of cold temperature stress on corals (Saxby et al. 2003, Hoegh-Guldberg et al. 2005), despite knowledge of increased temperatures causing a breakdown of the symbiotic relationship (Lesser 1996, Brown 1997a, Thornhill et al. 2006b).

Early evidence showed that, while some corals are adapted for cooler conditions (Roberts et al. 1982), others are killed by severe declines in temperature (Saxby et al. 2003, Hoegh-Guldberg et al. 2005). Cold temperature stress causing light-dependent photoinhibition of *Symbiodinium* cells has been documented in the coral *Montipora digitata,* following short-term cold exposure (Saxby et al. 2003). Following exposure to 12°C and 14°C, in symbionts usually subject to temperatures from 23 - 26°C, irreversible photodamage was reported, evident in chronically-depressed photochemical efficiency several hours after temperatures were returned to their original state. These findings parallel

the present study, with photochemical efficiency depressed following cold temperature exposure, however the LHI symbionts were still able to photosynthesize at 63% of original capacity after 72 h in low light, suggesting a degree of tolerance to cold stress. Symbiont-type specific responses to cold stress have been observed in cultured *Symbiodinium* cells, with clade B2 from the temperate western Atlantic found to be physiologically cold tolerant and able to rapidly recover from prolonged exposure to low temperatures (10°C), unlike *Symbiodinium* types from tropical environments (A3, B1 and C2, Thornhill et al. 2008). The cold-tolerance of B2 was suggested to contribute to its distribution in temperate habitats (Thornhill et al. 2008); a pattern which may be emulated by the symbionts present at LHI. Although no attempt was made to assess the potential for recovery in the present study, the reduced impact of cold temperature after 32 h suggests that the symbionts can tolerate cold conditions, and thus may explain why these novel types are present in corals at LHI.

While the effect of cold stress on the symbiotic relationship in corals has received some attention, little is known of the impact on the photosynthetic apparatus of *Symbiodinium* cells. The present study used a plant efficiency analyzer (PEA) to determine the impact of temperature stress on the condition of PSII and the redox state of the primary (Q_A) and secondary (Q_B) electron acceptors in symbionts; a method previously used to show the site of impact of high temperature stress on symbionts (Hill et al. 2004a, Ulstrup et al. 2008; Hill and Ralph 2006). Our results suggest that cold temperatures impact a similar site to that impacted by warm temperatures, with a decrease in the J step along fast induction curves (FICs) indicating a decline in the rate of Q_A reduction and the reduced capacity for Q_A to transport electrons through the electron transport chain to Q_B, due to

photoinhibition (Chylla & Whitmarsh 1989, 1990, Hill & Ralph 2006). A primary cause for this response could be an increase in the proportion of inactive PSII centres, whereby Q_B cannot oxidize Q_A^-, and as a consequence the photochemical capacity of PSII declines (Hill & Ralph 2006). The cold-induced reduction in photochemical efficiency, specifically the capacity to transport electrons, may also be linked to the impact of cold temperatures on rates of reactions, via the effect on the kinetic energy of enzymes and substrates (Hoegh-Guldberg & Pearse 1995, Saxby et al. 2003), including those of the Calvin-Benson cycle (Nobel 1991, Saxby 2003). Finally, Takahashi et al. (2009) reported that acceleration of photoinhibition by moderate heat stress is attributed primarily to inhibition of the repair of photodamaged PSII, and that the photoinhibition sensitivity of *Symbiodinium* to heat stress is determined by the thermal sensitivity of the PSII repair processes. The same mechanisms may play a part in cold temperature stress; however further study is required to determine if this is the case.

While the responses to cold temperature stress reported here are similar to those reported for increased thermal stress, both in the present study and in multiple comparable studies (Iglesias-Prieto et al. 1992, Warner et al. 1996, Warner & Fitt 1999, Robison & Warner 2006), the effect of a 3°C temperature decrease from annual minima on the photochemical efficiency, maximum excitation pressure (Q_m) and the FIC steps of symbionts appears to be less than a 3°C increase in temperature from annual maxima. Additional evidence for limited cold stress is seen in the higher symbiont density and chlorophyll concentration per cell in the cold treatment compared to the warm one. Cold shock (3 - 11°C below ambient) has been shown to trigger the loss of dinoflagellate symbionts from some sea anemones and corals (Shinn 1966, Coles & Jokiel 1977, Steen &

Muscatine 1987, Saxby et al. 2003, Hoegh-Guldberg et al. 2005), particularly observed at lower latitudes (Shinn 1966, Coles & Jokiel 1977, Hoegh-Guldberg et al. 2005) where symbionts would have been less adapted to cooler and variable conditions than those present at LHI. For example, Hoegh-Guldberg et al. (2005) reported winter bleaching in corals at Heron Island (23°26'S), when corals were subject to a 2-day period of 14 - 15°C, when annual average temperatures usually range from 17 - 25°C. Based on the findings of the present study, it is clear that photosynthetic damage can ensue from cold temperature stress, however the continued photosynthetic activity of symbionts at LHI suggests they may be adapted to these cool and variable conditions.

5.4.3 Additive effect of low temperature and high irradiance

Most bleaching observed in the field is caused by elevated temperatures combined with solar radiation, with excessive light causing the impairment of PSII by a loss of functional PSII centres (Fitt & Cook 2001, Lesser & Farrell 2004). The present study found an additive effect of both increased and lowered temperatures with heightened irradiance, causing a decline in photochemical efficiency, and increased maximum excitation pressure over PSII (Q_m). An additive effect of low temperature and high irradiance was demonstrated by Saxby et al. (2003), whereby exposure of corals to 14°C in low light had little effect on photochemical efficiency while corals exposed to 14°C and full sunlight were heavily impacted. Variation in the photochemical responses to light intensities and temperatures are also evident from the FICs in the present study. A greater decline in the J step is evident in high light treatments compared to low light, which equates to a decrease in the rate in Q_A reduction, and an increase in non-photochemical quenching (Hill & Ralph

2005). Diurnal patterns in the FIC in the present study are similar to those observed by Hill and Ralph (2005), however the daily recovery reported in that study was not observed in the high and low temperature treatments in the present study, particular in high light, suggesting that photodamage has occurred (Demmig-Adams et al. 1989). The lack of a morning rise in the P step in all the high-light treatments, irrespective of temperature, provides further evidence of permanent damage to PSII; in optimal conditions nighttime reduction in the PQ pool occurs and is followed by a morning rise in the P step when there is a greater capacity for the electron transport chain to oxidize the PQ pool (Hill & Ralph 2008). Although corals in the experiment were at a similar water depth to that they would experience at low tide, the high light treatments used may have been too intense, and thus may have confounded results. However, the additive effect of light and temperature stress was consistant among temperatures, and therefore the results remain valid.

5.4.4 Ecological implications

While many high latitude corals can withstand seasonal variations in sea temperature of more than 12°C (Kleypas et al. 1999), small excursions above and below geographically associated maxima or thresholds lead to stress in both tropical and high latitude corals (Hoegh-Guldberg 1999, Hoegh-Guldberg & Fine 2004, Hoegh-Guldberg et al. 2005). The flexibility of *P. damicornis* to associate multiple symbiont types as opposed to the previously reported fidelity to C1 in the Indo-Pacific, and its ability to cope with low temperatures by hosting apparently thermally-resilient symbionts, helps to explain the success of this coral at the marginal location of LHI. However, the detrimental effect of a 3°C increase above the summer maximum suggests that *P. damicornis* may be on the

boundary limits of its survival, and that future warming may lead to coral bleaching events. Further, Ulstrup et al. (2006) found *P. damicornis* hosting C1 on the GBR to be highly sensitive to elevated temperatures (< 2°C), and therefore, even if *P. damicornis* colonies at LHI did associate with C1 (present in other host species), this may not confer a greater thermal tolerance. As *P. damicornis* is a dominant species at Lord Howe Island (Harriott et al. 1995), its loss would have a considerable effect on the community. Questions remain as to whether these novel high latitude coral-*Symbiodinium* associations will have the ability to acclimate to rapidly increasing temperatures, despite their ability to cope with cooler temperatures. Furthermore, despite predictions that high latitude reefs could act as refuges in a future of warming seas (Glynn 1996), the proximity of these high latitude corals to their upper thermal threshold suggests this is unlikely, unless they prove to have the ability to rapidly adjust their thresholds.

Chapter 6

General Discussion

Collectively, the chapters in this thesis examine the persistence of corals in marginal environments, and highlight the roles that the symbiotic dinoflagellates and the environment play in shaping these communities. Contrary to the expectation that symbiont diversity would be low at high latitude sites because of the challenging conditions faced, major findings include high diversity and reduced host specificity of symbionts in corals at the high latitude site of Lord Howe Island (LHI), Australia. Physiological assessment of novel LHI symbionts (*Symbiodinium* spp.) led to the suggestion that they may be specialised for cooler and more variable temperatures, so contributing to the success of corals at this marginal location. In contrast, a low diversity of generalist symbionts was uncovered at the equatorial site of Palmyra Atoll.

Local environmental conditions, primarily sediment regimes, were found to affect the distribution of coral species at Palmyra Atoll, with no discernible effect on symbiont distribution (in terms of the holobiont). On a wider scale, local environment conditions (specifically wave exposure) were shown to influence coral community structure at the Kermadec Islands (KI), New Zealand, as opposed to the low and variable temperatures associated with high latitude sites. By assessing the diversity and physiological tolerances of symbionts in marginal environments, and their associated control of host distribution, this study was able to gain a better understanding of how corals survive in marginal

environments, which will provide greater insight into how corals may respond to climate change.

Three major questions arose from the results of this study, and these will be considered in detail here. First, how and why do symbiont communities differ at marginal sites compared to 'optimal' sites? Second, why is there such a difference in the diversity of corals and symbionts, and the degree of host-specificity of symbiont communities between marginal sites? The potential causes of this variability will be discussed. Finally, the susceptibility of these marginal reefs to future climate change will be assessed, and their potential to act as refuges for corals in a future of warming seas.

6.1 Marginal *vs.* optimal sites

Marginal reefs or communities are defined as those that occur where conditions are close to the environmental thresholds for coral survival (Kleypas et al. 1999, Harriott & Banks, 2002). These thresholds include high or low temperatures, salinities or nutrient levels, or low light penetration or aragonite saturation state (Kleypas et al 1999). Marginal communities also occur within the tropical belt where environmental conditions are suboptimal for reef development, such as in areas influenced by upwelling, heavy runoff, or river deltas (Perry & Larcombe 2003). It is traditionally viewed that optimal environmental conditions lead to high coral diversity (Guinotte et al. 2003), evident in the decline of coral diversity and cover with increasing latitude and the associated decline in temperature (Harriott & Banks 2002). Assessing differences in coral community structure between optimal and marginal sites in the present study, clear differences exist, with reduced

diversity and cover at high latitude LHI and marginal regions of Palmyra Atoll, compared to optimal sites/regions.

However, of interest to the present study is the contrasting high diversity of symbionts at high latitudes (19 types in 13 genera at LHI, and 10 types in 9 genera at the KI) to those on the optimal sites such as the Great Barrier Reef (GBR, see Chapter 3, LaJeunesse et al. 2003, LaJeunesse et al. 2004a). The high symbiont diversities at high latitudes may be (1) a potential adaptation of the host to the seasonally variable conditions of high latitude sites, and (2) linked to the isolation of the high latitude sites.

High latitude sites exhibit wide ranges in their annual temperatures, for example Lord Howe Island annually varies between 18 – 26°C (chapter 3), whereas areas of the Central GBR only fluctuate approximately 2°C annually (Lough & Barnes 2000). To persist under these variable conditions, hosts at LHI and the KI may have evolved the ability to harbour a range of symbiont types, with varying physiologies to cope with temperature fluctuations. For example, *Acropora cuneata* hosted 5 different symbiont types at LHI, but across the range of the GBR (15°) the greatest number of types found in an *Acropora* sp. is only four (LaJeunesse et al. 2004a). The local scale niche diversification of symbionts at LHI contributes to the high diversity of symbionts, and has been observed in many other cnidarian hosts at multiple locations (Rowan & Knowlton 1995, Rowan et al 1997, Baker 2001, Iglesias-Prieto et al 2004, Sampayo et al. 2007, Venn et al 2008b). By hosting multiple types, varying with local environmental conditions, these corals persist and indeed thrive in these 'stressful' marginal environments, with potentially little cost to the host. Further information is required on the physiological diversity of these novel symbionts, in order to assess the benefits derived from hosting them.

The presence of multiple novel types at LHI and the KI may also be attributable to their biogeographic isolation. LHI is extremely isolated, located over 1000 km from the GBR, which is proposed as its the source of larvae (Wolanski 1994). Long distance dispersal to LHI has been found to be rare and populations are genetically isolated (Ayre & Hughes 2004), with a lack of migratory 'stepping-stones' acting as a barrier to genetic exchange between symbionts. It is suggested that isolation can lead to the co-evolution of regionally distinctive host–symbiont combinations (LaJeunesse 2005), such as the multiple regionally endemic types of *Symbiodinium* at LHI. Comparatively, the GBR exhibits high gene flow between reefs (Ayre & Hughes 2004), thus negating the need or capacity for the evolution of endemic types. Interestingly, LHI and the KI do share some novel, potentially high-latitude specific symbiont types, despite being located 2,200 km apart. Furthermore, symbiont type C3w is present in hosts at LHI, the KI and Thailand (LaJeunesse, in prep). Separate evolutionary events may have occurred in these symbiont types, however further examination of the evolutionary events leading to *Symbiodinium* diversification is required to fully understand current distribution patterns.

Of note, some divergent symbionts were present in horizontal strategists at LHI, a scenario that is rare and has only been reported in *Fungia scutaria* that contains C1f in Hawaii (Weis et al. 2001, Rodriguez-Lanetty et al. 2004). The presence of novel symbionts that have not co-evolved in vertically transmitting species may be attributable to their evolution under long-term isolation in stressful environmental conditions, whereby a particular symbiont is selected over a generalist type from the external environment.

6.2 Marginality – a variable term

Considerable variability existed in the community structure of the three marginal communities examined in the present study, both in terms of corals and symbionts. LHI and the KI both exist on the thresholds for coral growth, with low mean annual and seasonal temperatures, and a low aragonite saturation state (Buddemeier 1997; Kleypas et al. 1999). Yet, when assessing the variability in coral community structure among these sites and other high latitude sites, distinct differences were established based on local scale conditions (i.e. wave exposure), rather than latitude. Furthermore, the gradient of marginality at Palmyra Atoll, associated with the variation in local scale sediment regime, led to variation in the coral community structure, with reduced diversity and cover in highly sedimented areas, and thus marginal sites. The considerable variability in coral diversities and distributions among marginal sites (whether marginal due to extremes of temperatures, or any other wide scale variable) suggest that local scale factors should also be considered when assessing the parameters controlling the structure of the communities.

Looking more closely at symbiont diversity, and distributions among and within marginal sites, scale becomes an important factor in assessing the degree of marginality. The scale of marginality at LHI and the KI would be deemed large-scale, whereas Palmyra is subject to local scale marginality, and a vast difference in symbiont diversity is seen between the two sites. Within the localised, marginal, regions of Palmyra, and across the atoll as a whole, symbiont diversity was low and unaffected by the extent of sedimentation. Conversely, LHI, the epitome of marginality due to its high latitude location, exhibits a high diversity of symbionts, despite the variable and extreme conditions experienced, and

perhaps as a factor of this variability. At Palmyra Atoll, the lack of correlation between symbiont distribution and potential environmental stressors suggests that the symbionts present are robust enough to cope with the current environmental regime.

It is clear from the vast sub-cladal diversity discovered in the present study, that sub-cladal distinctions of *Symbiodinium* need to be the standard in studies of distributions and physiology of symbionts, as physiological differences at a sub-cladal level have now been established (Tchernov et al. 2004, Sampayo et al. 2007, Sampayo et al. 2008). Further, it is estimated that 25% of scleractinian coral species can host more than one clade of *Symbiodinium* (Goulet 2007), but this may greatly underestimate the flexibility of the host, because the relevant question is: how many species can host more than one sub-clade (type)? A greater understanding of the mechanisms underlying these differences is required, assessing the full genetic and physiological diversity within the genus *Symbiodinium,* particularly in marginal environments.

6.3 Future of 'marginal' reefs and communities

Predictions on the rate of global change at their most conservative expect a rise of 2°C by 2050 – 2010, in addition to atmospheric carbon increasing to twice pre-industrial measures (Hoegh-Guldberg et al. 2007). It has been proposed that marginal reefs/communities, predisposed to broad environmental variability and/or on the boundary limits of survival, may in the future act as refuges in an environment modified by global climate change (Glynn 1996, Riegl & Piller 2003). Furthermore, it is suggested that reefs under 'optimal' conditions may become more marginal through shifts in environmental variables (Kleypas et al. 1999, Guinotte et al. 2003, Perry & Larcombe 2003). However, a wide range of

environmental and physiological requirements need to be satisfied in order for these communities to act as refuges (Fig. 6.1). Additionally, the fact that the rate of change from 'optimal' to 'marginal' conditions is likely to exceed the rate of acclimation/adaptation (Hoegh-Guldberg et al. 2007, Edmunds & Gates 2008) causes further concern for the future of coral reefs.

A key point when assessing the appropriateness of high latitude sites as refuges is the dissimilarity of the coral species compositions at high latitude sites compared to 'optimal' sites, and the cause(s) of these dissimilarities. Many important coral species on tropical reefs are absent or rare at high latitudes, for example massive *Porites* and branching *Acropora* spp. (Veron & Done 1979). Their dearth at high latitudes is correlated with the absence of reef accretion capacity caused by the reduced temperatures (Glynn & Stewart 1973, Crossland 1988). If the water temperatures increase, as they are predicted to, perhaps accretion would occur at the high latitude sites which at present lack a reef framework, such as the KI, however many factors argue against the establishment of tropical species in subtropical locations. For example, for low latitude coral species to successfully colonise at high latitudes, they must first get there. However, many high latitude islands are isolated, with rare larval input as a result of most corals having short dispersal distances (Hughes et al. 2002, Ayre & Hughes 2004). Furthermore, vast variability in the physical setting of these sites exists. For instance, the KI are exposed to high wave energy, which deems them challenging for settlement and growth. Most reef-building species are absent there, due to the low temperatures, the lack of larval supply, and the difficulty associated with growth and establishment in high wave-energy environments (Menge & Olsen 1990, Harriott & Banks 2002). Add to this the impacts of thermal stress

which reduce reproductive output and gamete production (Szmant & Gassman 1990, Lasker & Coffroth 1999, Baird & Marshall 2002), and the associated decrease in carbonate ion concentrations at higher latitudes as atmospheric carbon dioxide levels spiral upwards (Caldeira & Wickett 2003, Hoegh-Guldberg 2005, Hoegh-Guldberg et al. 2007), and the chance that these remote high-latitude sites will act as refuges becomes highly unlikely. The complexities of these potential refuges would need to be assessed on a site-by-site basis, to allow such predictions to be made.

In recent times, many studies have concentrated on the ability of corals to acclimate or adapt to changes in their environment by varying the identities and/or composition of their algal symbionts (Buddemeier & Fautin 1993, Fautin & Buddemeier 2004, Berkelmans & van Oppen 2006). Physiological distinctiveness of community symbiont types (Tchernov et al. 2004, Frade et al. 2008a, Takahashi et al. 2009), and adaptive shifts in symbiont communities have been demonstrated in a number of studies, both under experimental conditions (Berkelmans & van Oppen 2006, Sampayo et al. 2008) and *in situ* (Suwa et al. 2008, Venn et al. 2008b). Corals present at marginal locations, such as LHI, may be predisposed to broad environmental variability, perhaps through their flexibility to associate with a range of thermally-tolerant symbiont types. However, these types probably evolved over long periods, with slowly changing environmental conditions. The likelihood of these symbionts being available at optimal sites as they become more marginal is low; future rises in temperature are, at their most conservative, 2°C by 2050, much greater than the absolute change at any one location over the last 20 yr of < 0.5°C (Hoegh-Guldberg 1999, IPCC 2007). Thus, as the climate rapidly changes, natural selection of corals and

their symbionts is likely to occur, leading to changes in the composition of reefs, and favouring tolerant holobionts over stress-susceptible ones.

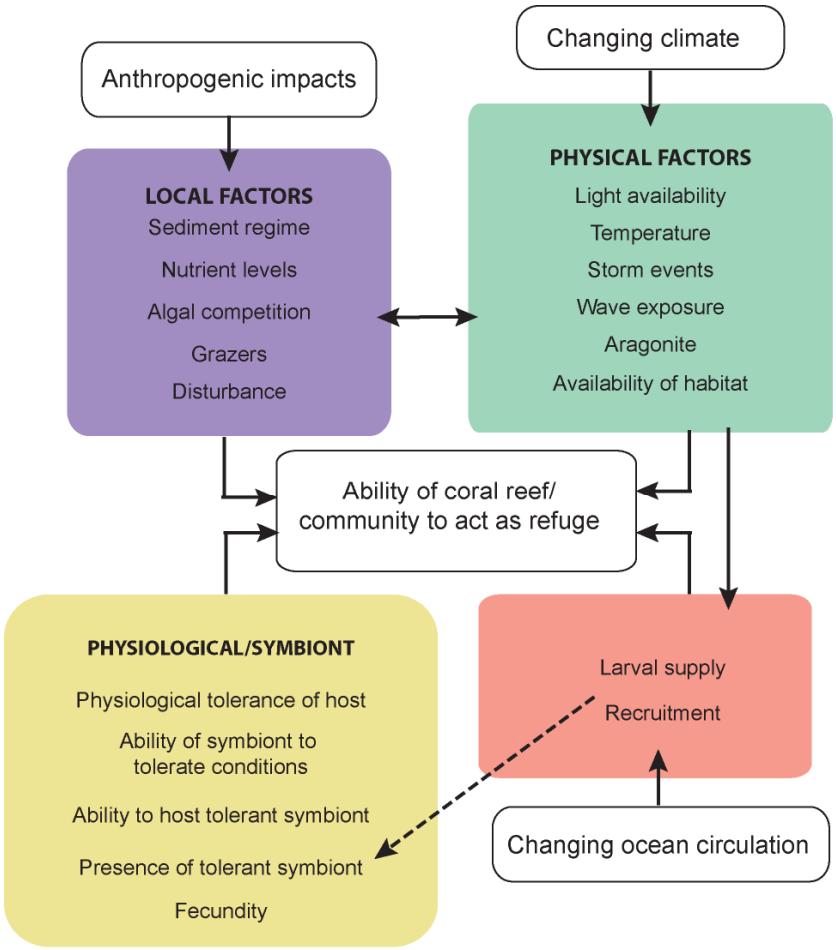


Figure 6.1 Environmental and physiological factors and processes that will influence the ability of a marginal reef/community to act as a refuge. Factors are grouped on the basis of similarity. Potential impacts on these processes are indicated in the outer white boxes. Arrows between boxes indicate where factors/processes interact. Dotted line indicates a single factor is affected.

Crucially, at sites that are already marginal, tolerant symbionts may be favoured in the future as conditions change, but only corals that have the ability to associate with thermally-tolerant symbiont types will theoretically be able to survive. Additionally, corals that can harbour tolerant symbionts may do so as a trade-off to other attributes that favour them less in the future, such as increased susceptibility to disease (Stat et al. 2008, Correa et al. 2009), and low growth rates (Little et al. 2004). Furthermore, contrary to the original definition of the 'adaptive bleaching hypothesis' (Buddemeier & Fautin 1993), the flexibility of a coral to associate with multiple *Symbiodinium* types does not mean that it has the ability to switch its symbionts for completely new ones. Indeed, long-term fidelity to certain symbiont types has been demonstrated in colonies of various species of coral analyzed over many years (Goulet & Coffroth 2003, LaJeunesse et al. 2004a, Thornhill et al. 2006a, Thornhill et al. 2006b), and it is suggested that permanent changes in the symbiont composition are more likely to occur over generations than within the life-cycle of an individual host (Thornhill et al. 2006b, Baird et al. 2007, Sampayo et al. 2008). Although the flexibility of corals to harbour multiple symbiont types is important for their future, physiological differences in these symbionts should be assessed, as should the long-term effect on the host of harbouring these different symbiont types.

Assessing the susceptibility of the sites studied here to future abiotic changes, tentative predictions can be made. Palmyra Atoll may be most at risk. Potentially if the clade D symbionts already present in some hosts at this site are 'stress-tolerant' clade D symbionts (Baker et al. 2004, Mostafavi et al. 2007), and if conditions change to the extent that current symbionts cannot cope, only hosts able to harbour either this 'tolerant' clade D or other tolerant types will survive. At both LHI and the KI, the considerable symbiont diversity and

the flexibility of many hosts to associate with multiple, potentially physiologically distinct, symbiont types may aid these communities in their survival. Further, physiological testing indicated an ability for the symbionts to tolerate a range of conditions, and their presence in thermally-variable environments stands them in good stead for dealing with future environmental changes. However, predictions like this will depend on the rate of change of the global conditions, as well as the physiological tolerance range of both symbiont types and coral species.

In conclusion, much remains to be learnt of the ability of corals and their dinoflagellate symbionts to adapt to changing environmental conditions, and the role that marginal communities may play as refuges. Questions remain as to whether the symbionts present in marginal environments are indeed physiologically distinct, the physiological cost of harbouring tolerant symbionts on the host, and most importantly, whether corals and their symbionts do indeed have the capacity to adapt or acclimate to new environmental conditions, and whether these changes can occur quickly enough to prevent the future degradation of coral reefs and communities on a global scale.

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Appendices

Appendix 1

Kermadec Islands coral community analysis

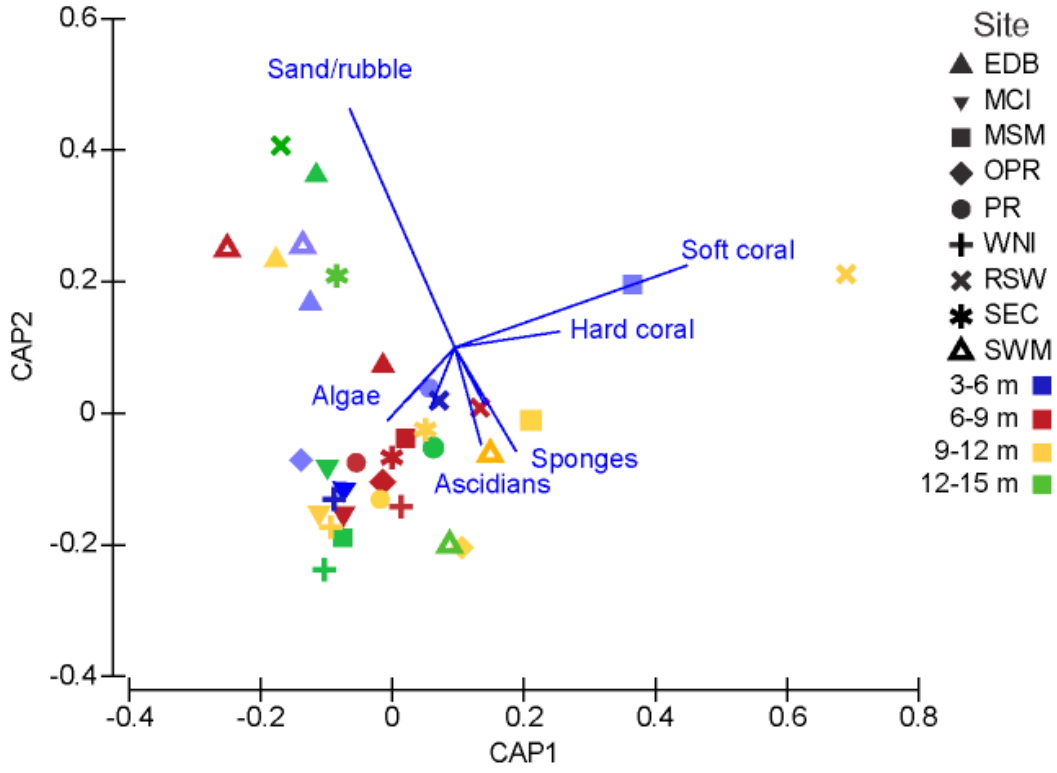

Figure A1.1 Canonical analysis of principal components examining differences in benthic community cover at 9 sites at the Kermadec Islands. Shapes represent sites, colours represent depths. Benthic groups driving the separation of sites shown as vectors. Similarities calculated using dispersion weighted zero-adjusted Bray-Curtis resemblance measure.

Appendix 2

Physiological capacity of *Symbiodinium* to facilitate survival of scleractinian corals at environmentally marginal sites

A2.1 INTRODUCTION

Reef-building corals contain symbiotic, unicellular dinoflagellate microalgae of the genus *Symbiodinium*, which play a significant role in host nourishment and physiology. As a reflection of the high genetic diversity of the genus (8 clades and numerous sub-clades), considerable physiologically diversity of *Symbiodinium* has been observed at both a cladal and subcladal level, in terms of tolerance to irradiance and/or temperature (Rowan & Knowlton 1995, Baker & Rowan 1997, Iglesias-Prieto & Trench 1997b, Rowan et al. 1997, Baker et al. 2004, Rowan 2004). In many cases, external environmental conditions promote certain pairings between partners, including combinations with regard to depth, irradiance or temperature gradients, latitude and longitude, and host ontogeny (Rowan et al. 1997, LaJeunesse et al. 2003, Coffroth & Santos 2005, Frade et al. 2008b). The physiological variation in *Symbiodinium* types has been suggested as a basis for coral tolerance of bleaching stressors, including elevated temperature. Specifically, corals are proposed to enhance their bleaching resistance by associating with thermally tolerant *Symbiodinium* genotypes, either by uptake from the external environment or by increasing their population of cryptic symbionts (Buddemeier & Fautin 1993, Fautin & Buddemeier 2004). The

capacity of corals to shift or "shuffle" symbiont populations towards different host-symbiont combinations has been observed in response to coral bleaching (Baker et al. 2004), transplantation to a different light habitat (Baker 2001, Toller et al. 2001b), living in marginal habitats (Toller et al. 2001b,Venn et al. 2008b), or variability within a species on regional scales (van Oppen et al. 2001). It has been suggested that flexibility in host-symbiont partnerships and physiological responses to changing environmental conditions are likely to be most evident at high latitudes, where the coral-symbiont partnerships are subject to far more variable environmental conditions than at tropical locations. Considering the more conservative temperature increases of 1 - 2°C by 2030 (Hoegh-Guldberg 1999), understanding the potential for adaptive responses of *Symbiodinium* to changing environmental conditions, and thus to future climate change is of critical importance. Thus, the aim of the present study was to assess the capacity for shuffling of corals living in a marginal environment. Specifically, we aimed to address the following questions:

(1) Do corals at high latitude host symbionts specifically adapted to their extreme and variable environment?

(2) Are corals from high latitudes specifically adapted to modify their dominantly expressed symbiont type (achieved by either external acquisition or from internal shuffling)?

(3) Following transplantation can the holobiont survive in the long term and maintaining growth rates equivalent to the holobiont optimal for that specific environment?

(4) Are there any changes in symbiont density or photosynthetic performance after transplantation into the new environment in comparison to the control individuals?

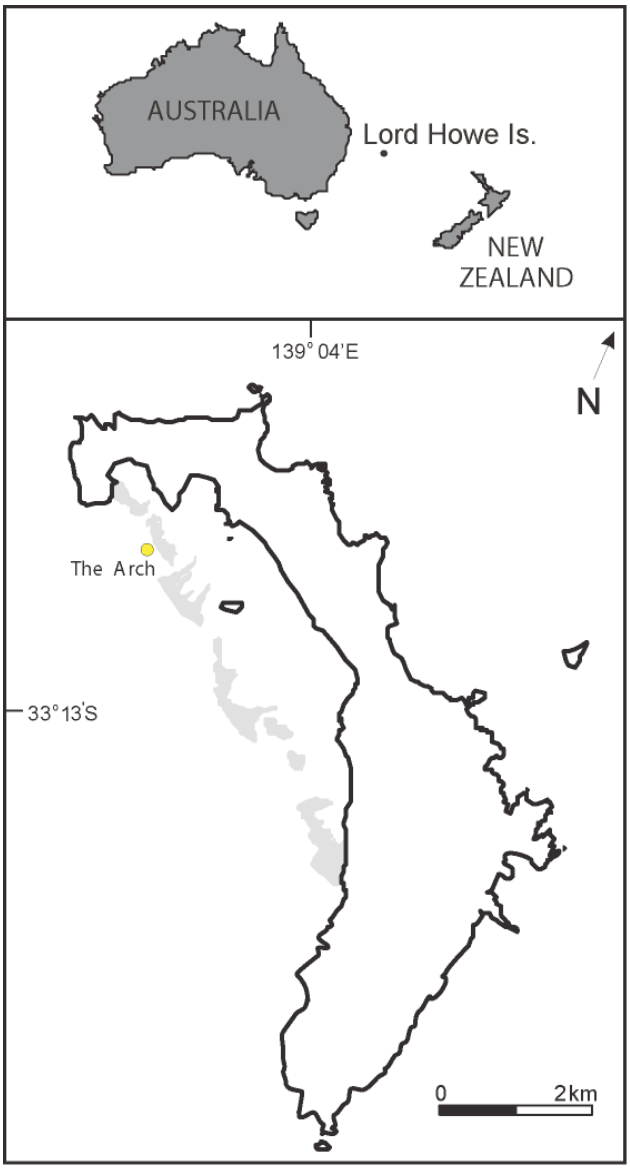

Figure A2.1 Location of Lord Howe Island, Australia, and backreef site, The Arch, at which coral transplants were conducted.

A2.2 METHODS

A2.2.1 Study site and experimental setup

Situated over 1000 km from the southern Great Barrier Reef (GBR), Lord Howe Island (LHI; 31"33'S, 159"05' E, Fig. A2.1) is home to the southernmost coral reef in the world. Coral reefs occur at Lord Howe Island due to the influence of the warm-water East Australian Current (EAC). The boundary between the EAC and the southern temperate Tasman Current varies from 30°S in winter to 34°S in summer and runs close to LHI (Martinez 1994). The EAC maintains relatively high mean annual sea-surface temperatures at the island of between 18°C and 26°C (Veron & Done 1979, Chapter 4), the average low temperature similar to that of the southern GBR and subtropical coastal coral communities of south-eastern Australia. Considering its geographical isolation, latitude and small size (11 km by 2.5 km), LHI sustains surprisingly high coral species diversity with 83 hermatypic coral species (from 33 genera) and coral cover comparable to tropical sites.

Samples of two common corals at Lord Howe Island, *Acropora cuneata* (15 colonies) and *Pocillopora damicornis* (10 - 15 colonies), were collected at two depths: 5 - 7 m and 12 - 15 m at The Arch, on the western backreef of the island (Fig. A2.1). Two nubbins (4 - 5 cm diameter of base) were removed from each individual colony, and placed into seedling trays and fixed in place with fast-drying underwater cement (Mapei Granirapid), and left cure in aquaria overnight. During this period the nubbins were stained with Alizarin red (100 mg l^{-1}) to allow for determination of growth over the transplant period. The seedling trays containing the coral fragments were fixed securely

underwater to the existing reef using cable ties, and placed at both collection depths (Fig. A2.2). Following deployment, photographs were taken of each tray in situ to allow additional determination of growth rates at the end of the experimental period. The experimental setup entailed half of the fragments (8 fragments) from each colony being placed in seedling trays and returned to their collection depth (i.e. controls), and the other eight fragments were placed in seedlings trays and returned to the opposite depth (i.e. treatment - shallow to deep, or deep to shallow). The *A. cuneata* transplants were set up in August 2007, and the *P. damicornis* transplants in January 2008, both to be retrieved in September 2008.

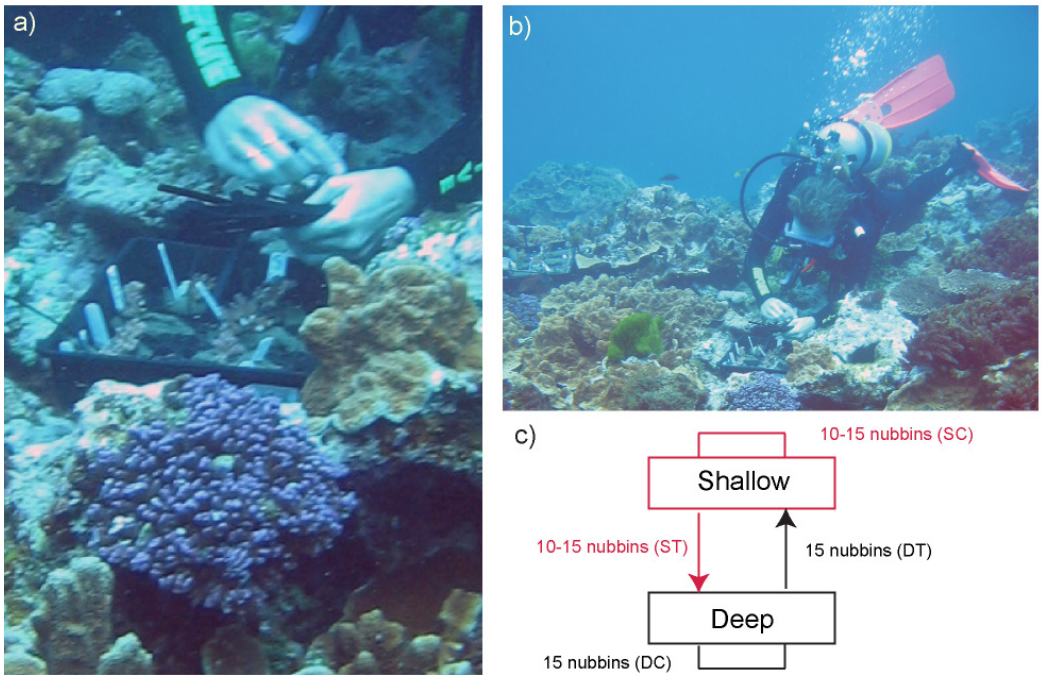

Figure A2.2 Overview of the transplantation experiment, showing setup (a, b) and sampling design (c). SC = shallow controls, DC = deep controls, DT = nubbins transplanted from the deep to the shallow, ST = nubbins transplanted from the shallow to the deep.

A2.2.2 Environmental parameters

During the experimentation period, in situ data loggers (ONSET Hobo loggers) were attached securely to the seedling trays at each depth to record water temperature and irradiance at each depth at hourly intervals. The mean, maximum and minimum daily temperatures were to be calculated for each depth as well as the difference between the minimum and maximum temperature at each depth. Paired t-tests between sites were to be done on the raw data (daily average) to determine if the two depths experienced significantly different average daily temperatures. Data from the light loggers was only to be used for the first 5 days after deployment to exclude the effect of fouling on the light sensors.

A2.2.3 Genetic identification and physiological characteristics of *Symbiodinium*

Samples were collected at the beginning of the experiment for identification of the original *Symbiodinium* type harboured by the colonies. The fragments were processed the following day according to Loh et al. (2001), and preserved using 20 % DMSO solution. Symbiont identity was established using ITS2-DGGE, following protocols outlined in Chapter 3. Effective quantum yield of the colony (ΔF/Fm', see Chapter 5 for details) was recorded using a Diving PAM fluorometer (Walz, Germany) at the beginning of the experiment, prior to sample collection. The yield was recorded at three different locations on the same coral fragment approximately 1 cm below the tip of the branch. Due to the difficulties of working at such a remote location, dark adapted maximum quantum yield could not be recorded, but effective quantum yield was deemed suffice for comparing photosynthetic

efficiency between depths.

A2.3 RESULTS

Upon return to Lord Howe Island in September 2008, it was discovered that the experimental set-up had been destroyed by the worst storms experienced by the Island in 20 years. No transplants were remaining and the experiment could not be repeated due to time constraints.

Appendix 3

Symbiodinium diversity by sampling effort

Table A3.1 Diversity of *Symbiodinium* types in various locations in relation to no. of hosts, families and genera sampled. LHI: Lord Howe Island, GBR: Great Barrier Reef

Site	No types	No. hosts	No. family	No. genera	Types per host	Types per family	Type per genus	Reference
LHI	19	16	7	13	1.19	2.71	1.46	Present study
Southern GBR	20	79	16	34	0.25	1.25	0.59	LaJeunesse et al. 2003
Central GBR	25	140	23	64	0.18	1.09	0.39	LaJeunesse et al. 2004a
Kermadec Is.	5	5	4	5	1.00	1.25	1.00	Present study
Palmyra	10	34	9	13	0.29	1.11	0.77	Present study
Hawaii	16	24	8	12	0.67	2.00	1.33	LaJeunesse et al.2004b
Caribbean	20	37	8	19	0.54	2.50	1.05	LaJeunesse 2002
Okinawa, Japan	17	67	13	27	0.25	1.31	0.63	LaJeunesse et al. 2004a

Appendix 4

Palmyra Atoll coral diversity, distribution and photosynthetic health

A.4.1 Coral diversity at Palmyra Atoll

Table A4.1 Species list of scleractinian cnidarians reported at Palmyra Atoll from 1987-2005 (Williams et al. 2008). ITS2-DGGE *Symbiodinium* types identified in coral species shown following reference. *Montipora* species identified to group level (Table A4.2) in survey therefore *Symbiodinium* type not shown.

Acropora abrotenoides (Lamarck, 1816)	
Acropora aculeus (Dana, 1846)	
Acropora acuminata (Verrill, 1864)	C3
Acropora aspera (Dana, 1846)	
Acropora cerealis (Dana, 1846)	C3
Acropora cf. *clathrata* (Brook, 1891)	
Acropora cf. *robusta* (Dana, 1846)	
Acropora cytherea (Dana, 1846)	C3
Acropora digitifera (Dana, 1846)	
Acropora divaricata (Dana, 1846)	C3
Acropora elseyi (Brook, 1892)	C3
Acropora florida (Dana, 1846)	C3
Acropora gemmifera (Brook, 1892)	
Acropora globiceps (Dana, 1846)	
Acropora granulosa (Milne Edwards & Haime, 1860)	
Acropora humilis (Dana, 1846)	
Acropora hyacinthus (Dana, 1846)	
Acropora latistella (Brook, 1891)	
Acropora longicyathus (Milne Edwards & Haime, 1860)	
Acropora monticulosa (Brüggemann, 1879)	
Acropora multiacuta Nemenzo, 1967	
Acropora muricata Linnaeus, 1758	
Acropora nana (Studer, 1878)	
Acropora nasuta (Dana, 1846)	C3
Acropora paniculata Verrill, 1902	
Acropora polystoma (Brook, 1891)	

Acropora robusta (Dana, 1846)		
Acropora rosaria (Dana, 1846)		
Acropora samoensis (Brook 1891)		
Acropora selago (Studer, 1878)		
Acropora sp. Oken, 1815		
Acropora spicifera (Dana, 1846)		
Acropora squarrosa (Ehrenberg, 1834)		
Acropora subulata (Dana, 1846)		
Acropora tenuis (Dana, 1846)		
Acropora valida (Linnaeus 1758)	C3	
Acropora variabilis (Klunzinger, 1879)		
Acropora vaughani Wells, 1954		
Acropora verweyi Veron & Wallace, 1984	C3	
Alveopora verrilliana Dana, 1872		
Astreopora expansa Brüggemann, 1877		
Astreopora gracilis Bernard, 1896	C1	
Astreopora listeri Bernard, 1896		
Astreopora myriophthalma (Lamarck, 1816)		
Astreopora sp. Blainville, 1830		
Astreopora suggesta Wells, 1954		
Balanophyllia sp. [small cups] Searles, Wood, 1844		
Ctenactis echinata (Pallas, 1766)		
Cycloseris cyclolites (Lamarck, 1801)		
Cycloseris patelliformis (Boschma, 1923)		
Cycloseris sp. Milne Edwards & Haime, 1849		
Cyphastrea serailia (Forskål, 1775)		
Echinophyllia aspera (Ellis & Solander, 1788)		
Echinophyllia sp. Klunzinger, 1879	D1	D1a
Edwards & Haime 1848		
Edwards & Haime 1848		
Favia favus (Forskål, 1775)		
Favia matthaii Vaughan, 1918	C3	
Favia pallida (Dana, 1846)		
Favia rotumana (Gardiner, 1899)		
Favia rotundata (Veron & Pichon, 1977)	C3	
Favia sp. Oken, 1815		
Favia speciosa Dana, 1846		
Favia stelligera (Dana, 1846)	C3	
Favites abdita (Ellis & Solander 1786)		
Favites chinensis (Verrill, 1866)		
Favites flexuosa (Dana, 1846)		
Favites halicora (Ehrenberg, 1834)		

Favites pentagona (Esper, 1794)	
Favites russelli (Wells, 1954)	
Favites sp. Link, 1807	
Fungia concinna Verrill, 1864	
Fungia danai Milne Edwards & Haime, 1851	
Fungia fungites (Linnaeus, 1758)	
Fungia granulosa Klunzinger, 1879	
Fungia horrida Dana, 1846	
Fungia moluccensis Horst, 1919	
Fungia paumotensis Stutchbury, 1833	C1, $C1_{xx}$
Fungia repanda Dana, 1846	
Fungia scutaria Lamarck, 1801	
Gardineroseris planulata (Dana, 1846)	C1
Goniastrea edwardsi Chevalier, 1971	
Goniastrea pectinata (Ehrenberg, 1834)	
Goniastrea retiformis (Lamarck, 1816)	
Halomitra pileus (Linnaeus 1758)	
Herpolitha limax Esper, 1797	
Heteractis crispa[2] (Ehrenberg, 1834)	
Heteractis malu[2] (Haddon & Shackleton, 1893)	
Hydnophora exesa (Pallas, 1766)	
Hydnophora microconos (Lamarck, 1816)	
Hydnophora pilosa (Veron, 1985)	
Hydnophora rigida (Dana, 1846)	
Isopora brueggemanni (Brook, 1893)	
Isopora cuneata (Dana, 1846)	
Isopora palifera (Lamarck, 1816)	
Leptastrea agassizi Vaughan 1907	
Leptastrea bewickensis Veron & Pichon, 1977	
Leptastrea pruinosa Crossland, 1952	
Leptastrea purpurea (Dana, 1846)	C1 C1b
Leptastrea sp. A [large angular calices] Milne	
Leptastrea sp. B Milne Edwards & Haime 1848	
Leptastrea sp. C [small round calices] Milne	
Leptastrea transversa Klunzinger, 1879	
Leptoria phrygia (Ellis & Solander, 1786)	
Leptoseris mycetoseroides Wells, 1954	
Lobophyllia corymbosa (Forskå1, 1775)	
Lobophyllia hemprichii (Ehrenberg, 1834)	
Merulina ampliata (Ellis & Solander, 1786)	$C3_{xx}$
Montastraea annuligera (Milne-Edwards & Haime, 1849)	

Montastraea curta (Dana, 1846)	
Montastraea sp. Blainville, 1830	
Montipora aequituberculata Bernard, 1897	
Montipora caliculata (Dana, 1846)	
Montipora capitata (Dana, 1846)	
Montipora cf. *incrassata* (Dana, 1846)	
Montipora danae (Milne Edwards & Haime, 1851)	
Montipora dilatata Studer, 1901	
Montipora efflorescens Bernard, 1897	
Montipora flabellata Studer, 1901	
Montipora foliosa (Pallas, 1766)	
Montipora foveolata (Dana, 1846)	
Montipora hoffmeisteri Wells, 1954	
Montipora informis Bernard, 1897	
Montipora millepora Crossland, 1952	
Montipora monasteriata (Forskå1, 1775)	
Montipora patula Verrill, 1864	
Montipora peltiformis Bernard, 1897	
Montipora sp. Blainville, 1830	
Montipora spongodes Bernard, 1897	
Montipora tuberculosa (Lamarck, 1816)	
Montipora venosa (Ehrenberg, 1834)	
Montipora verrilli Vaughan 1907	
Pachyclavularia violacea[1] (Quoy & Gaimard 1833)	
Pachyseris sp. Milne Edwards & Haime 1849	
Pavona cactus	
Pavona chiriquiensis Glynn, Mate & Stemann, 2001	
Pavona clavus (Dana, 1846)	
Pavona duerdeni Vaughan, 1907	
Pavona explanulata (Lamarck, 1816)	
Pavona frondifera (Lamarck, 1816)	
Pavona maldivensis (Gardiner, 1905)	
Pavona minuta Wells, 1954	
Pavona varians Verrill, 1864	C1
Platygyra daedalea (Ellis & Solander, 1786)	
Platygyra lamellina (Ehrenberg, 1834)	
Platygyra pini Chevalier, 1973	
Platygyra ryukyuensis Yabe & Sugiyama, 1936	
Platygyra sp. Ehrenberg, 1834	
Platygyra sinensis (M. Edwards & Haime, 1849)	
Plesiastrea versipora (Lamarck, 1816)	
Pocillopora brevicornis Lamarck, 1816	

Pocillopora damicornis (Linnaeus, 1758)	C1c, C42a
Pocillopora capitata Verrill, 1864	
Pocillopora eydouxi Milne Edwards & Haime 1860	C1c, C42a
Pocillopora meandrina Dana 1846	C1c
Pocillopora sp. Lamarck, 1816	
Pocillopora verrucosa (Ellis & Solander, 1786)	C1c, C42a
Pocillopora zelli Veron 2000	
Porites annae Crossland, 1952	
Porites australiensis Vaughan, 1918	
Porites evermanni Vaughan, 1907	
Porites lichen Dana, 1846	
Porites lobata Dana, 1846	C15/b
Porites lutea Milne Edwards & Haime, 1851	
Porites murrayensis Vaughan, 1918	
Porites rus (Forskå1, 1775)	
Porites solida (Forskå1, 1775)	
Porites sp. [nodular] Link, 1807	
Porites sp. Link, 1807	
Porites superfusa Gardiner, 1898	
Porites vaughani Crossland, 1952	
Psammocora contigua (Esper, 1797)	
Psammocora haimeana Milne Edwards & Haime, 1851	
Psammocora nierstraszi Horst, 1921	C1
Psammocora profundacella Gardiner, 1898	
Psammocora stellata Verrill, 1864	
Psammocora verrilli Vaughan, 1907	
Rhodactis howesii[4] (Ehrenberg, 1834)	
Sandalolitha robusta Quelch, 1886	
Sarcophyton sp.[1] Gosliner, Behrens & Williams, 1996	
Sinularia sp.[1] Gosliner, Behrens & Williams, 1996	
Stereonephthya sp.[1] Gosliner, Behrens & Williams, 1996	
Stichodactyla mertensii[2] Brandt, 1835	
Stylaster elegans.[5] Verrill, 1864	
Stylophora pistillata Esper, 1797	
Symphyllia recta (Dana, 1846)	
Tubastraea coccinea Lesson, 1831	
Turbinaria reniformis Bernard 1896	D1a
Turbinaria frondens (Dana, 1846)	
Turbinaria sp. Oken, 1815	

Table A4.2 Groups of *Montipora* species found at Palmyra Atoll, remote Central Pacific based on their growth-form and skeletal characters. Adapted from Veron (2000)

No.	Group Description
2	Laminar species without conspicuous coenosteum ridges
3	Encrusting or massive species with prominent coenosteum tuberculae
4	Encrusting or massive species with prominent thecal papillae
6	Encrusting species with very small corallites
7	Species with funnel-shaped (foveolate) corallites
8	Species with large coenosteum tuberculae formin

A4.2 Coral distributions at Palmyra Atoll

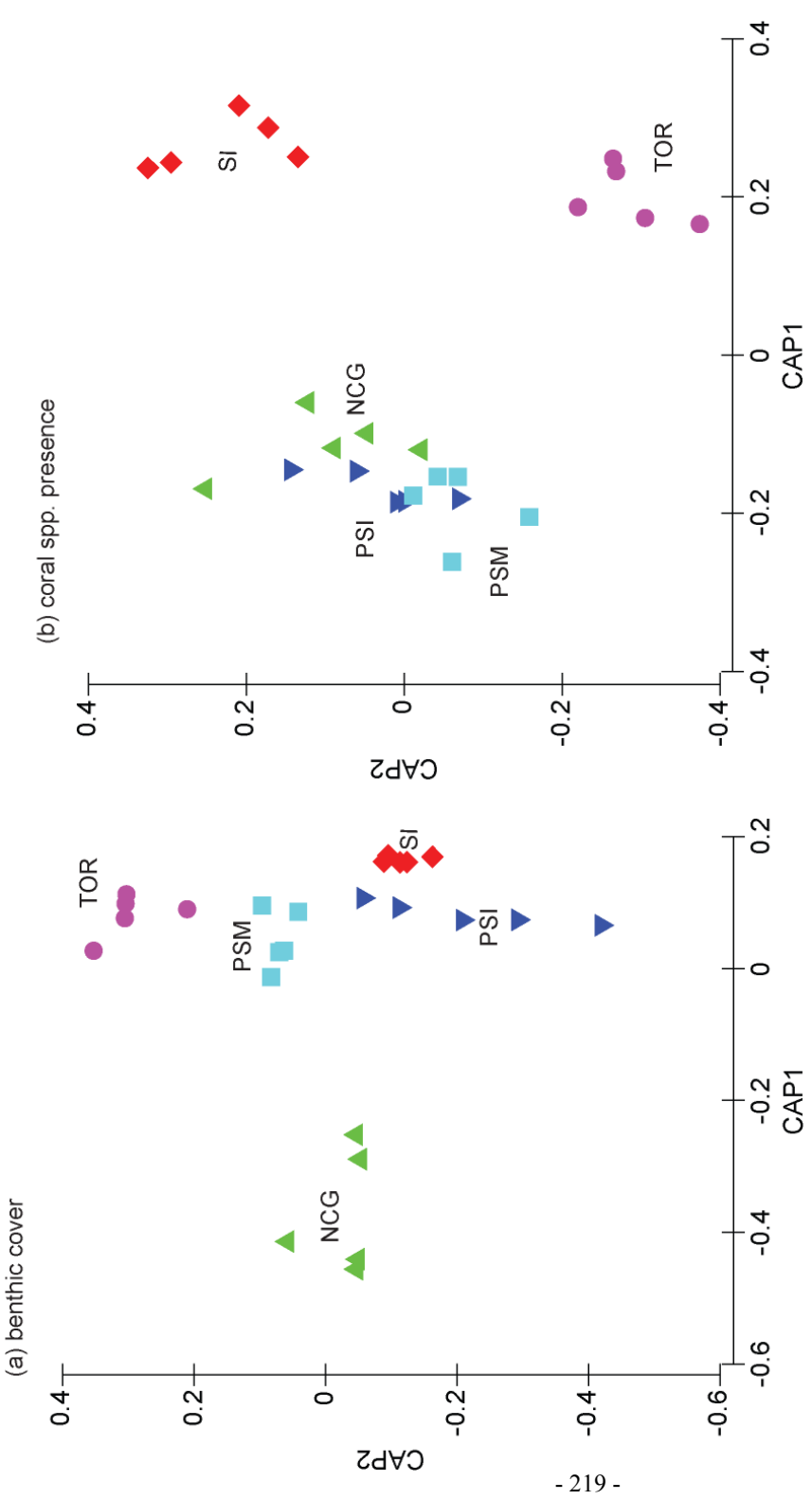

Figure A4.1 CAP ordination of (a) benthic cover and (b) coral species assemblages at Palmyra atoll among five sites. Ordinations based on a zero-adjusted Bray-Curtis coefficient, with a presence/absence transformation used for coral species and dispersion-weighting pretreatment for benthic cover. Site codes shown near symbols for clarity. NC: North Coral Gardens, PSI: Penguin Spit Inner, PSM: Penguin Spit Middle, SI: Sand Island, T: Tortugonas.

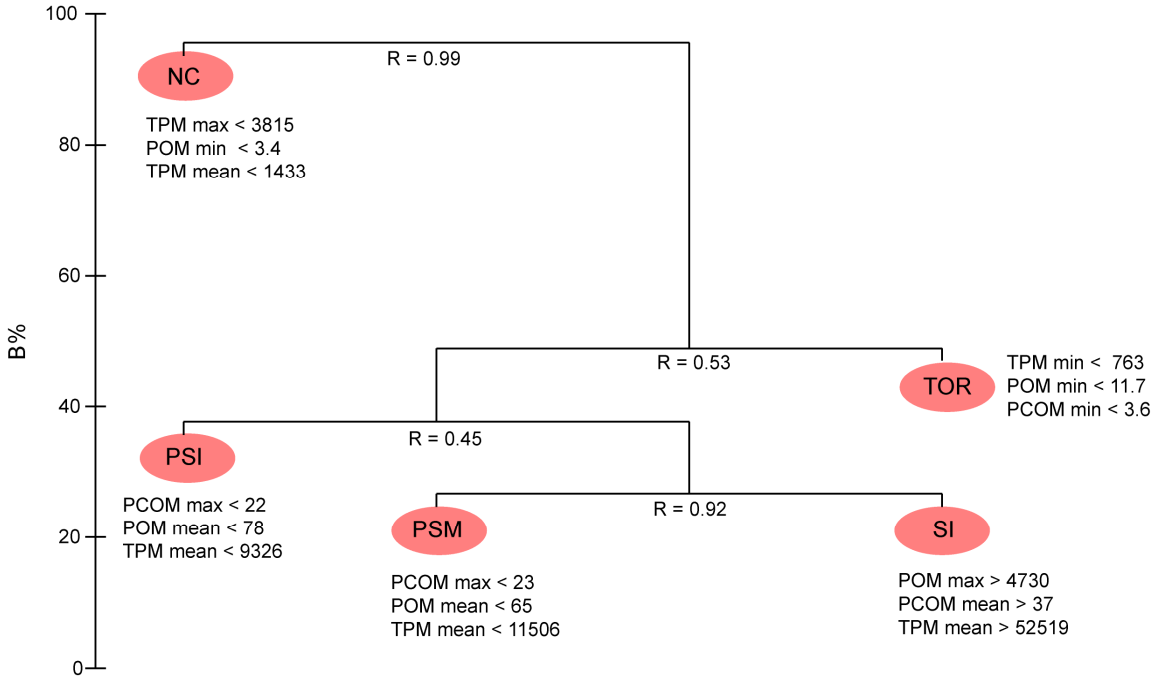

Figure A4.2 LINKTREE classification and regression tree analysis (with Simprof test of 1000 permutations) showing major splits in benthic cover between sites in link-tree dendrogram by most significant environmental variable(s). B% is the absolute measure of group differences. For each split the ANOSIM test statistic R is shown. NC: North Coral Gardens, PSI: Penguin Spit Inner, PSM: Penguin Spit Mid, SI: Sand Island, T: Tortugonas. POM IN %, TPM/POM in mg m^{-2} d^{-1}.