INTER- AND INTRA-SPECIFIC VARIATION IN BLEACHING SUSCEPTIBILITY AMONG SCLERACTINIAN CORALS

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Statement of contributions of others

This thesis includes collaborative work undertaken with my supervisors Professors Morgan Pratchett and Andrew Baird. My collaborators also provided intellectual guidance, financial support, field assistance and equipment, technical instruction and editorial assistance.

Chapter 5 resulted from analysis of data originally compiled by Professor Morgan Pratchett and was reliant on external input from Dr Jeff Maynard and Dr Scott Heron. The original concept for this paper arose from discussions between Pratchett and Maynard, but I was responsible for compiling all relevant data and testing for temporal declines in bleaching susceptibility among common coral genera in Moorea.

For all remaining chapters, I was responsible for the project concept and design, data collection, analysis and ecological interpretation, and the final synthesis of results into a form suitable for publication. Full references are provided for all sources when data was extracted from published papers and used in meta-analyses.

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Abstract

Coral bleaching is the disassociation (either expulsion or degradation) of zooxanthellae and the coral host, and it is a general stress response of corals. Anomalous sea surface temperatures have caused widespread incidence of bleaching referred to as mass bleaching; however, chronic, longer-term stress from global climate change is also potentially increasing coral vulnerability to subsequent stress. Sustained and ongoing increases in sea surface temperatures are expected to result in greater incidence of mass bleaching of scleractinian corals, assuming that corals are incapable of acclimating or adapting at required rates. Acclimation is a short-term relief from stress, such as producing heat shock proteins; whereas, adaptation works on populations, hence would use natural selection to produce corals that are more tolerant to stress. A significant limitation in assessing the fate of corals subject to changing environmental conditions is a precise quantitative metric for measuring incidence and severity of coral bleaching. In the field, bleaching is often reported based on the conspicuous "paling" of individual coral colonies, species, or assemblages, but there is not currently a clear and unambiguous definition that can be used to say exactly when individual corals (or populations) are bleached. The purpose of this thesis was to compare among alternative methods used to quantify the incidence and severity of coral bleaching, both at the level of individual colonies and local populations or species, to establish a rigorous quantitative definition for coral bleaching. Bleaching, therefore, can be defined as a loss of greater than half of the zooxanthellae population density, concurrent with rapid changes in physiological quenching efforts, and often displayed as a colour change of 2-3 shades. This metric was then used to explore taxonomic, spatial (geographical), and temporal variation in bleaching susceptibility among scleractinian corals.

Chapter 2 of the thesis focused on measures of zooxanthellae density, specifically testing for intraspecific variation in zooxanthellae densities of the common reef coral,

Acropora millepora, in the Palm Islands, inshore Great Barrier Reef. Various methods are available to quantify zooxanthellae densities; however, a direct comparison of these techniques has yet to be done. Here, we compared estimates of zooxanthellae densities obtained using conventional airbrushing coupled with post-tissue-blasting surface area determination, versus a technique whereby zooxanthellae densities are quantified from a known area (0.25 cm^2) of tissue after corals have been fixed and decalcified. Estimates of zooxanthellae densities obtained using the two different methods were significantly correlated ($R=0.40$, $n=81$, $p<0.01$), such that both techniques revealed similar patterns of variation among locations. The main benefit of the decalcification technique was reduced handling time, because the technique eliminates the time-consuming process of tissue blasting and retrospective estimates of surface area. We estimate that decalcification halves the handling time per sample, and produces a more accurate estimate of zooxanthellae density.

Chapter 3 analysed published estimates of zooxanthellae densities for a wide range of different corals and locations, testing whether there are consistent thresholds that distinguish bleached versus unbleached corals. Moreover, zooxanthellae densities are naturally regulated (e.g. due to season, light availability), so an important point to this chapter was to determine if bleaching could be distinguished from these natural variations in zooxanthellae densities. Normal zooxanthellae densities ranged from $0.1x10^6$ cells/cm^2 up to 18.0×10^6 cells/cm^2; whereas, zooxanthellae densities reported for bleached corals were between 0.001 and 6.5×10^6 cells/cm^2. Marked variation in published estimates of zooxanthellae densities was largely attributable to differences in the methods among studies (e.g. size of tissue sample, method of tissue removal and surface area determination), though there were significant and consistent differences among coral species, with growth form and with depth. It is not possible therefore, to establish a single threshold density of zooxanthellae that distinguished

bleached and unbleached corals. However, after accounting for taxa (genera) it does appear that relative changes in zooxanthellae densities are a good indication of the fate of individual corals. In the absence of distinct bleaching events, natural variation in zooxanthellae densities (e.g., among seasons) was typically <50% of the mean. During bleaching events however, zooxanthellae loss within individual corals often ranged from 55-100%. Moreover, corals that experienced >78% zooxanthellae loss almost invariably died, whereas those corals that lost 55-77% of zooxanthellae were bleached, but generally recovered. Sub-lethal bleaching caused by pollutants did not adhere to the bleaching definition, as conspicuous loss of zooxanthellae density was often observed at levels of what is considered "natural variation". For other stresses however, (and particularly, thermal stress) it may be possible to define when corals have bleached, and predict their fate based on proportional declines in zooxanthellae densities.

To specifically test for inter- and intra-specific variation in bleaching susceptibility, Chapter 4 exposed twenty whole colonies of *Acropora nasuta* and *Pocillopora damicornis* to controlled warming in experimental facilities (with carefully controlled light and temperature environments) at Orpheus Island. Corals, after acclimated to laboratory conditions, were subjected to a simulated warm water anomaly, with a slow rate of increase of 0.5^{o}C every third day until they reached 31.6^{o}C, which is equivalent to the 1998 temperature anomaly that lead to extensive mass bleaching of scleractinian corals in the central Great Barrier Reef, Australia. Daily observations of coral health were made with coral colour charts and Pulse-Amplitude Modulated Fluorometry measurements; corals were considered to have bleached when marked changes in the quenching analyses occurred simultaneously with a change in 2-3 shades of colour. *Post hoc* measurements of zooxanthellae densities were used to confirm when bleaching occurred. There was marked variation in the time to bleaching both within and among coral species. For *A. nasuta*, the mean time to bleach was 8 days, but ranged 12

days, while, for *P. damicornis*, mean time to bleach was 12 days and ranged 15 days. Moreover, both corals showed phenotypic variation in the timing of bleaching responses, therefore there may be underlying genetic variation upon which the corals could adapt.

Chapter 5 explored temporal changes in bleaching susceptibility among key genera of reef-building corals in Moorea, French Polynesia, comparing bleaching incidence of four genera (*Acropora*, *Montipora*, *Pocillopora* and *Porites*) during mass-bleaching events in 1991, 1994, 2002 and 2007. *Acropora* and *Montipora* consistently bleached in far greater proportions (up to 98%) than *Pocillopora* and *Porites*. However, there was an apparent and sustained decline in the proportion of colonies that bleached during successive bleaching events, especially for *Acropora* and *Montipora*. Coral genera that are highly susceptible to coral bleaching, and especially *Acropora* and *Montipora*, exhibited temporal declines in their susceptibility to thermal anomalies at Moorea, French Polynesia. One possible explanation for these findings is that gradual removal of highly susceptible genotypes (through selective mortality of individuals, populations, and/ or species) is producing a coral assemblage that is more resistant to sustained and ongoing ocean warming.

Chapter 6 tests whether taxonomic variation in bleaching susceptibility and mortality is spatially consistent among geographic regions, comparing extensive data sets from the Indian, Pacific and Atlantic oceans. Data was compiled from 105 distinct studies, spanning the Pacific, Indian and Atlantic Oceans, and from 1982 to 2013. Differences in bleaching susceptibility and mortality were apparent among different coral genera, but the hierarchy of bleaching susceptibility differed on geographic scales, among ocean basins. These large-scale differences may be attributable to inherent differences in biology (e.g., geographic variation in associations between corals and their symbionts), but may also reflect taxonomic differences in the capacity of corals to acclimate or adapt when facing extreme environmental changes. Among decades, it is apparent that bleaching susceptibility and mortality have

generally declined over time, possibly reflecting increased bleaching resistance at the level of populations or communities due to selective removal of highly susceptible phenotypes.

This thesis shows that there is phenotypic variation at many scales within and among corals. For instance, phenotypic variation was found in mean zooxanthellae densities, both within and among species. Then, phenotypic variation was observed as marked variation in the timing of the bleaching response within and between two commonly susceptible coral species. Next, phenotypic variation was observed for a bleaching event, where the proportion of susceptible corals decreased over the course of time. Most notably, however, there is marked variation in bleaching susceptibility among different coral taxa, which is likely to lead to directional shifts in the structure of coral assemblages with increasing incidence of mass-bleaching. Establishing exactly how these assemblages will change is, however, critically dependent on understanding species-specific susceptibility to bleaching and recovery capacity of these corals in the aftermath of periodic bleaching events. Future research needs to focus much more on the longer-term fate of coral colonies, populations and species subject to ongoing bleaching.

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Chapter 1: General Introduction

1.1 Climate change and reef-building corals

The biology and ecology of all organisms are influenced to a greater or lesser extent by local environmental conditions, especially temperature. Accordingly, sustained and ongoing changes in the global climate are having significant effects on the distribution, abundance, survivorship, development, phenology, physiology, behaviour, and fitness of organisms (Hughes 2000, Walther et al. 2002, Parmesan and Yohe 2003, Root et al. 2003, Harley et al. 2006). Climate change is also implicated in dramatic shifts in species composition and community structure across a number of important and highly sensitive ecosystems (e.g., arctic, arid, tropical rainforests and coral-reef ecosystems), as well as contributing to species extinctions within these systems (Brown 1997, Walther et al. 2002, Thomas et al. 2004). Effects of climate change on individual organisms, populations, and species are apparent across virtually all ecosystems ranging from polar terrestrial to tropical marine environments (Walther et al. 2002). Moreover, it has long been known that coral reefs are highly susceptible to changes in environmental conditions (Williams & Bunkley-Williams 1990, Glynn 1991, Walther et al. 2002).

Climate change affects coral reef ecosystems in a multitude of ways, including: i) increased frequency or intensity of severe tropical storms (Emanuel 2005), which increases sedimentation and eutrophication (Prosper 2005) as well as freshwater run-off (Prosper 2005), ii) increased ocean acidification and reduced carbonate saturation, which will directly affect coral growth (Knowlton 2001, Hoegh-Guldberg et al. 2007), and iii) increased ocean temperatures, which may subject coral reef organisms to unprecedented or unbearable temperature extremes (Hoegh-Guldberg 1999). To date, it is the direct effects of increased ocean temperatures that have had the greatest impacts (Hoegh-Guldberg 1999), and generated the most concern (Knowlton 2001) for coral reefs. Most notably, increased ocean

temperatures have been linked to large-scale and multi-specific bleaching of scleractinian corals (Williams & Bunkley-Williams 1990, Glynn 1991, Walther et al. 2002, Parmesan 2006), as well as increased occurrence and virulence of coral disease (Willis et al. 2004), both of which contribute to widespread mortality of scleractinian corals (Hoegh-Guldberg 1999, Wilkinson 1999) and extensive degradation of coral reef environments (Hughes et al. 2003).

Mass-bleaching is expected to occur when corals are exposed to temperatures of $\geq 1^{o}C$ above the long-term summer weekly maxima for $\geq$ 1 week (Goreau and Hayes 1994). However, exposure to greater temperatures for shorter periods (e.g. $\geq 2^{o}C$ above the long-term summer weekly maxima for 1/2 week) may also lead to mass-bleaching, leading to the notion of Degree Heating Week (DHW). The DHW is the number of weeks multiplied by the extent to which average weekly temperatures exceeded local bleaching thresholds (Jokiel and Coles 1990, Goreau and Hayes 1994). This concept was established and widely accepted in the 1990's, but is increasingly being challenged (e.g. Maynard et al. 2008b, Berkelmans 2009). Berkelmans (2002) showed that the relationship between temperature thresholds that cause bleaching and necessary exposure times are non-linear, and short-term exposure to very hot temperature will more likely cause bleaching compared to prolonged exposure to moderate temperatures (see also Jokiel and Coles 1990). Berkelmans (2009) compared the thermal histories and DHW's of several locations on the GBR with the observed effects during bleaching events and found that at some locations, thermal thresholds have increased throughout repeated bleaching exposure. It is also clear that corals vary greatly in their susceptibility to bleaching, such that some corals may bleach at 1DHW, but other corals (both con-specifics and con-generics) may only bleach at either higher temperature or prolonged exposure to extreme temperatures (Maynard et al. 2008b).

Corals have a variety of mechanisms (e.g. mucous secretion, tentacle retraction, down regulation of pigments, zooxanthellae and/ or mycosporine-like amino acids), to cope with

natural variations in environmental parameters (e.g. seasonal changes in thermal and photic regimes); however, the general response of corals to most major extrinsic threats (e.g. mass bleaching due to anomalous thermal regimes, e.g. Goreau and Hayes 1994) is to expel the zooxanthellae from the gastrodermal cells of coral tissue through one of many mechanisms (reviewed in Chapter 3, e.g. host-cell detachment). Isolated incidences of coral bleaching (limited to one or a few different coral colonies) have been reported in the scientific literature for over a century (e.g., Vaughan 1916) and are not unexpected given that bleaching may simply reflect the general poor health of individual colonies. Simultaneous bleaching of a large number of different colonies, however, is indicative of large-scale environmental stress (Williams et al. 1987, Glynn 1991), and increasing incidence of mass-bleaching events is major cause for concern (e.g. 16% global mortality in the 1997-98 mass bleaching event, Wilkinson 2002). Moreover, bleaching events are predicted to increase in frequency and/ or severity over coming years (Hoegh-Guldberg 1999, Sheppard 2003, Donner et al. 2005), and cause changes in the relative abundance of different corals (e.g. Baird and Marshall 2002, Hughes et al. 2003).

The bleaching process is highly variable within and among coral species (e.g. Marshall and Baird 2000), and while extreme bleaching events ultimately result in host-coral mortality (e.g. Baird and Marshall 2002), there is the possibility that bleaching, and subsequent symbiont shuffling, may be an adaptive strategy to increase resilience to changing environmental conditions (e.g. Meiog et al. 2007). The adaptive bleaching hypothesis (first proposed by Buddemeier and Fautin 1993) suggests that the rapid expulsion of resident zooxanthellae during exposure to extreme or unprecedented environmental conditions may provide the opportunity for exogenous uptake of more thermally tolerant clades or types of zooxanthellae (Baker 2001, Baker et al. 2004, Buddemeier and Fautin 1993, Buddemeier et al. 2004, Fautin and Buddemeier 2004). Furthermore, recent findings suggest that reefs that

have experienced recurrent episodes of thermal stress often had lessened effects (lower incidence or severity of bleaching) over time (Brown et al. 2000, Maynard et al. 2008a, Middlebrook et al. 2008, Pratchett et al. 2013, Thompson and van Woesik 2009). Temporal declines in bleaching incidence during successive episodes of equivalent temperature stress point to acclimation or adaptation, but these patterns may be attributed to i) selective removal (filtering) of more susceptible individuals, ii) acclimatization of individual corals (e.g., due to changes to a more tolerant symbiont community, or iii) changes in the energy reserves (condition) of corals during each specific stress event (Thompson and van Woesik 2009, Maynard et al. 2008a, Pratchett et al. 2013).

Although there has been a significant amount of work relating to regulation of zooxanthellae densities (e.g. Jones and Yellowlees 1997, Fitt et al. 2001), the cladal composition of zooxanthellae population (e.g. Rowan et al. 1997, Berkelmans and van Oppen 2006) and variation in the sizes and population turnover of zooxanthellae among different coral hosts (e.g. Jones and Yellowlees 1997; Wilkerson et al. 1988), bleaching has yet to be rigorously and unambiguously linked to changes in zooxanthellae (e.g., what proportion of zooxanthellae are lost before a coral is considered bleached), which undermines the capacity to determine whether coral bleaching is adaptive, morbid or both.

1.2 Variation in susceptibility to bleaching

Since the earliest mass-bleaching events, it was apparent that different corals vary in their susceptibility to bleaching (Williams and Bunkley-Williams 1990), whereby the proportion of colonies that are affected varies among species, genera and families. Several studies have put forward hierarchies of bleaching susceptibility among different corals (mostly genera) for single locations (e.g., Loya et al. 2001), or tested for consistent differences across

geographical locations (McClanahan et al. 2004). Corals generally considered winners are those with high biomass, low population turnover rates and the ability to concentrate stress to produce partial mortality, while opposing traits confer susceptibility (e.g. Baird and Marshall 2002, Loya et al. 2001). The generality of these biological traits is however, increasingly questioned given spatial and temporal variation in hierarchies of bleaching susceptibility among coral taxa (e.g., Guest et al. 2012). On the Great Barrier Reef (GBR), Pocilloporidae corals, especially *Stylophora* and *Pocillopora,* are often the first to bleach and the most severely affected corals, whereas Fungiidae tend to be resistant to all but the most severe bleaching episodes. In French Polynesia, however, Pocilloporidae corals are much more resistant to bleaching compared to *Acropora* (e.g., Penin et al. 2007); to the extent that recurrent bleaching is causing a shift towards *Pocillopora*-dominant coral assemblages (Berumen and Pratchett 2006). Moreover, the bleaching hierarchy is inconsistent between bleaching years (Penin et al. 2013). The prediction of increased frequency of coral bleaching events and known differential susceptibility among coral genera (Loya et al 2001; McClanahan et al. 2004) has led to predictions of marked changes in the structure of coral assemblages (e.g., Baker et al. 2008).

Many studies have documented local and geographical variability in bleaching susceptibility among coral species (e.g. >300 papers in the bleaching susceptibility, mortality/recovery database). However, there is often marked intra-specific variation in bleaching susceptibility among colonies that occupy ostensibly the same habitat and have been subject to very similar thermal regimes (Hueerkamp et al. 2001). For example, bleaching episodes rarely cause 100% mortality across all colonies of a given species (Baird and Marshall 2002). Intra-specific variation in bleaching susceptibility is important because it demonstrates an inherent level of phenotypic plasticity, which, if heritable, may provide the capacity for adaptation to ongoing climate change (e.g., Pandolfi et al. 2011). However,

because of the potential for very fine scale differences in environmental conditions, and the multitude of extrinsic factors that may influence the susceptibility of individual colonies to local environmental stresses (e.g. West and Salm 2003), it remains unknown whether the phenotypic variation in the bleaching response of conspecifics is a result of environmental heterogeneity or intrinsic differences in bleaching susceptibility.

Many different factors contribute to variation in bleaching susceptibility within and among coral species, including genotype, depth, habitat, colony size, morphology, and/or the clade of zooxanthellae that predominates within each coral colony (Edmunds 1994, Marshall and Baird 2000, Loya et al. 2001, Stimson et al. 2002). As a first step towards assessing whether corals are adapting to climate change, it is necessary to separate the influence of extrinsic factors (e.g., contrasting micro-climates) from intrinsic differences (e.g., mycosporine-like amino acids) in bleaching susceptibility among individual coral colonies (Jokiel 2004). The extrinsic factors are generally controllable in a laboratory setting. However, considerable work is still required to establish and quantify variation in intrinsic factors that may influence bleaching susceptibility. If the variance in intrinsic factors (e.g., microbiology of the holobiont) that influence bleaching susceptibility have a genotypic basis, and are determined to be heritable, then this provides considerable scope for adaptation (Csaszar et al. 2010).

1.3 Quantifying coral bleaching

While awareness of coral bleaching has increased, and it is often very obvious when mass bleaching has occurred, there is not currently a clear and unambiguous method or measurement that can be used to say exactly when individual corals (or populations) are bleached. In the field, bleaching is often reported based on the conspicuous “paling” of

individual coral colonies, species, or assemblages (e.g., Brown 1997). Given that mass bleaching of corals is almost invariably due to declines in the density of zooxanthellae (Hoegh-Guldberg and Smith 1989a), explicit measures of zooxanthellae densities over time provide the most reliable and unambiguous definition of coral bleaching (Fitt et al. 2001). However, densities of zooxanthellae are highly variable spatially, temporally and taxonomically, such that the absolute densities of zooxanthellae cannot be used to infer that a coral has bleached. Rather there may be an explicit requirement for ongoing monitoring of individual coral colonies, such that the only unequivocal indicator that bleaching has occurred is a rapid or pronounced decline in zooxanthellae densities.

1.4 Research aims and objectives

The overarching aims of my thesis were two-fold. Firstly, I wanted to establish a more comprehensive definition of coral bleaching, so I explored the methods used to define coral bleaching in field observations and experiments. This information was then used to determine taxonomic, spatial (geographical), and temporal variation in bleaching susceptibility among scleractinian corals. My thesis is comprised of five independent research studies (Chapters 2-6), with formative chapters (2-4) mostly aimed at improving the methods and metrics used to establish when bleaching has occurred, followed by studies (chapters 4-6) that use this information to explore variation bleaching susceptibility within and among coral taxa, as well as in time and space. More specifically, **Chapter 2** compares two commonly used methods of determining average zooxanthellae population densities, with the hypothesis that the methods will produce similar results that are comparable, to later find that means varied significantly between the two methods, and that trends between locations were consistent between methods. **Chapter 3** utilises a database of healthy and bleached zooxanthellae densities to attempt to answer the question of whether bleaching can be differentiated from natural variations in healthy zooxanthellae population densities. We determined that mean

zooxanthellae density cannot be used to define bleaching due to variability in methods and natural fluctuations in zooxanthellae population densities; however, bleaching can be defined through mean per cent zooxanthellae population loss (loss of 55-77% nonlethal, while >78% likely lethal). **Chapter 4** is an experiment designed to simulate a mass bleaching event in order to i) define bleaching using non-invasive techniques supported by post hoc invasive techniques (which were discussed in detail in Chapters 2 and 3) ii) determine if there is variability in the timing of bleaching relative to days at a constant thermal stress iii) consider bleaching mechanisms in relation to recovery potential. We compared a common non-invasive visual estimate of bleaching, coral colour cards (Siebeck et al. 2006), a common invasive laboratory method of determining bleaching, mean zooxanthellae density loss (McCowan et al. 2011) and a more recent non-invasive field method of determining coral health, PAM Fluorometry, to determine that bleaching is best defined by PAM Fluorometry with quenching analysis and a change to the holobiont scale of observation. Moreover, inter- and intra-specific bleaching variation in the timing of the bleaching response exists such that approximately two weeks had past after the bleaching of the first colony to that of when the last colony bleached; this suggests intrinsic variation that could be heritable. Furthermore, the observation was made that 40% of corals that sloughed tissue recovered after approximately one month after being returned to the field. Finally, the first corals to bleach (not tissue slough) were the ones observed to symbiont shuffle, while the last corals to bleach exhibited chronic photoinhibition of the zooxanthellae population followed by rapid expulsion of incompetent zooxanthellae and rapid return to average photosynthetic yield values, but with very apparent quenching efforts maintained for at least a month post-bleaching. **Chapter 5** explores temporal variation in the bleaching susceptibility of scleractinian corals in Moorea, French Polynesia, comparing taxonomic variation in bleaching susceptibility recorded in 2007 to previously documented bleaching events in 1991, 2002, and 2007, and attempting to

determine if there has been a change to the bleaching susceptibility of corals through time. Moorea is an interesting location for studying responses of corals to climate-induced coral bleaching because bleaching has been recorded every 4-7 years throughout the last 2 decades. Accordingly, this study revealed temporal declines in the susceptibility of corals, especially *Acropora* and *Montipora*, to thermal anomalies. One possible explanation for these findings is that gradual removal of highly susceptible genotypes (through selective mortality of individuals, populations, and/ or species) is producing a coral assemblage that is more resistant to sustained and ongoing ocean warming. **Chapter 6** examines spatial and taxonomic variation in bleaching susceptibility and mortality based on a database compiled from global data on the proportion of colonies within each genus that bleach and/ or die during mass-bleaching episodes, predominantly caused by prolonged exposure to extreme temperatures. Specifically, we tested whether taxonomic (generic) variation in bleaching susceptibility and mortality is consistent among broad geographic regions (ocean basins), and through time (decades), and found that generic variation in bleaching susceptibility and mortality was not consistent geographically, and there is a general decrease through time, excluding recent episodes, which suggests that susceptible corals have recovered to become susceptible again.

Results of the independent studies in each of the aforementioned chapters are brought together and discussed in **Chapter 7**, which considers the importance and ramifications of marked inter- and intra-specific variation in bleaching susceptibility among scleractinian corals. Most notably this thesis shows high levels of inter- and intra-specific variation in bleaching susceptibility among reef-building corals. This indicates that there is likely to be significant capacity for adaptation to climate change, both at population and community levels (*sensu* Hughes et al. 2003). The concern however, is that the coral taxa most susceptible to recurrent coral bleaching (e.g., *Acropora*, Pratchett et al. 2013; Chapter 5) are

important contributors to reef growth and habitat structure, such that selective mortality of these corals may have devastating effects on the ecosystem function and diversity of coral reef ecosystems (Pratchett et al. 2008). Moreover, coral reefs globally are facing increasing anthropogenic disturbances, which may ultimately constrain or undermine adaptation to climate-related increases in ocean temperatures. Furthermore, this thesis does not deal with the issue of ocean acidification, which has potential to further destroy reef accretion.

Chapter 2: A comparison of two methods for measuring densities of zooxanthellae in *Acropora millepora*[1]

2.1 Abstract

Quantification of zooxanthellae densities in tissues of reef-building corals aids in the assessment of the extent and severity of coral bleaching. Various methods are available to quantify zooxanthellae densities; however, a direct comparison of these techniques has yet to been done. Here, we compare estimates of zooxanthellae densities obtained using conventional airbrushing coupled with post-tissue-blasting surface area determination, versus a technique whereby zooxanthellae densities are quantified from a known area (0.25 cm^2) of tissue after corals have been fixed and decalcified. Estimates of zooxanthellae densities obtained were correlated across replicate colonies ($R=0.40$, $n=81$, $p<0.01$), and both techniques revealed similar patterns of variation among locations. The airbrush method is useful for few measurements which can be completed within a few hours, whereas the main benefit of the decalcification technique was reduced handling time, this is especially advantageous with larger sampling sizes, where the reduction in overall time used in the laboratory equates to more time available for other ventures. The decalcification technique eliminates the time-consuming process of tissue blasting and retrospective estimates of surface area. We estimate that decalcification halves the processing time per sample, and potentially produces a more accurate estimate of zooxanthellae density.

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2.2 Introduction

Many scleractinian corals and other marine invertebrates have an obligate symbiosis with zooxanthellae, whereby the endo-symbiotic dinoflagellates provide up to 90% of the energy requirement of the coral host, while the corals protect and provide inorganic nutrients to the zooxanthellae (Muscatine and Porter 1977). This relationship has been fundamental to the diversification and success of scleractinian corals in building coral reefs. Importantly, the tight nutrient cycling between corals and zooxanthellae has allowed for accelerated coral calcification in the nutrient poor tropical oceans (Pearse and Muscatine 1971, reviewed in Hallock 2001). However, the relationship between corals and their zooxanthellae is also very tenuous during periods of rapid environmental change, leading to increased incidence and severity of coral bleaching (Hoegh-Guldberg et al. 2007).

Bleaching is a general response to environmental stress, such as changes in temperature, salinity, sedimentation, and aerial exposure (Glynn 1996; Brown 1997; Hoegh-Guldberg 1999; Douglas 2003), caused by the loss of zooxanthellae and/or their associated pigments (Hoegh-Guldberg and Smith 1989a). When the stressor is too frequent or severe, as is the case during mass bleaching events, extensive zooxanthellae loss will occur, which has significant consequences for the host corals, leading to reduced growth (Jokiel and Coles 1990; Porter et al. 1989), reproductive failure or reduced fecundity (Szmant and Gassman 1990), and ultimately whole-colony mortality (Yonge and Nicholls 1931; Hoegh-Guldberg 1999; Jones 2008).

The occurrence and severity of bleaching among natural coral populations is often quantified using indirect proxies for zooxanthellae densities, such as conspicuous paling of coral tissues (Marshall and Baird 2000). Non-intrusive techniques are useful to quantify major changes in coral health and condition, and facilitate rapid sampling across a significant number and high diversity of corals (Fitt et al. 2001). There is however, a critical need to

validate indirect proxies of zooxanthellae loss (Siebeck et al. 2006). For example, paling or whitening of coral tissues provides limited resolution to assess changes in zooxanthellae density, which might be necessary to predict and forewarn the occurrence of bleaching-related mortality (Jones 2008).

The purpose of this study was to explore the utility of a relatively novel method for directly measuring zooxanthellae densities in host coral tissues based on fixing and decalcifying host coral tissues. This method has been used previously by Drew (1972) and Stimson (1997), Stimson et al. (2002), and is fundamentally different from the more common and widespread method, where coral tissue is removed from intact skeletons using water or air jets (termed waterpiking or airbrushing, e.g., Muscatine 1980; Porter et al. 1984; Hoegh-Guldberg and Smith 1989a, b; Falkowski et al. 1993). The decalcification technique eliminates the need to retrospectively measure the surface area of coral samples from which tissues were removed, therefore, potentially limiting error. Stimson (1997) used the decalcification technique to measure densities in *Pocillopora damicornis* and results obtained were not dissimilar to estimates obtained using airbrushing (D'Croz and Mate 2004; Schloder and D'Croz 2004) and waterpiking (Li et al. 2008). In this study, we directly compared estimates of zooxanthellae densities obtained for paired coral samples using both the decalcification technique and airbrushing. The two techniques will be directly compared in terms of the relative measure of zooxanthellae densities, as well as the overall time required to process and handle coral samples.

2.3 Materials and Methods

In order to compare the two methods of measuring zooxanthellae densities, i) airbrushing tissues from intact coral skeletons and ii) fixing and decalcifying coral samples, two replicate

nubbins were collected from each of 81 tagged colonies of the stony coral *Acropora millepora* from between 1-3 m depth in July 2007. Colonies were sampled from three sites; two from Orpheus Island (Pioneer Bay and Cattle Bay), and one at the southwest corner of Pelorus Island, all part of the Palm Islands Group, Great Barrier Reef, Australia (18o35'S, 146o29'E). All coral nubbins were snap frozen in liquid nitrogen, and maintained at -30oC until further laboratory analysis.

For airbrushed samples, tissues were taken from frozen coral nubbins using a modified airgun connected to a dive cylinder containing compressed air. Coral tissues were airbrushed into a plastic bag filled with 15mL of 0.5 µm filtered seawater until all tissue was removed (time for this varied based on sample size; from five to fifteen minutes). The resultant slurry was then homogenized at 11 rotations/minute for thirty seconds. Nine mL of the suspension were immediately fixed in 1 mL of formaldehyde. Each of the 8 replicate subsamples were processed in the following manner: the vial was shaken vigorously. Then, using a clean pipette, the sample was placed onto a Neubauer Improved Tiefe Depth Profoundeur (0.100mm) haemocytometer, and viewed at 400x magnification with an Olympus CX31 light microscope. To mitigate 'edge effects' (i.e. counting cells lying on quadrat margins more than once) only the cells that touched the top and left-hand side of each square were counted, thus ignoring those that touched the bottom and right-hand side. There were eight replicate counts from each branch.

Zooxanthellae densities (number per cm^2) were determined by multiplying the number of zooxanthellae counted in each sample (N) by 10^4 (to account for 0.0001ml sampled in haemocytometer chamber) and 16.67 (to account for dilution with 15ml of water used when airbrushing), and then divided by the estimated surface area (cm^2) of the branch from which tissues were removed. The surface area of respective nubbins was determined using the aluminium foil method (Marsh 1970), whereby nubbins were carefully wrapped

with a uniform single layer of aluminium foil, which was then weighed to establish the surface area of the foil. A calibration curve of the surface area to mass ratio was constructed based on pieces of aluminium foil with known area ($y= 0.34x$, $r^2 = 0.99$, $n=15$), which was then used to back calculate the surface area of aluminium pieces wrapped around each coral sample.

For decalcified samples, nubbins were removed from the freezer and fixed in 10% buffered formalin for 4 days. Each sample was then placed in an individual container with 5% HCL solution to gently decalcify the sample over the period of 5 days. The HCL within each container was refreshed on days 3 and 4. Once the skeletons were dissolved, the remaining tissue samples were triple rinsed and stored in 70% ethanol. Two replicate 5x5mm sections were cut from the surface of each coral sample. Sections were taken 1-2cm from the apical tip, thereby avoiding areas of tissue that may be devoid of zooxanthellae (Gladfelter et al. 1989, Li et al. 2008). These sections were then placed in individual vials with 1 mL of 70% ethanol. The sample was then mixed with an Ultra Turrax T25 Basic homogenizer (Crown Scientific) for two minutes. 0.0025ml aliquots of this homogenate were immediately placed on to Neubauer Improved Tiefe Depth Profoundeur (0.100mm) haemocytometer to quantify zooxanthellae densities as described previously for the airbrushed samples ($n = 8$ for both methods).

A paired T-test was used to test for differences in estimates of zooxanthellae densities obtained using i) airbrushing tissues from intact coral skeletons and ii) fixing and decalcifying coral samples, directly comparing between samples obtained from each coral colony. The relationship between the two techniques was also tested using correlation analysis. Finally, resolution of the two methods was compared based on the detection of significant differences in zooxanthellae densities among 3 coral populations from distinct locations. Data were square root transformed and ANOVA with Tukey's post hoc

comparisons were conducted to test for differences in zooxanthellae densities of corals from each of three locations (Cattle Bay, Pioneer Bay and Southwest Pelorus) based on estimates obtained using each technique.

2.4 Results and Discussion

This study revealed highly significant differences in zooxanthellae estimates obtained using standard airbrushing of coral samples collected from replicate colonies of *A. millepora*, versus estimates obtained following decalcification of coral samples. The latter technique (decalcification) provided significantly higher estimates of zooxanthellae densities, compared to the standard airbrushing technique (Paired T-test, $t = 11.92$, $df = 80$, $p < 0.01$). These differences are most likely caused by differences in the extent of tissue sampled using each technique. Following decalcification a small ($0.25cm^2$) section of coral tissue was taken from well below the apical tip, whereas during airbrushing, tissue was removed from the entire length of coral branches (including the tip). This can cause discrepancy, because the zooxanthellae densities in *Acropora* are generally much lower towards the tip (Gladfelter et al. 1989, Li et al. 2008), leading to lower estimates of zooxanthellae densities when averaging over the entire branch length. Further, differences may arise because water-blasting and airbrushing does not remove tissues that perforate throughout the coral skeleton of *Acropora* corals (and other corals with perforate skeletons), though there are probably very few zooxanthellae contained within these deep coral tissues.

Estimates of zooxanthellae densities obtained from decalcified coral samples versus those samples from the same colonies that were airbrushed were highly correlated ($R = 0.40$, $n = 81$, $p<0.01$, Figure 2.1). However, the estimated zooxanthellae densities were much higher for decalcified coral samples, and this discrepancy increased with increasing densities

of zooxanthellae (Figure 2.1). Consequently, the two techniques are not interchangeable, but either technique could be used independently to test for changes in zooxanthellae densities within and among coral populations. The maximum density of zooxanthellae ($3.85x10^6$ zooxanthellae per cm^2), as well as the range in estimates of zooxanthellae densities ($3.06x10^6$ zooxanthellae per cm^2), were much higher for the decalcification technique than for the airbrushing method ($2.77x10^6$ and $2.37x10^6$ zooxanthellae per cm^2, respectively), which may increase resolution for detecting differences in zooxanthellae densities. For this study, both decalcification (ANOVA, $F_{(2,78)} = 5.690$, $p<0.01$) and airbrushing (ANOVA, $F_{(2,78)} = 13.621$, $p<0.01$) revealed significant variation in zooxanthellae densities among corals at each location, whereby the average zooxanthellae densities for corals from Southwest Pelorus, was significantly higher than for corals in Cattle Bay or Pioneer Bay (Figure 2.2).

The primary benefit of using the decalcification technique over more commonly used tissue blasting techniques (e.g., D'Croz and Mate 2004, Li et al. 2008), is the time it takes to handle samples. Decalcifying *Acropora* samples in mild hydrochloric acid takes up to 5 days, but there is very limited handling time during this process. Following decalcification, the time taken to section tissues, prepare a homogeneous solution, and count the zooxanthellae in 3 replicate aliquots was <10 minutes. Importantly, this process removes the time-limiting step of carefully blasting tissues from intact coral skeletons, which in itself takes 10-15 minutes per sample. Moreover, it negates the need to retrospectively measure the surface area of the intact coral sample. There are numerous methods available to measure the surface area of coral samples, which vary in their accuracy (Jones et al. 2008, Naumann et al. 2009), but all are fairly time-consuming. This study used the foil wrapping technique (Marsh 1970), which aside from developing the required calibration curve, took up to 8 minutes to wrap and cut, and then weigh the foil for each coral branch.

Irrespective of the potentially increased efficiency in processing samples, the fewer steps involved in the decalcification technique may reduce inaccuracies in measuring zooxanthellae densities in coral tissues. The primary concern identified in the decalcification process, is the accuracy with which small sections can be cut from the decalcified tissues, due to their elasticity and flexibility. Increasing the size of the coral sample that is taken (up to 1cm^2) will reduce extrapolation of errors when scaling up to number of zooxanthellae per cm^2, but embedding tissue sections in paraffin wax, prior to cutting precise sections, could also make further improvements. Furthermore, some of the ethanol may have evaporated, which would inflate the mean zooxanthellae population density. In comparison, there are a number of potential inaccuracies associated with standard tissue blasting methods, including loss of zooxanthellae due to spillage, and incomplete tissue removal during waterpiking and airbrushing (Johannes and Wiebe 1970). Methodologies used to retrospectively measure the surface area of intact coral samples may also introduce further sources of error. In foil wrapping, the surface area of irregular coral samples is likely to be overestimated due to difficulties in getting smooth, non-overlapping coverage of the entire sample (Hoegh-Guldberg 1988), which would further reduce the estimate of mean zooxanthellae density.

Accurate quantification of zooxanthellae densities in tissue samples from corals (and other zooxanthellate organisms) is critical for establishing the extent and severity of bleaching, which is increasingly becoming the major threat to coral reefs, globally (Hughes et al. 2003). This study presents an effective method for measuring zooxanthellae densities based on decalcification of coral samples, which requires less handling-time, and is potentially much more accurate, compared to currently widespread techniques based on blasting tissues from intact coral samples. Moreover, tissue samples can be immediately fixed in 10% buffered formalin (rather than freezing) prior to processing, and much less tissue is required for analyses, which is important if repeatedly sampling corals through time. Further

refinements of this technique may be required to obtain accurate estimates of zooxanthellae densities that are comparable within and among corals, especially for non-*Acropora* corals. However, this study has shown that it can be more efficient to estimate zooxanthellae densities in coral tissues that have been decalcified, rather than physically removed from intact coral skeletons, particularly when completing large quantities of zooxanthellae population density samples. If, however, sample sizes are small and laboratory space is limited, airbrushing samples may be a better choice. Moreover, future studies should use repeated measures as well as resolution and sensitivity testing to test for differences in methods.

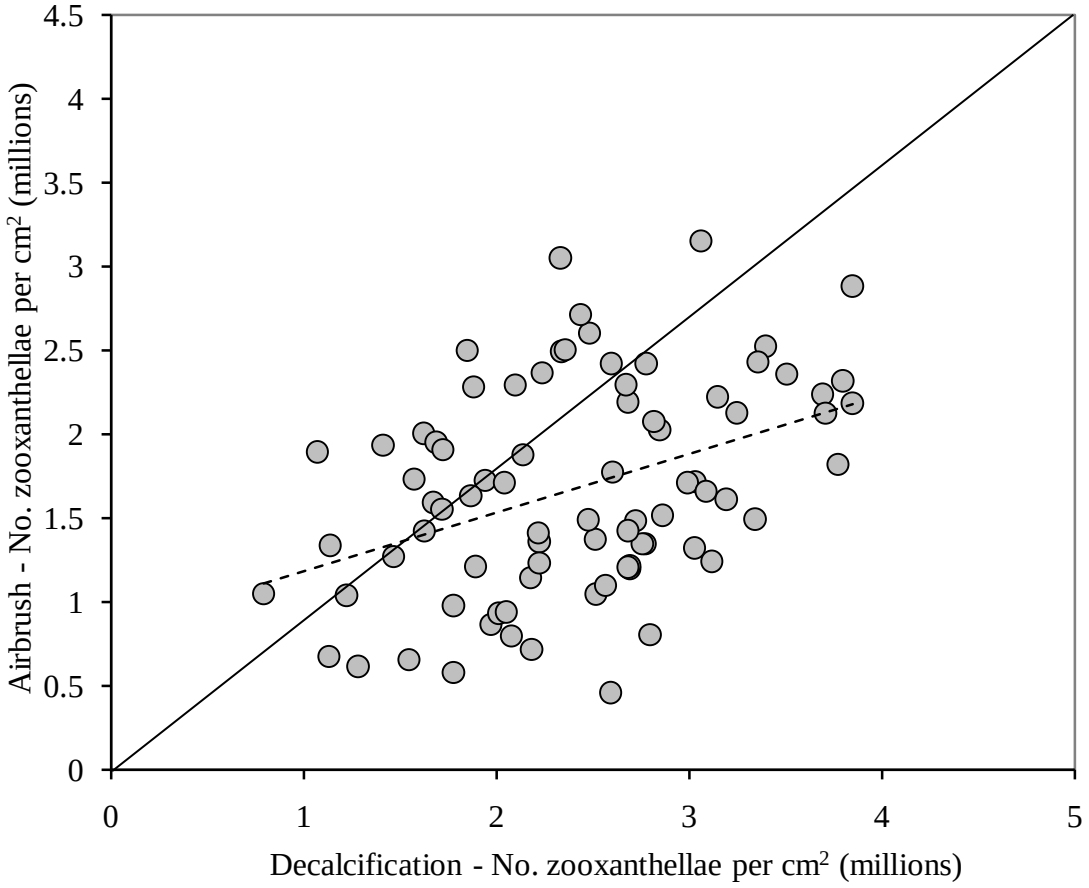

Figure 2.1: Comparative estimates of replicate branches of *A. millepora* zooxanthellae densities obtained using standard airbrushing of coral samples versus decalcification. While there was a significant correlation in the two estimates ($R = 0.41$, $n = 81$, $p<0.01$), the line of best fit (dashed line) diverges greatly from a 1:1 relationship (solid line).

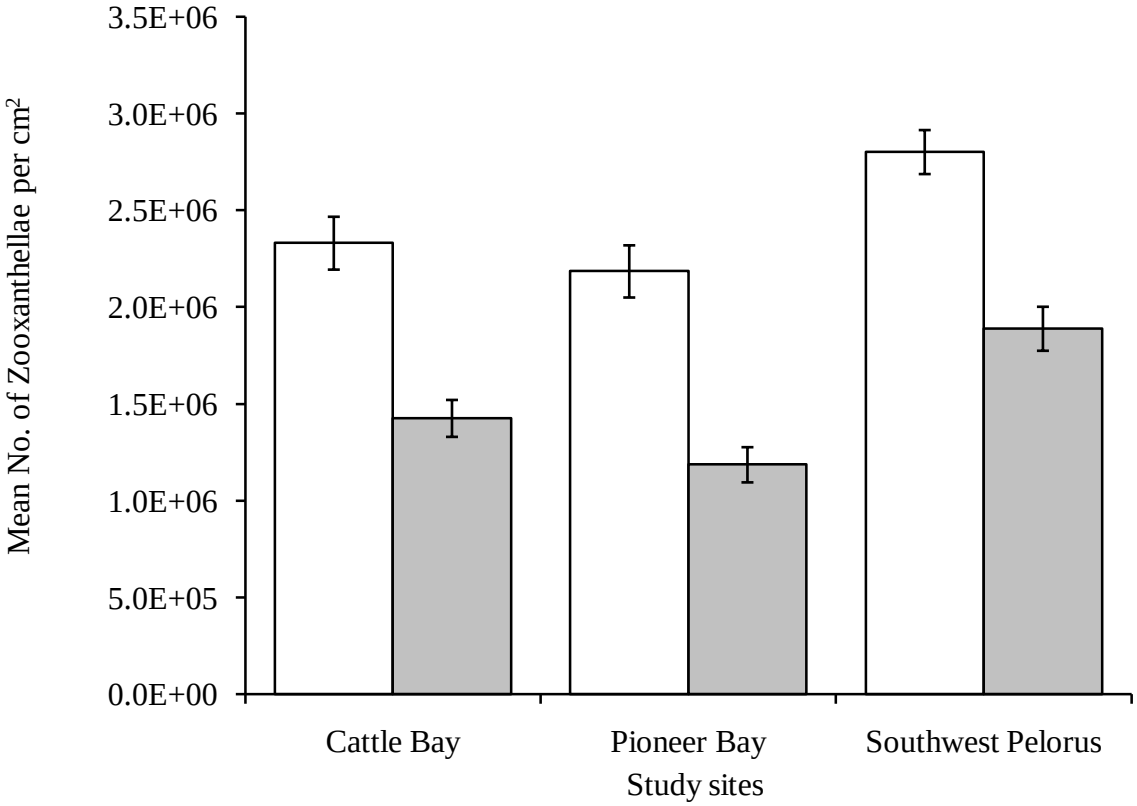

Figure 2.2: Mean (± SE) zooxanthellae densities for replicate colonies of *A. millepora* from three different locations in the Palm Islands, central Great Barrier Reef; paired samples were collected from each colony (N = 81 colonies) and standard airbrushing (grey bars) versus a decalcification technique (white bars) were compared.

Chapter 3: Distinguishing coral bleaching from background variation in zooxanthellae densities

3.1 Abstract

While coral bleaching can occur due to declines in the concentration of photosynthetic pigments, severe episodes of bleaching caused by acute environmental disturbances almost invariably result in declines in the density of zooxanthellae from host coral tissues. Accordingly, quantitative measures of zooxanthellae loss provide one of the most reliable and unequivocal indicators of bleaching. However, densities of zooxanthellae are highly variable spatially, temporally and taxonomically, suggesting that it is necessary to monitor changes in zooxanthellae densities within individually identified coral colonies rather than rely on an absolute threshold of zooxanthellae densities that can be used to infer whether or not a coral has bleached. This study combined data from 147 studies, comparing reported estimates of zooxanthellae densities between nominally healthy (unbleached) and bleached corals. This study showed that bleached corals had generally lower zooxanthellae densities compared to nominally healthy corals, but there was a large overlap in the range of values reported for bleached versus unbleached corals. Normal zooxanthellae densities recorded across 120 species of scleractinian corals ranged from $0.1x10^6$ cells/cm^2 up to 18.0 x 10^6 cells/cm^2; whereas, zooxanthellae densities reported for bleached corals (e.g. experiencing known environmental stresses) were between 0.001 and 6.5 x 10^6 cells/cm^2. Marked differences in published estimates of zooxanthellae densities appeared to be due, at least in part, to differences in the methods used to quantify zooxanthellae densities (e.g. size of tissue sample, method of tissue removal and surface area determination), though there were significant and consistent differences among coral species, with growth form and with depth. Results from these analyses suggest that when zooxanthellae population densities are below 1 million cells/cm^2 the colony can be considered degraded or at least sublethally bleached. Moreover,

after accounting for taxa (genera) it does appear that relative changes in zooxanthellae densities are a good indication of the fate of individual corals. In the absence of distinct bleaching events, natural variation in zooxanthellae densities (e.g., among seasons) was typically <50% of the mean. During bleaching events however, zooxanthellae loss within individual corals often ranged from 55- 100%. Moreover, corals that experienced >78% zooxanthellae loss almost invariably died, whereas those corals that lost 55-77% of zooxanthellae were bleached, but generally recovered. Rigorous quantitative estimates of zooxanthellae loss may therefore provide clear indications as to when coral have bleached, as well as predicting the likelihood of recovery.

3.2 Introduction

Corals have a variety of mechanisms to cope with stress (e.g. mucous secretion, tissue retraction) but the most common response to extrinsic disturbances (e.g., environmental extremes and pollutants) is significant and rapid decline in the density of zooxanthellae and/ or declines in the concentration of photosynthetic pigments within the zooxanthellae, (e.g., Vaughan 1916, Glynn 1991, Chapter 4), both of which, reduce photosynthetic yield. Declines in the concentration of photosynthetic pigments, rather than loss of zooxanthellae *per se*, is often related to prolonged exposure to low-level stresses (e.g., low light, Yonge and Nicholls 1931; herbicides, Jones 2004, Jones 2005) or precedes expulsion of zooxanthellae (Chapter 4). Acute stress, such as extreme temperature anomalies, may result in the rapid expulsion of zooxanthellae from host coral tissues (e.g. via host cell detachment, Gates et al. 1992). Recovery of the zooxanthellae pigmentation takes days to weeks (Le Tissier and Brown 1996) compared to loss in the zooxanthellae population density, which takes weeks to months to recover (Baird and Marshall 2002, Drollet et al. 1994, Drollet et al. 1995, Jiminez et al. 2001, Obura 2001). Increasing incidence of mass-bleaching episodes, involving simultaneous

bleaching across colonies and species, is strongly linked to increasing incidence and severity of temperature anomalies, which are ultimately caused by sustained and ongoing global climate change (Williams et al. 1987, Glynn 1991). Sustained increases in ocean temperatures bring baseline temperatures much closer to the maximum thermal tolerances for reef corals, such that even moderate temperature anomalies may cause bleaching. Mass-bleaching events are now commonplace on many reefs throughout the world (Wilkinson 1999), and are expected to increase in frequency and severity throughout this century (Hoegh-Guldberg 1999).

While awareness of coral bleaching has increased and it is often very obvious when mass-bleaching has occurred (e.g., Wilkinson 1998, 2002), there is not currently a clear and unambiguous definition that can be used to say exactly when individual coral colonies or populations are bleached. In the field, bleaching is often reported based on the conspicuous "paling" of individual coral colonies, species, or assemblages (e.g., Brown 1997), though the severity of bleaching within individual corals is sometimes measured based on direct comparison of physiological parameters, such as Pulse Amplitude Modulated (PAM) fluorometry and/or zooxanthellae densities (e.g., Jones 1997b and Okamoto et al. 2005). In experimental studies, bleaching is established and quantified using explicit measures of temporal changes in one or more of the abovementioned parameters, but these measurements may be taken at a variety of physiological levels (e.g., fragments, colonies). Given that mass coral bleaching is almost invariably due to declines in the density of zooxanthellae (Hoegh-Guldberg and Smith 1989a), explicit measures of zooxanthellae densities over time provide the most reliable and unambiguous definition of coral bleaching (Fitt et al. 2001). However, densities of zooxanthellae are naturally variable (spatially, temporally and taxonomically), which suggests that there is no absolute threshold of zooxanthellae densities that can be used to infer that a coral has bleached. This further suggests that there is an explicit requirement

for ongoing monitoring of individual coral colonies, such that the only unequivocal indicator that bleaching has occurred is a rapid or pronounced decline in zooxanthellae densities within individual coral colonies or populations. While there are at least 147 published studies that have quantified zooxanthellae densities among scleractinian corals, this data has never been formally analysed in order to test whether bleached corals can be distinguished from unbleached corals based on zooxanthellae densities.

Densities of zooxanthellae can vary greatly even for healthy coral colonies (e.g. Fagoonee et al. 1999), varying both within (Jones and Yellowlees 1997, Moothien-Pillay et al. 2005) and among coral colonies (e.g. Drew 1972, Dunstan 1979, Fitt et al. 2000, Li et al. 2008, and Stimson et al. 2002), among seasons and with location (Brown et al. 1995, Brown et al. 1999, Centeno 2002, Chen et al. 2005, Costa et al. 2005, Fagoonee et al. 1999, Fitt et al. 2000, Moothien-Pillay et al. 2005, Stimson 1997). Most notably, densities of zooxanthellae within an individual coral can be 2-3 times higher in winter, compared to summer months; though the extent of seasonal variation varies by location (Mwaura et al. 2010); corals from the tropics exhibit less seasonal variation in their zooxanthellae densities compared to corals from subtropical areas (e.g. Coles et al. 1976). Among taxa, bleaching susceptible corals (e.g. *Acropora*) are often noted as having lower average zooxanthellae densities than more resistant corals (e.g. *Porites*) (Li et al. 2008). Moreover, they suggest that branching morphologies have lower initial zooxanthellae densities, which may make them more susceptible to bleaching than massive morphologies. Similarly, Fitt et al. (2001) suggested that the mean summer zooxanthellae densities of corals are regularly lower than winter densities, making corals more susceptible to bleaching in summer months. This raises the question whether corals with naturally lower densities of zooxanthellae are more or less susceptible to bleaching.

The purpose of this study was twofold. The first objective was to quantify variation in zooxanthellae densities among seemingly healthy corals to test for differences among taxa, among locations and among habitats. The second aim was to compare absolute zooxanthellae densities documented during acute bleaching episodes to naturally observed variation in zooxanthellae densities (or background zooxanthellae variation) reported among seemingly healthy scleractinian corals, to test whether there is a consistent absolute density or proportional loss of zooxanthellae that can be used to characterise bleached corals. The ultimate aim was to assess whether there are specific thresholds of zooxanthellae loss below which a coral can be considered bleached and/ or committed to whole-colony mortality. If so, this will greatly improve the resolution possible in distinguishing between healthy and bleached coral colonies, which is a critical measure in assessing whether coral populations and communities have become more or less resilient to increased thermal maxima.

3.3 Materials and Methods

This study combined data from 147 published papers (Table 3.1), as well as estimates of zooxanthellae densities from an independent experiment conducted at Orpheus Island on the inshore Great Barrier Reef (Chapter 4). In the controlled experiment, replicate colonies of two common coral species (*Acropora spathulata* and *Pocillopora damicornis*) were subjected to a simulated bleaching event equivalent to the 1998 thermal anomaly (as experienced at Orpheus Island), recording the time taken until first evidence of bleaching, all the while recording changes in colour (*sensu* Siebeck et al. 2006) photo inhibition (via PAM Fluorometry with standardised quenching methods), and zooxanthellae densities. Zooxanthellae densities were quantified throughout the experiment to record absolute and relative changes in zooxanthellae densities associated with apparent bleaching (e.g., relating

this to observed colour loss), and were used here to compare with published estimates of absolute and relative zooxanthellae loss associated with sublethal versus lethal bleaching.

Densities of zooxanthellae in host coral tissues are expressed in various forms (e.g. per mL homogenate, Muscatine et al. 1989; per mg protein, Cook et al. 1988; per unit area of host coral tissue, Porter et al. 1984, 1989; per polyp, Lesser et al. 1990). Wherever possible, published densities of zooxanthellae were converted to the number of zooxanthellae within $1cm^2$ of coral tissue (cells/cm^2), following Porter et al. (1984). This measurement fails to take account of varying tissue depth, but for most corals there is a fairly consistent depth of tissue over the surface of skeleton, and/ or zooxanthellae are generally concentrated in surface tissues. Where it was not possible to convert published estimates of zooxanthellae densities (e.g., number of zooxanthellae per mg protein), data was excluded from analyses of absolute zooxanthellae densities, but were used (where possible) in analyses based on the proportional changes in zooxanthellae densities between healthy versus bleached corals.

3.3.1 Variation in zooxanthellae densities among healthy (unbleached) corals

For each study, data was extracted on the mean density of zooxanthellae for each individual coral species that was studied (as reported within text, tables or estimated following digital recalibration of graphical values). To capture variation within studies, we also recorded the maximum and minimum zooxanthellae density recorded for each coral species, whether it be the minimum recorded across different replicate colonies or the minimum density recorded through time for a single coral colony. This reduced the bias towards studies that extensively studied just one or two species over a vast areas or periods of time, focussing instead on variation among different studies, which may be attributable to differences in method, location or inherent differences among different coral taxa.

A series of independent 1-way ANOVAs were run for each variable (with Tukey's *post-hoc*, where appropriate), which included all appropriately categorized estimates of zooxanthellae density (where n > 5). ANOVA was used to establish the level (and significance) of variation in zooxanthellae estimates attributable to the methods (sample size, tissue removal and surface area determination), taxa (family, genus and species), morphology, and location (ocean basin). All data were square root transformed to meet assumptions, and *post hoc* test results are shown, where applicable. To compare among variables, F ratios are used (Figure 3.2), which already account for imbalances in the amount od data used to test each variable and give a relative indication of the importance of the hierarchy for causes of variation in mean healthy zooxanthellae population densities. Regression analyses were conducted to directly compare healthy versus bleached zooxanthellae densities for well-studies species.

3.3.2 Defining bleaching based on absolute zooxanthellae densities

In an attempt to distinguish between bleached and unbleached corals (e.g., Hueerkamp et al. 2001, Li et al. 2011) mean zooxanthellae densities were recorded for both healthy and bleached colonies of each coral species. For corals that were bleached, changes in zooxanthellae densities were expressed as the per cent loss compared to the mean (highs and lows recorded) and wherever possible, were separated into those that ultimately recovered ("sublethal bleaching") versus those that died ("lethal bleaching"). Studies that did not follow (or report) the ultimate fate of bleached coral colonies were used to extend the range of estimates of zooxanthellae for healthy corals, but the data on zooxanthellae densities of bleached corals was not used. For studies that reported bleaching due to species environemental stresses, only those that considered either i) changes in temperature (including experimental tests of temperature exposure, as well as transplants between depths

or latitudes), ii) changes in light (darkness, irradiance, light, light transplants, UVB and UVR), iii) changes in salinity, or iv) specific pollutants (antifoulant, copper, cyanide, herbicide, insecticide, lubricants, metal pollution) were included. Studies on the effects of added nutrients (e.g., Fagoonee et al. 1999) were excluded because they can have positive effects on zooxanthellae densities.

3.3.3 Proportional loss in zooxanthellae densities

Due to the diversity of methods used in quantifying zooxanthellae densities, there were very low numbers of studies that provide directly comparable absolute estimates of zooxanthellae densities (i.e. using similar methods across all aspects of the process). However, many studies report proportional declines in zooxanthellae densities (e.g., McCowan et al. 2011), based on estimates of zooxanthellae densities obtained using exactly the same methods before and after acute disturbances. Assuming that there is no inherent bias between methods in measuring absolute estimates of zooxanthellae densities across high (normal) and low (bleached) levels, then the proportional declines in zooxanthellae densities may be compared, regardless of the method used. Therefore, proportional differences in the zooxanthellae densities between healthy versus nominally bleached corals from the same population, was used to test for variation in the bleaching susceptibility among coral taxa, among locations, and among different causes of coral bleaching.

3.4 Results

3.4.1 Variation in zooxanthellae densities among healthy (unbleached) corals

A total of 403 records of zooxanthellae densities (cells/cm^2) for scleractinian corals were extracted from published sources (Table 3.1), to be used to assess variation in zooxanthellae densities across healthy (unbleached) corals (Table 3.2). The mean zooxanthellae density across all corals (regardless of species, habitat or location) was $3.93x10^6$ ($\pm$ $2.0x10^5$ SE

zooxanthellae per cm^2), ranging from $3.4x10^5$ zooxanthellae per cm^2 for *Favia favus* in the Red Sea, Eilat (Levy et al. 2003) up to $2.6x10^7$ zooxanthellae per cm^2 for *Coeloseris mayeri* in Thailand (Brown et al. 1999) (Figure 3.1).

Amongst the variables studied, most variation in published estimates of zooxanthellae densities for scleractinian corals was attributable to differences in the methods used to estimate zooxanthellae densities, including the size of the sample unit, method for tissue removal, and method of tissue surface area determination (Table 3.2, Figure 3.2, Figure 3.3). The size of the sampling unit used to determine mean zooxanthellae densities resulted in greatest variation in zooxanthellae densities (Figure 3.2), such that it is not really viable to compare between studies that use nubbins or small branches versus those that take *in situ* measurements to characterise bleaching at the level of entire colonies. Tukey's *post hoc* tests revealed three distinct groups, with the mean values ($1.25x10^7 \pm 1.99x10^6$ SE zooxanthellae per cm^2) obtained from coring samples from massive or encrusting corals (e.g., Agariciidae, Faviidae, Oculiniidae, and Poritidae) being significantly higher than estimates derived from entire coral colonies (mean = $4.78x10^6 \pm 1.88x10^5$ SE zooxanthellae per cm^2), where replicate branches were sampled to give an average across the entire colony. Lowest densities of zooxanthellae were obtained from experimental studies that focussed on small individual fragments of corals, such as nubbins (mean = $2.73x10^6 \pm 5.62x10^5$ SE zooxanthellae per cm^2), where typically there is only a single estimate of zooxanthellae density obtained by removing all tissue across the entire fragment or nubbin (Figure 3.3).

Alternative methods used to separate coral tissue form the underlying carbonate skeleton (i.e., decalcification, airbrushing and waterpiking) also caused significant variation in resulting estimates of zooxanthellae densities (Figure 3.2). Tukey's *post hoc* tests revealed that zooxanthellae densities were significantly higher based on estimates derived from decalcified samples (mean = $5.41x10^6 \pm 3.55x10^5$ SE zooxanthellae per cm^2, n=152),

compared to airbrushing (mean = $3.51x10^6$ $\pm 6.73x10^5$ SE zooxanthellae per cm^2, n=41) or waterpiking (mean = $2.59x10^6$ $\pm 1.97x10^5$zooxanthellae per cm^2, n=146). There was however, no significant difference in estimates obtained using airbrushing or waterpiking (Figure 3.3).

Methods of surface area determination (313/369 records) caused a significant amount of variation in mean zooxanthellae densities (Figure 3.2). Tukey's *post hoc* revealed significantly higher results for leaf-area image analysis (n=11), which had a mean zooxanthellae density of $1.24x10^7$ ($\pm 2.45x10^6$ SE zooxanthellae per cm^2). Modification of the paraffin wax method (n=13) and calipers (n=140) were also separated by Tukey's *post-hoc*, but not significant; the mean healthy zooxanthellae density for the modified paraffin wax method was $5.90x10^6$ ($\pm 1.88x10^6$ SE zooxanthellae per cm^2) and for calipers it was $4.95x10^6$ ($\pm 2.81x10^5$ SE zooxanthellae per cm^2). Mean healthy values from studies that calculated surface areas (mixture of graphing paper and vague descriptions, such as "measured") were $2.76x10^6$ ($\pm 4.34x10^5$ SE zooxanthellae per cm^2). Mean healthy zooxanthellae densities that were normalized to coral polyps (but presented in cells/cm^2) were $2.55x10^6$ ($\pm 7.01x10^5$ SE zooxanthellae per cm^2). Aluminum foil used for surface area determination produced a mean healthy zooxanthellae density of $2.66x10^6$ ($\pm 2.50x10^5$ SE zooxanthellae per cm^2). The original paraffin wax method produced mean values of $2.36x10^6$ ($\pm 2.30x10^5$ SE zooxanthellae per cm^2). For those interested, a separate ANOVA was run to compare the paraffin wax method with the modified version of this method, which produced significantly ($F_{(1,47)}=4.577$, $p<0.05$) higher estimates when modified.

3.4.2 Taxonomic differences in zooxanthellae densities

Aside from methodological differences, there were inherent differences in zooxanthellae densities apparent among different coral taxa. Tukey's *post hoc* revealed three distinct groups, where *Goniastrea aspera* had the highest mean healthy zooxanthellae density.

Acropora nasuta, *Pocillopora damicornis*, and *Porites lutea* had intermediate values. The third grouping, corals with low mean zooxanthellae densities, included *Montastrea annularis, Seriatopora hystrix, Montastrea faveolata, Porites cylindrica, Acropora millepora, Porites lobata* and *Stylophora pistillata*.

The bleaching susceptibility and mortality database had 40 published studies, which was comprised of 158 records for bleaching susceptibility of the species presented above and 123 records of mortality. There was significant variation attributed to species for bleaching susceptibility (ANOVA, $F_{(8,149)} = 3.155$, $p > 0.01$) and for bleaching-related mortality (ANOVA, $F_{(8,114)} = 2.838$, $p > 0.01$). Bleaching susceptibility of species was in the order of *Porites lobata*, *Porites lutea*, *Pocillopora damicornis*, *Acropora millepora*, *Montastrea annularis*, *Montastrea faveolata*, *Stylophora pistillata*, *Seriatopora hystrix* and most susceptible *Goniastrea aspera*, which was not coincident with the hierarchy of mean healthy zooxanthellae densities (Figure 3.4), and there was no clear relationship between bleaching susceptibility and healthy zooxanthellae densities (Figure 3.5). It could be however, that variation in zooxanthellae densities attributable to methodological differences among studies have obscured clear and consistent taxonomic differences. Moreover, bleaching-related mortality observed a different hierarchy than either mean zooxanthellae densities or bleaching susceptibility; *P. lobata* had the least amount of mortality, followed by *M. annularis, P. lutea, M. faveolata, G. aspera, S. pistillata, A. millepora, P. damicornis* and the species which has experienced the most mortality, *S. hystrix*.

Pocillopora damicornis data were analysed to establish effects of unit size (branch, fragment orcolony) on estimates of zooxanthellae densities, which yielded significantly different results. (ANOVA, $F_{(2, 58)} = 50.728$, $p<0.00$). Tukey's *post hoc* separated each of the categories, with branches (n=11) having the lowest average density of ($1.24x10^6 \pm 3.4x10^5$ SE zooxanthellae per cm^2, fragments (n=11) having medium healthy density of $3.01x10^6$

(±6.72x10^5 SE zooxanthellae per cm^2) and colonies (n=37) having the highest mean healthy zooxanthellae density of 3.01x10^6 (±1.22x10^5 SE zooxanthellae per cm^2). These were later pooled into branch or colony (Figure 3.3).

Zooxanthellae densities were clearly different among coral genera and families (Figure 3.2), although due to the all of the variation in methods (and likely geographic variation in zooxanthellae densities which were not accounted for), the counts were too low to determine a mean healthy genus zooxanthellae density. Tukey's *post-hoc* separated the lower mean zooxanthellae densities of the genera *Acropora*, *Pocillopora*, *Pavona*, and *Goniastrea*. For family data, Tukey's *post-hoc* test grouped Agariciidae and Oculiniidae as having higher (not significant) healthy zooxanthellae densities. Pocilloporidae had the lowest zooxanthellae densities, closely followed by Poritidae, Acroporidae and Faviidae. Agariciidae and Oculiniidae had higher average zooxanthellae densities then the other families.

Growth form (n=309/369 records) had a significant effect on variation in zooxanthellae densities amongst healthy corals (Figure 3.2). Encrusting growth forms (n=10) had the highest mean zooxanthellae density, 5.41x10^6 (±1.46x10^6 SE zooxanthellae per cm^2), and was separated by Tukey's *post hoc*, but not significant. Branching corals (n=191) had an average zooxanthellae density of 3.59x10^6 (±1.71x10^5 SE zooxanthellae per cm^2), with high values (n=133) averaging 4.0x10^6 (±2.1x10^5 SE zooxanthellae per cm^2) and low values (n=133) averaging 3.3x10^6 (±2.1x10^5 SE zooxanthellae per cm^2). Massive corals had an average zooxanthellae density of 5.38x10^6 (±6.05x10^5 SE zooxanthellae per cm^2), with high values (n=35) averaging 6.73x10^6 (±1.09x10^6 SE zooxanthellae per cm^2) and low values (n=35) averaging 5.12x10^6 (±9.22x10^5 SE zooxanthellae per cm^2). Foliose corals (n=15) had the lowest mean zooxanthellae density, 1.94x10^6 (±3.26x10^5 SE zooxanthellae per cm^2) and were separated by Tukey's *post host*, although non-significant.

Depth had a significant effect on mean zooxanthellae densities; however, the relationship was such that corals decreased in zooxanthellae densities until >20m, but increased thereafter. Mean healthy zooxanthellae densities for <3m were $3.99x10^6$ ($\pm 5.35x10^5$ SE zooxanthellae per cm^2). Depths ranging between 3-6m had average zooxanthellae densities of $4.50x10^6$ ($\pm 2.37x10^5$ SE zooxanthellae per cm^2). From 7-10m, zooxanthellae densities averaged $2.48x10^6$ ($\pm 4.80x10^5$ SE zooxanthellae per cm^2). From 11-19m, zooxanthellae densities averaged $1.94x10^6$ ($\pm 2.60x10^5$ SE zooxanthellae per cm^2). At depths >20m, zooxanthellae densities averaged $3.38x10^6$ ($\pm 6.46x10^5$ SE zooxanthellae per cm^2).

The ocean basin did not have a significant effect on mean healthy zooxanthellae densities; however, within ocean basins, bleaching was significantly different from mean healthy zooxanthellae population densities (ANOVA, $F_{(2,178)} = 14.434$, $p<0.001$). Tukey's *post hoc* showed a significant difference for bleached zooxanthellae densities from the Pacific Ocean compared to the Atlantic and Indian Oceans. Mean healthy and bleached zooxanthellae densities of pair-wise data for the Atlantic Ocean (n=31) healthy: $2.69x10^6$ ($\pm 3.17x10^5$ SE zooxanthellae per cm^2), bleached: $4.85x10^5$ ($\pm 9.36x10^4$ SE zooxanthellae per cm^2). For the Indian Ocean (n=19) healthy densities average $5.24x10^6$ ($\pm 1.24x10^6$ SE zooxanthellae per cm^2) and bleached densities were $1.01x10^6$ ($\pm 2.65x10^5$ SE zooxanthellae per cm^2). In the Pacific Ocean (n=128), healthy densities averaged $7.43x10^6$ ($\pm 2.13x10^6$ SE zooxanthellae per cm^2) and bleached densities averaged $2.78x10^6$ ($\pm 3.89x10^5$ SE zooxanthellae per cm^2).

3.4.3 Defining bleaching based on mean zooxanthellae densities

103 records of zooxanthellae densities (cells/cm^2) in experimentally bleached corals were entered into the database. The average zooxanthellae densities for these bleached corals was

$1.26x10^6$ ($\pm 8.78x10^5$ SE zooxanthellae per cm^2), and ranged from $5.00x10^5$ zooxanthellae per cm^2 for thermal stress/seasonal variation in *Montastrea annularis* (Fitt et al. 2001) up to $3.8x10^6$ zooxanthellae per cm^2due to solar irradiance for *Goniastrea aspera* (Le Tissier and Brown 1996) (Figure 3.1). Despite inherent taxonomic variation in the zooxanthellae densities, direct (pair-wise) comparisions between healthy and bleached corals of each species revealed a strong positive relationship (r^2= 0.509, $F_{(1, 186)}$ = 194, $p<0.001$), and bleached corals had an average of 71% lower densities of zooxanthellae compared to healthy colonies of the same species.

Efforts were made to find an absolute density of zooxanthellae that could be used to clearly distinguish between bleached versus unbleached corals (e.g. Figure 3.1). It appears that if corals have a zooxanthellae population density of below $1x10^6$ cells/cm^2 it should be interpreted as at least sublethal stress, potentially bleaching. However, given marked variation in published estimates of zooxanthellae densities the highest factors (analysed in this study) were pooled into categories based on Tukey's *post hoc* tests and then they were nested based on F-ratios (Figure 3.2) and graphed (Figure 3.3). Due to the variability in the methods employed, the counts were too few to continue to find a definition of bleaching based on mean zooxanthellae densities, therefore the percent difference from the mean was used.

3.4.4 Bleaching defined through proportional loss in zooxanthellae density

Bleaching is clearly distinguishable from natural variation in zooxanthellae densities based on the relative change in zooxanthellae densities. Even in the most extreme cases of natural fluctuations in zooxanthellae densities for individual corals or populations, zooxanthellae densities varied by less than 50% of the overall mean; fluctuations below the mean (33%) were greater than above the mean (27%). In contrast, proportional declines in zooxanthellae

densities reported for corals that bleached, but recovered, were generally >55%. Moreover, in cases where corals bleached and died, proportional declines in zooxanthellae densities were generally >78%.

Given marked variation in the natural densities of zooxanthellae among scleractinian corals, it makes sense that proportional loss in zooxanthellae densities (rather than absolute or threshold densities of zooxanthellae) provides the most reliable indication of the timing and severity of coral bleaching. Variability in the percent loss associated with both sublethal and lethal bleaching of corals was affected by the ocean basin, methods for tissue removal, method for sample size, causal agent and timeframes. Sublethal bleaching was significantly different among ocean basins (ANOVA, $F_{(2, 182)} = 3.369$, $p<0.05$) with increased percentage loss from the Atlantic (n=38, mean=49%) to the Pacific (n=106, mean=58%) and the Indian (n=39, mean=57%). Methods for tissue removal caused significant variability (ANOVA, $F_{(2, 137)} = 22.217$, $p<0.05$): decalcification (n=62) was significantly higher (Mean 67±1.3) than airbrushing (n= 24, mean = 55±3.5%) or waterpiking (n=52, mean = 50±2.2%). Methods for sample size caused significant variability (sublethal, $F_{(6,165)} = 2.423$, $p<0.05$): samples (n=10) and fragments (n=20) were lower (separated by Tukey's *post hoc*) than other sizes (49% and 52%, respectively), while core samples (n=13) were higher than other sizes (72%). The range of sublethal bleaching from other categories of methods SS (branch (n=33), colony (n=65) and subsamples (n=11)) was 55-60%. Lethal bleaching percentage loss was also affected by methods SS ($F_{(5, 92)} = 9.553$, $p<0.05$). Samples (n= 28) were separated by Tukey's *post hoc* as lower (64%), while colonies (n=18) were separated as higher (89%): the range of other categories (anemone (n=9), branch (n=12), fragment (n=20), subsample (n=6)) was 68-83%.

For the causal agent of sublethal bleaching ($F_{(4,151)} =12.074$, $p<0.000$), Tukey's *post-hoc* showed that the percent loss associated with light, temperature and multiple stressors (most often representing temperature and another variable) was significantly higher than the

percent loss associated with cold shock or pollutants (both of which are within "natural variation"). For lethal bleaching (ANOVA, $F_{(4, 91)} = 6.466$, $p<0.000$), the percent loss associated with cold-shock was significantly lower than the other causes. Zooxanthellae loss associated with sublethal bleaching due to increased temperature averaged 59.1±1.60%, for sublethal bleaching (n = 79) and 77.6±2.20 for lethal bleaching (n = 48). Synergistic stressors showed an average loss of 58.5% (±3.44 SE) for sublethal bleaching (n = 19), and an average loss of 85.9% (±4.17 SE) associated with lethal bleaching (n = 8). For light stress, the average loss associated with sublethal bleaching (n = 17) was 56.9% (±4.71 SE), while lethal bleaching (n = 9) averaged a loss of 82.0% (±4.76 SE). Pollutants caused an average loss of 41.9% (±2.42 SE) for sublethal bleaching (n = 23), while for lethal bleaching (n = 15), the loss averaged 77.4% (±2.78 SE). Cold-shock caused an average loss of 33.7% (±5.14 SE) for sublethal bleaching (n = 14), and 53.8% (±5.37 SE) for lethal bleaching (n = 12).

The timeframes of experiments/observations also had a significant impact on the mean zooxanthellae loss. Importantly, very few studies accounted for changes in the condition of coral colonies over time (but see Chapter 4). Zooxanthellae loss associated with sublethal (ANOVA, $F_{(5,182)} = 7.541$, $p<0.001$) and lethal (ANOVA, $F_{(5, 117)} = 6.108$, $p<0.001$) bleaching significantly increased with time and in both cases, Tukey's *post hoc* separated the lower mean values of one day experiments, which were significantly lower for sublethal stress. Experiments that were ≤1 day averaged 40.1% (±3.54 SE) loss associated with sublethal bleaching (n = 32) and 64.2 (±0.67 SE) for lethal bleaching (n = 28). Experiments that were a few days to a month averaged 52.7% (±2.03 SE) for sublethal bleaching (n = 46), and for lethal bleaching (n = 35), bleached values averaged 75.9% (±0.34 SE). Experiments that were one to three months averaged 49.2% (±2.13 SE) for sublethal bleaching (n = 22) and 85.7% (±0.53 SE) for lethal bleaching (n = 42). Finally, experiments or observations over

three months averaged 64.0% (±2.01 SE) for sublethal bleaching (n = 42), and lethal bleaching (n = 33) averaged 84.8% (±0.35 SE).

3.5 Discussion

3.5.1 Variation in zooxanthellae densities among healthy (unbleached) corals

Coral bleaching is most apparent due to the white skeleton becoming visible due to the acute loss of zooxanthellae from within host coral tissues (e.g., Glynn 1996). This is in contrast to natural variations in zooxanthellae populations densities (e.g. Jones and Yellowlees 1997), which have a slower rate of change than acute bleaching (Chapter 4). This study shows that densities of zooxanthellae vary greatly even among seemingly healthy (unbleached) corals (Figure 3.1). Moreover, absolute estimates of zooxanthellae densities within host coral tissues are highly conditional upon the size of the sample unit (branch, fragment or colony), methods used both to separate coral tissues from the underlying skeleton (decalcified or stripped), and to estimate surface area of the coral sample (Figure 3.2), as well as taxon (species and genus) and habitat (specifically, depth). Further differences in estimates of zooxanthellae densities probably also relate to differences in experimental protocols. For instance, the time that corals are left to acclimate to aquarium conditions ranges from hours to weeks (although they were not fully recorded or analysed, but see Coles and Jokiel (1978) and Berkelmans and Willis (1999), and another likely source of variation is the replication of homogenized subsamples, which ranged from 2-12. More importantly, it seems that results obtained using the markedly different methods are often not comparable, thereby requiring standardisation of methods (Bucher and Fischer 2006) if we are to maximize interpretation across multiple disparate studies moving forward. For this reason, we advocate decalcifying coral tissues and

then estimating zooxanthellae densities within a specific section of tissue with known physical dimensions (see Chapter 2).

Despite differences in estimates of zooxanthellae densities, it is clear that bleached corals have lower zooxanthellae densities compared to healthy, but otherwise similar corals. However, the range of zooxanthellae densities recorded for corals that bleach, but do not die (sublethal bleaching) is well within the range of zooxanthellae densities recorded for seemingly healthy corals. Also, corals that bleached and did not recover (lethal bleaching) were reported to have $<1.3x10^6$ zooxanthellae per cm^2, which is clearly at odds with some earlier estimates of "normal" zooxanthellae densities; one million cells/cm^2 has commonly been cited as the average healthy zooxanthellae density (e.g. Drew 1972), but clearly this is dependent upon the methods and scale of observations. Glynn (1996) quoted the average healthy zooxanthellae density of $1\text{-}5x10^6$ cells/cm^2, while Li et al. (2008) found a range of healthy zooxanthellae densities from $0.67\text{-}\ 8.48\times10^6$ cell/cm^2 in the South China Sea. Dustan (1979) found a range of healthy zooxanthellae densities for *Montastrea annularis* that ranged from $2.65\text{-}8.76x10^6$ cells/cm^2. The full range of reported zooxanthellae densities ranges from $0.1\text{-}18.0 \times 10^6$ cells/cm^2, though some of these lower estimates are subject to some methodological issues. Even so, it is clear that "normal" densities for some corals and in some locations are well below that which characterises lethal bleaching in others.

Aside from methodological issues, zooxanthellae densities are clearly variable within and among coral taxa, as reported previously by Drew (1972) and Li et al (2008). The highest zooxanthellae density ($1.16x10^7 \pm 2.28x10^6$ SE zooxanthellae per cm^2) was recorded for *Goniastrea aspera*, a massive coral, while the lowest mean healthy zooxanthellae density was from *Stylophora pistillata*, a common branching coral ($1.87x10^6 \pm 5.92x10^5$ SE zooxanthellae per cm^2). Contrary to previous suggestions (e.g. Fitt et al. 2001, Li et al. 2008) variation in mean zooxanthellae densities of healthy coral species do not appear to correspond with inter-

specific differences in bleaching susceptibility. The results of this study suggest that morphology alone does not explain variations in mean healthy zooxanthellae densities, rather that variation in healthy zooxanthellae densities are also driven by morphologies within taxonomy, such as the effect observed on bleaching susceptibility and mortality (Chapter 6). It may be that differences in zooxanthellae densities are an important determinant of intra-specific variation in bleaching susceptibility, but there were generally too few comparable estimates of zooxanthellae densities for any one coral species to test this. Intraspecific variations in mean healthy zooxanthellae densities were observed for *P. damicornis*, the most commonly studied coral, which had a range of healthy zooxanthellae densities from $0.35x10^6$ cells/cm^2 (Muller-Parker et al. 1994) up to $10x10^6$ cells/cm^2 in Hueerkamp et al. (2001), but it does not appear that corals with highest initial zooxanthellae densities are any more or less susceptible to bleaching (see also Chapter 4), though such analyses will need to account for the type of zooxanthellae, as well as many other specific extrinsic factors that might influence bleaching.

In this study, there were no significant large-scale (geographic) differences in zooxanthellae densities, but there was a marked and consistent trend with depth, with shallower sites (1-3m) generally having higher zooxanthellae densities than deeper sites (up to 20m, where the pattern reversed and increases in mean healthy zooxanthellae densities were observed). This pattern has been reported previously (e.g., Dustan 1979, Li et al. 2008) and appears to be a response to changes in light and nutrient availability. Where less light occurs, fewer zooxanthellae are present; the opposite trend in deeper water corals (>20m) puzzled Dustan (1979), he explained the geometry of the photo-symbionts and how their shape affects light-scattering, so perhaps there is a different order present in deeper water corals to assist in maximising light capture.

3.5.2 Bleaching defined through proportional loss in zooxanthellae density

Although there was high variation in zooxanthellae densities recorded within and among seemingly healthy scleractinian corals (Figure 3.2 and 3.3), it appears that values below one million cells/cm^2 should be considered bleached corals. However, due to the variability in mean zooxanthellae population densities, the preferred method of distinguishing bleaching should be through use of baseline data and proportional difference in mean zooxanthellae density. In this study, natural variation in zooxanthellae densities averaged <50%, while bleaching of zooxanthellae was defined as >55% reduction in zooxanthellae densities. There are a few special cases where bleaching has been reported, but the declines in zooxanthellae densities are well below 55%, which tend to be associated with marked loss of pigments (e.g. Jones et al. 1997a, Kleppel et al. 1989, and Okamoto et al. 2005), rather than zooxanthellae loss. Importantly, corals that bleach due to general declines in pigment concentrations, but not zooxanthellae densities, can recover very rapidly (e.g. Le Tissier and Brown 1996), or ongoing bleaching will almost invariably result from loss of zooxanthellae. Therefore, these exceptions would not greatly alter conclusions about the likely fate of the corals.

In extreme cases, where proportional zooxanthellae loss is >78%, which occurs due to very severe or prolonged exposure to adverse conditions, it is very likely that the colonies cannot recover (e.g. Hueerkamp et al. 2001, Chapter 4), but this is dependent upon the level of colony integration (e.g. concentration of stress in massive colonies producing partial mortality, Baird and Marshall 2002). These results show that it may be possible to establish i) when corals have bleached, and ii) the fate of bleached corals, based on the proportional loss of zooxanthellae. However, this requires that either there is a very detailed baseline of zooxanthellae densities for individual coral populations (e.g., requiring extensive monitoring in time and space) or individual corals are followed through the course of predicted bleaching

events, the key being that there are some estimates of zooxanthellae densities prior to the bleaching occurring.

3.5.3 Spatial and temporal variation in zooxanthellae loss

While proportional declines in zooxanthellae densities (ideally, at the level of individual coral colonies) provide the most effective and unequivocal indication of coral bleaching, there was significant variation in proportional zooxanthellae loss in time and space, and depending upon the cause of bleaching (e.g., increasing temperature versus pollutants). Interestingly, the Indian Ocean had the highest mean zooxanthellae densities, and the Atlantic Ocean had the smallest loss associated with bleaching. This may suggest that baseline zooxanthellae densities in the Atlantic Ocean have been lowered (e.g. Pandolfi et al. 2005), in comparison to Pacific and Indian Ocean corals, and therefore, they have less to lose. It is important, however, to further explore these data to test whether marked differences in the structure of coral assemblages in each geographic location are the explanation for such findings. It may be that increased incidence of coral bleaching in the Atlantic (specifically, the Caribbean) has already caused selective filtering and directional shifts in the structure of coral assemblages, such that species with generally high zooxanthellae densities are already becoming less abundant.

Among different causes of coral bleaching, proportional declines in zooxanthellae densities were fairly consistent across thermal, photic, or synergistic stressors. However, average declines in zooxanthellae densities attributable to cold-shock events and pollutants were fairly moderate, and well within the range of natural variation. Importantly, most of the cold-shock data came from the work of Leonard Muscatine (e.g. Muscatine et al 1991), who monitored expulsion rates, and more than likely did not account for zooxanthellae that were

degraded *in situ*. Furthermore, one interpretation of the pollution data is that chronic stressors already lowered baseline zooxanthellae densities.

The only causal agent known to increase zooxanthellae densities is nutrient enrichment (e.g. ammonia), which has been shown to increase mean zooxanthellae densities an average of 79% (Fagoonee et al. 1999, Ferrier-Pages et al. 2001, Marubini and Davies 1996, Muller-Parker et al. 1994, Muscatine et al. 1989, Nordemar et al. 2003, Schloder and D'Croz 2004, Stambler et al. 1994, Stimson and Kinzie 1991, Stimson 1997). In corals, ammonia is regulated by urea, such that the measurement of urea has been suggested as an alternative method of diagnosing coral health (Bucher and Fischer 2006). It is likely that urea and ammonia from fish and invertebrate populations have an impact on mean healthy zooxanthellae densities (e.g. Meyer and Schultz 1985). This may provide added incentive to conserve coral reef habitats through the use of no-take reserves, whereby increasing local abundance of fishes may serve to increase health and condition of coral populations, through added nutrient inputs to the system (Fitt and Cook 2001, McGuire and Szmant 1997, Schloder and D'Croz 2004, Stambler et al. 1994, Stimson 1997).

3.5.4 Conclusions

Reported zooxanthellae densities for healthy (unbleached) corals vary enormously (Drew 1972, Muscatine 1980, Porter et al. 1984, Hoegh-Guldberg and Smith 1989a, b, Falkowski et al. 1993, Stimson 1997), ranging from 0.1 x 10^6 cells/cm^2 to18.0 x 10^6 cells/cm^2. In comparison, the mean densities of zooxanthellae recorded for bleached corals ranged from 0.001 x 10^6 cells/cm^2 in a massive *Porites* exposed to high temperature and high UVR (D'Croz et al. 2001) to 6.5 x 10^6 cells/cm^2 in *Plesiastrea versipora* following cyanide exposure (Jones and Hoegh-Guldberg 1999). Consequently, it is not possible to clearly distinguish bleached versus healthy coral colonies based on absolute densities of

zooxanthellae within the coral tissues; however, values < $1x10^6$ cells/cm^2 should be viewed as either degraded or bleached. A more accurate method, however, is to characterise bleaching based on the proportional change in zooxanthellae densities within individual coral colonies of populations; declines of >55% tend to indicate that coral have bleached but are likely to recover, whereas declines of >78% suggest that bleaching will likely be lethal. The proportional change in zooxanthellae densities does vary with the causal agent of bleaching, but abovementioned thresholds are generally applicable for climate-induced episodes of coral bleaching. Future research could be aimed at determining the relationships between tissue biomass, zooxanthellae densities and mortality potential.

Table 3.1: Comprehensive list of data sources (citations) used to obtain information on reported zooxanthellae densities in scleractinian corals, arranged by alternative causes of zooxanthellae fluctuations

Cause	Source
Aerial Exposure	Brown et al. 1995, Anthony and Kerswell 2007
Ammonium	Muscatine *et al.* 1989, Muller-Parker *et al.* 1994, Stambler *et al.* 1994, McGuire and Szmant 1997, Titlyanov *et al.* 2000a, Fitt and Cook 2001, Schloder and D'Croz 2004
Antifoulant	Smith et al. 2003
Branch Gradient	Gladfelter et al. 1989, Jones and Yellowlees 1997, McCowan et al. (Chapter 4)
Bacterial Infection	Toller et al. 2001, Rozenblat and Rosenberg 2004, Shenkar *et al.* 2006
Cold-shock	Steen and Muscatine 1987, Muscatine *et al.* 1991
Cyanide	Jones and Steven 1997, Jones and Hoegh-Guldberg 1999, Jones *et al.* 1999, Cervino *et al.* 2003
Depth	Drew 1972, Dunstan 1979, Li et al. 2008
Disease	Cervino *et al.* 2001, Toller *et al.* 2001, Cervino *et al.* 2004, Ravindran and Raghukumar 2006
Enrichment	Meyer and Schultz 1985, Musctaine *et al.* 1989, Stimson and Kinzie 1991, Falkowski *et al.* 1993, Marubini and Davies 1996, Stimson 1997, Dawson *et al.* 2001, Ferrier-Pages *et al.* 2001, Nordemar *et al.* 2003
Herbicide	Jones and Kerswell 2003, Jones 2004, Negri *et al.* 2005
Insecticide	Markey *et al.* 2007
Irradiance	Lesser *et al.* 1990, Le Tissier and Brown 1996, McCloskey *et al.* 1996, Salih *et al.* 2000, Bourne *et al.* 2007, Anthony et al. 2007
Light	Hoegh-Guldberg and Smith 1989a , Jones and Hoegh-Guldberg 1999, Titlyanov *et al.* 2000a, Toller *et al.* 2001, Titlyanov *et al.* 2002, Verde and McCloskey 2002, Levy *et al.* 2003, Talge and Hallock 2003, Dove 2004, Yakovleva and Hidaka 2004
Low light	Falkowski and Dubinsky 1981, Steen and Muscatine 1987, Hoegh-Guldberg and Smith 1989a, Mascarelli and Bunkley-Williams 1999, Titlyanov *et al.* 2001a, Abramovitch-Gottlib *et al.* 2005, Visram 2005, Titlyanov *et al.* 2006, Visram and Douglas 2007
Light (Natural Variation)	Porter et al. 1984, Kinzie III 1993, Titlyanov and Titlyanova 2002
Natural Bleaching	Hayes and Bush 1990, Szmant and Gassman 1990, Jones and Yellowlees 1997, Stimson et al. 2002, Brown and Dunne 2001, Lasker 2003, Leggat *et al.* 2003, Talge and Hallock 2003, Tseng 2004, Jones 2008
Pollutants	Harland and Brown 1989, Smith *et al.* 2003, Jones 2004, Mercurio *et al.* 2004, Mitchelmore *et al.* 2007

Salinity/Osmotic Shock	Hoegh-Guldberg and Smith 1989a, Titlyanov *et al.* 2000b, Dawson *et al.* 2001, Talge and Hallock 2003
Seasonal Variation	Fitt *et al.* 1993, Brown et al. 1995, Stimson 1997, Brown et al. 1999, Fitt et al. 2000, Dunne and Brown 2001, Centeno 2002, Warner et al. 2002, Costa et al. 2004, Chen et al. 2005, Costa et al. 2005, Moothien-Pillay et al. 2005, Winters et al. 2005, Verde and McCloskey 2007
Sedimentation	Peters and Pilson 1985, Philipp and Fabricius 2003
Size	Verde and McCloskey 1998, Shenkar et al. 2005
Starvation	Fitt *et al.* 1982, Clayton and Lasker 1984, Cook *et al.* 1988, Titlyanov *et al.* 1996, Titlyanov *et al.* 2000b, Fitt and Cook 2001, Titlyanov *et al.* 2001, Nordemar *et al.* 2003, Rodolfo-Metalpa *et al.* 2008
Temperature	O'Brien and Wyttenbach 1980, Steen and Muscatine 1987, Hoegh-Guldberg and Smith 1989a, Hoegh-Guldberg and Smith 1989b, Porter *et al.* 1989, Glynn and D'Croz 1990, Lesser *et al.* 1990, Fitt *et al.* 1993, Brown *et al.* 1995, Fitt and Warner 1995, Hoegh-Guldberg and Salvat 1995, Jones *et al.* 1998, Warner *et al.* 1996, Jones 1997, Fagoonee *et al.* 1999, Warner *et al.* 1999, Brown *et al.* 2000, Fitt *et al.* 2000, D'Croz *et al.* 2001, Dawson *et al.* 2001, Hueerkamp *et al.* 2001, Perez *et al.* 2001, Verde and McCloskey 2001, Brown *et al.* 2002a, Brown *et al.* 2002b, Centeno 2002, Dunn *et al.* 2002, Mise and Hidaka 2002, Warner *et al.* 2002, Abramovitch-Gottlib *et al.* 2003, Edmunds *et al.* 2003, Estes *et al.* 2003, Harithsa *et al.* 2003, Leggat *et al.* 2003, Talge and Hallock 2003, D'Croz and Mate 2004, Schloder and D'Croz 2004, Yakovleva and Hidaka 2004, Edmunds *et al.* 2005, Okamoto *et al.* 2005, Strychar *et al.* 2005, Visram 2005, Berkelmans and van Oppen 2006, Franklin *et al.* 2006, Rodolfo-Metalpa *et al.* 2006a, Rodolfo-Metalpa *et al.* 2006b, Bourne *et al.* 2007, Flores-Ramirez and Linan-Cabello 2007, Hill and Ralph 2007, Leutenegger *et al.* 2007, Visram and Douglas 2007, Hill and Ralph 2008, Rodolfo-Metalpa *et al.* 2008, Carilli et al. 2012
Transplant Depth	McCloskey and Muscatine 1984, Gleason and Wellington 1993, Edmunds and Gates 2002, Berkelmans and van Oppen 2006
UVR	Jokiel and York 1982, Lesser *et al.* 1990, Gleason 1993, Gleason and Wellington 1993, Kinzie 1993, Brown *et al.* 1995, Shick *et al.* 1999, Brown *et al.* 2000, D'Croz *et al.* 2001, Drohan *et al.* 2005, Hill and Ralph 2008, Rodolfo-Metalpa *et al.* 2008
Zooxanthellae observations	Verde and McCloskey 2001, Verde and McCloskey 2002, Titlyanov et al. 2006, Verde and McCloskey 2007

Table 3.2: Identified sources of variation in mean healthy zooxanthellae densities written as it was entered for statistical purposes

Source of Variation	Categories
Method for Sample Size	Branch, colony, core, fragment, nubbin, sample
Method for Tissue Removal	Airbrushing, decalcification, or waterpiking
Method for Surface Area Determination	Calculated, calipers, foil, image analysis, modified paraffin wax, paraffin wax
Species	*Acropora millepora, Acropora nasuta, Goniastrea aspera, Montastrea annularis, Montastrea faveolata, Pocillopora damicornis, Porites cylindrica, Porites lobata, Porites lutea, Seriatopora hystrix, and Stylophora pistillata*
Genus	*Acropora, Favia, Goniastrea, Montastrea, Montipora, Pavona, Pocillopora, Porites, Seriatopora, Goniastrea*
Family	Acroporidae, Agariciidae, Faviidae, Oculiniidae, Pocilloporidae and Poritidae
Morphology	branching, encrusting, foliose and massive
Depth	<3m, 4-6m, 7-10m, 11-19m, >20m

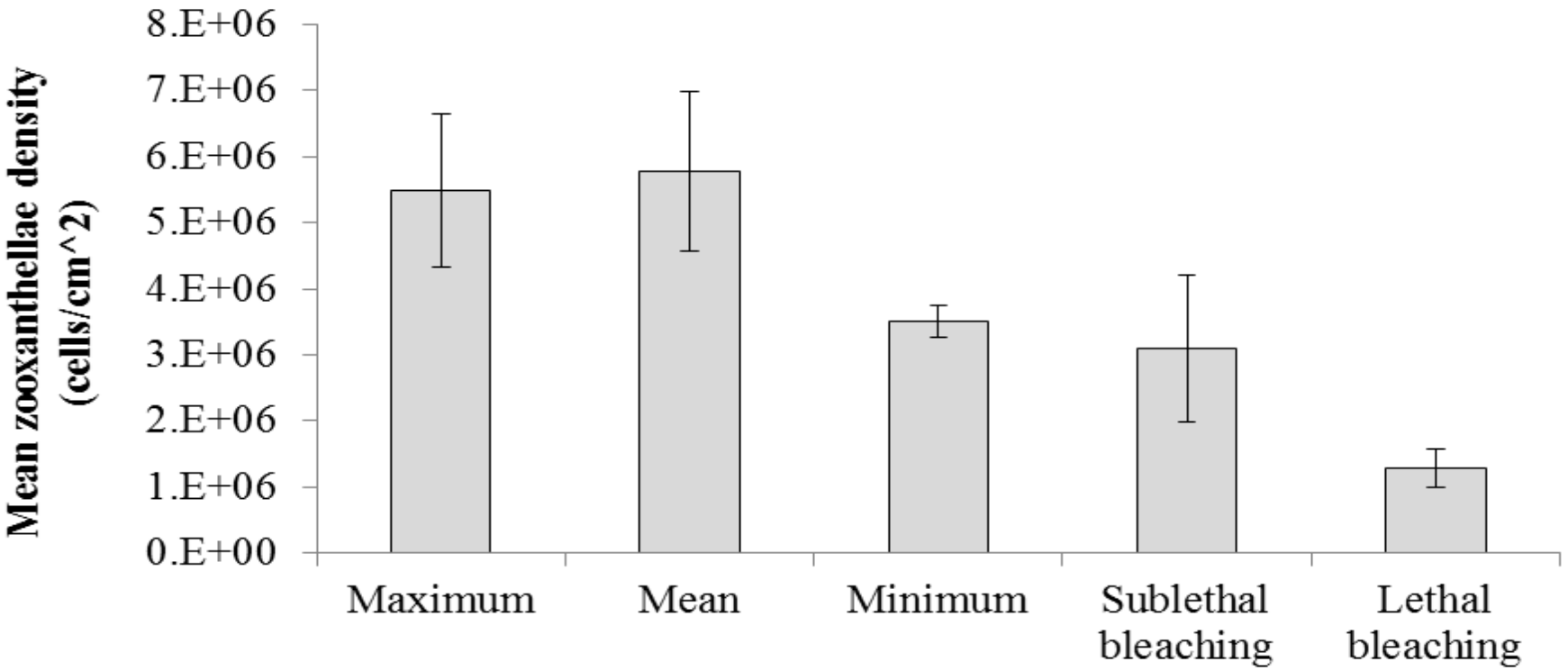

Figure 3.1: Average zooxanthellae densities for healthy (maximum, mean and minimum) and bleached (sublethal or lethal) corals. For corals surveyed through time (e.g., among seasons), the maximum, minimum and long-term average of zooxanthellae densities was recorded, such that the "maximum" is the average of maximum values recorded across all the distinct studies. If only single values were provided then these were included within estimates of "Mean" values, but excluded from "Maximum" or "Minimum".

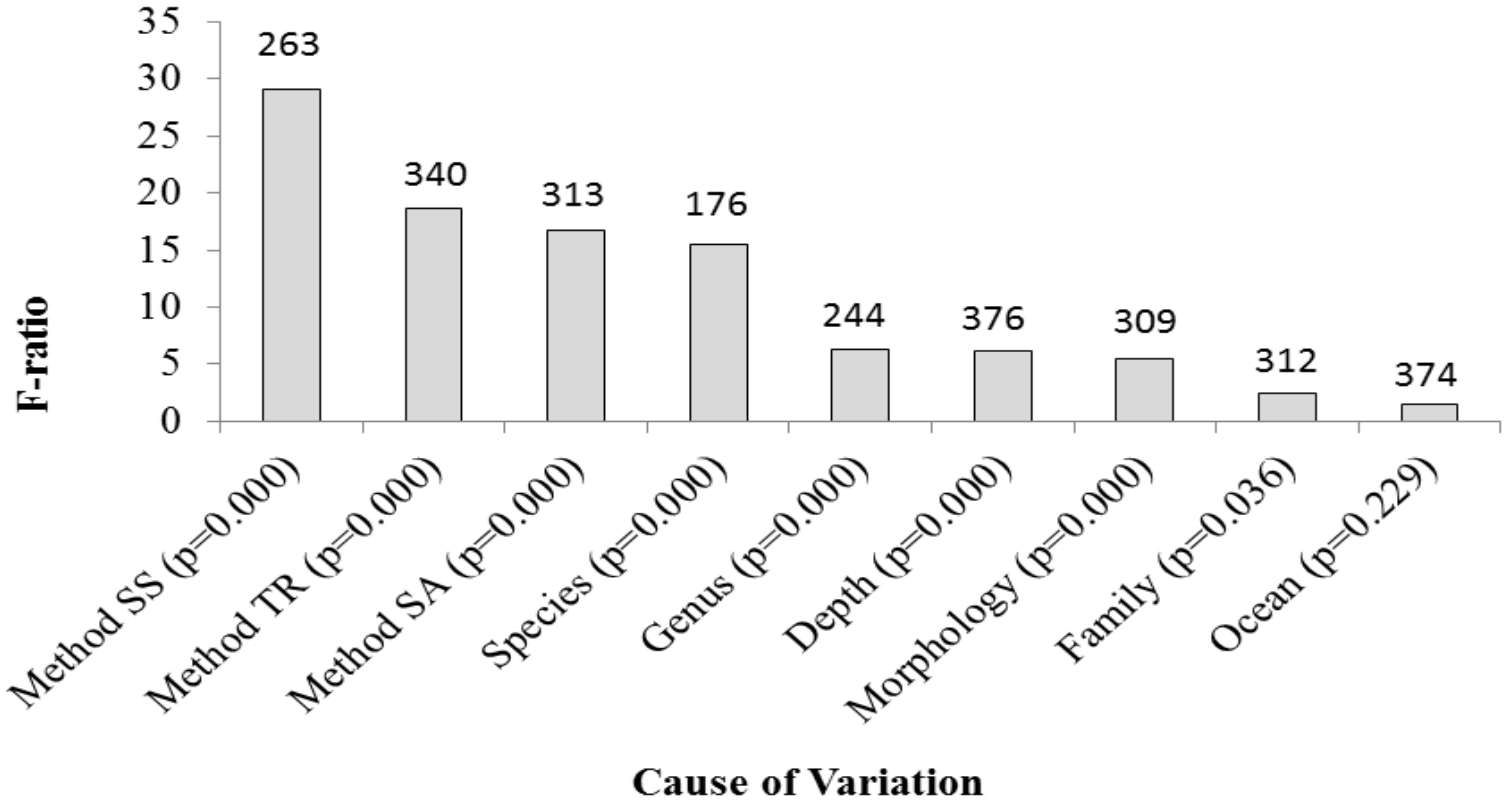

Figure 3.2: Comparison of the amount of variation (F ratios from multiple independent ANOVAs) in zooxanthellae densities due to different factors (p is shown below, n is shown on the graph). Methods SS stands for the sample size used, be it replication amongst branches or colonies, the Method TR stand for tissue removal, whether it was picked off or decalcified, lastly the Method SA stands for surface area – which was generally measured or inferred.

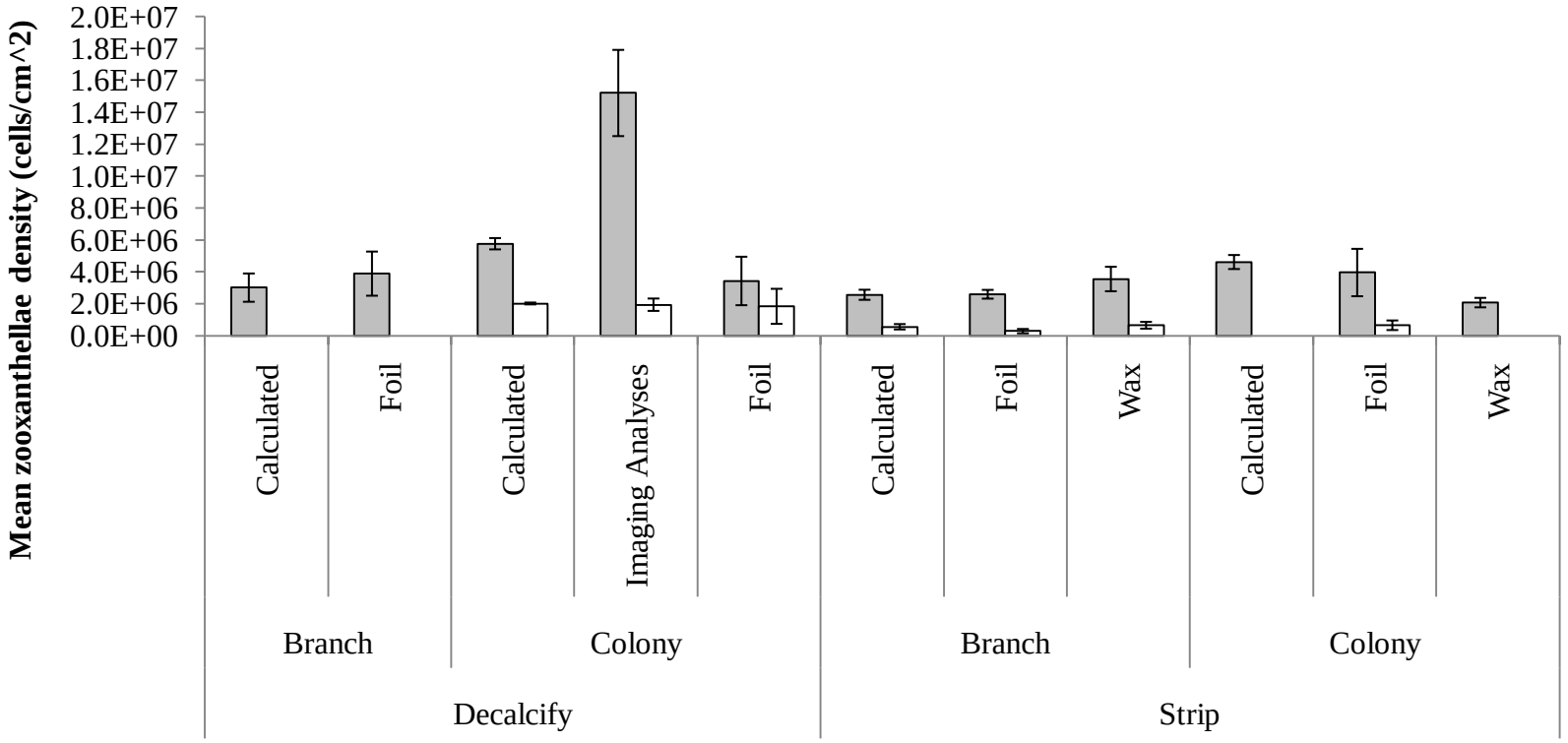

Figure 3.3: Mean (±SE) zooxanthellae densities for nominally healthy (grey bars) versus bleached (white bars) corals, categorized according to the methodological differences used to estimate zooxanthellae densities, including i) different methods for separating tissue (stripping = airbrushing or waterpiking, versus decalcifying), ii) the sampling unit (branch versus entire colony) and the method used to estimate surface area of the coral sample (calculated based on sample dimensions, foil wrapping, image analysis or wax dipping).

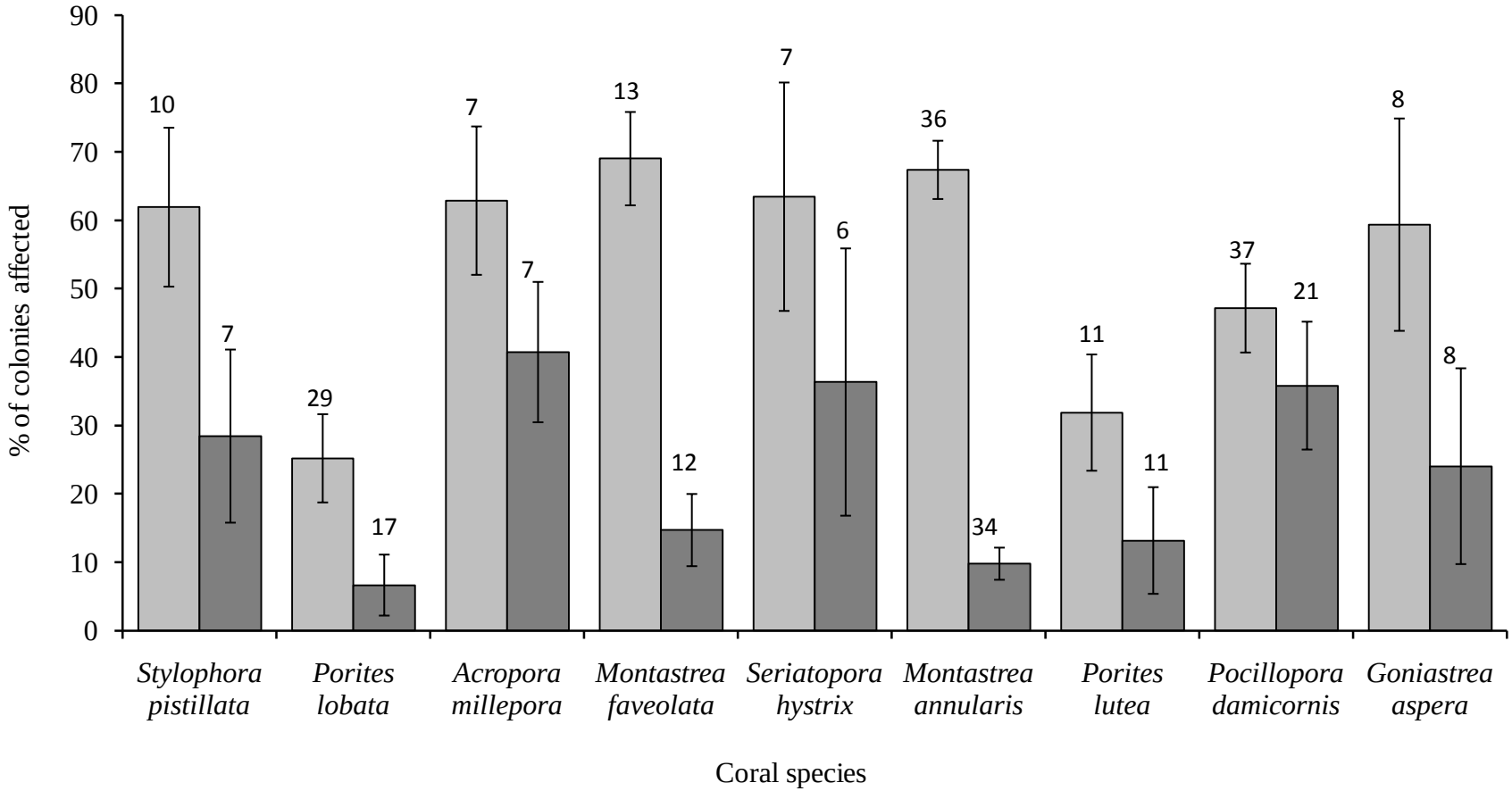

Figure 3.4: Bleaching susceptibility (light grey) and mortality (dark grey) of coral species in the order of lowest to highest "normal" healthy zooxanthellae densities. Numbers indicate the number of studies from which data was extracted to calculate bleaching susceptibility versus mortality for each coral genus.

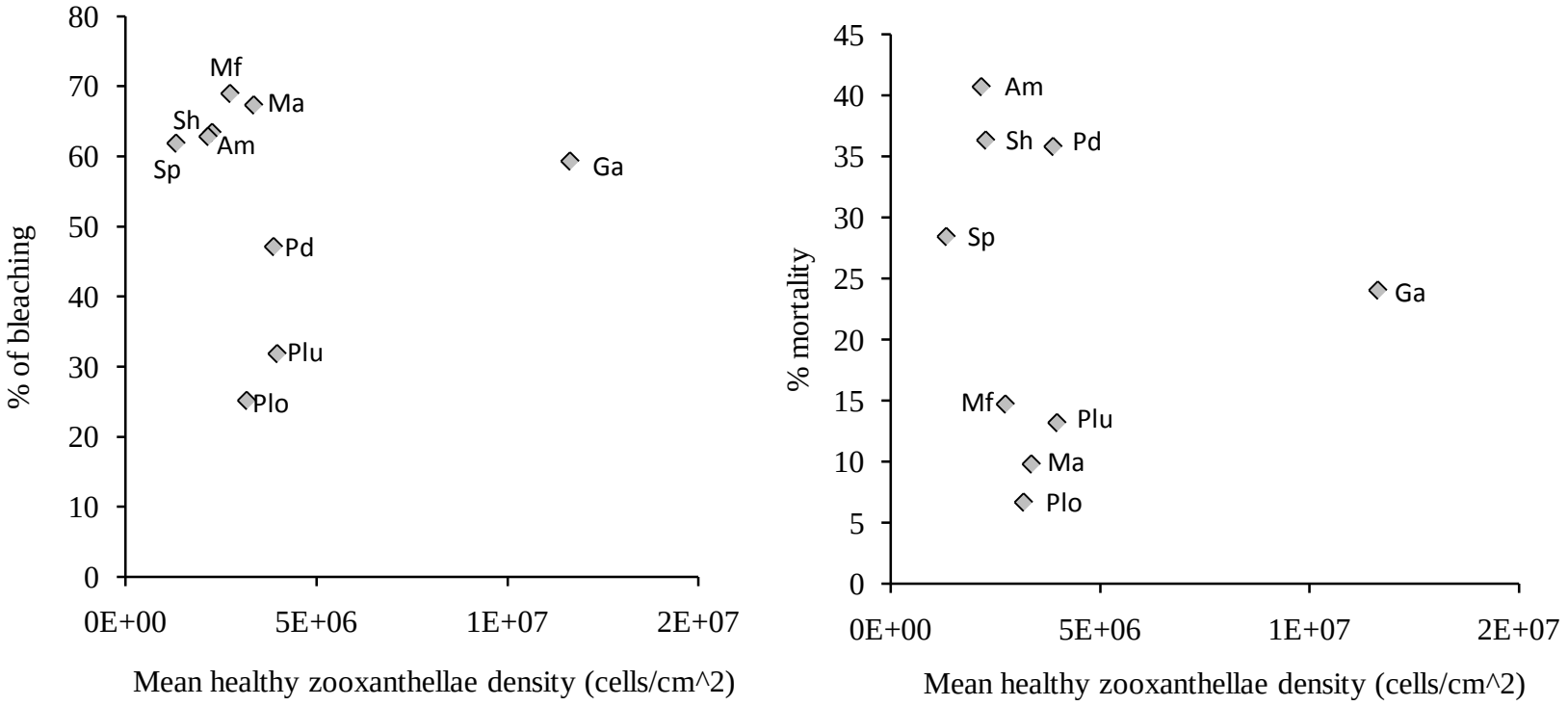

Figure 3.5: Relationship (or lack thereof) between healthy ("normal") zooxanthellae densities of common coral species versus their relative susceptibility to coral bleaching, calculated based on the mean proportion of colonies that bleached (left panel) or died (right panel). Labels refer to individual coral species: *Acropora millepora* (Am), *Goniastrea aspera* (Ga), *Montastrea annularis* (Ma), *Montastrea faveolata* (Mf), *Seriatopora hystrix* (Sh), *Stylophora pistillata* (Sp), *Pocillopora damicornis* (Pd), *Porites lobata* (Plo), *Porites lutea* (Plu)

Chapter 4: Inter- and intra-specific variation in bleaching susceptibility among common reef-building corals, *Acropora nasuta* and *Pocillopora damicornis*.

4.1 Abstract

Increasing ocean temperatures are causing increasing incidence of coral bleaching, which may lead to local or global extinctions of highly susceptible coral taxa. The fate of coral populations and species will largely depend on intra-specific variation in bleaching susceptibility, which has been poorly quantified. The purpose of this study was to test for intraspecific variation in the timing of the bleaching response and recovery among two common coral species. Corals were subjected to a simulated warm water anomaly, equivalent to the 1998 temperature anomaly that lead to extensive mass bleaching of scleractinian corals in the central Great Barrier Reef, Australia. Twenty whole colonies of contrasting reproductive modes, *Acropora nasuta* (spawning coral) and *Pocillopora damicornis* (brooding coral) were subjected to a gradual increase in temperature (0.5^{o}C/3rd day), and then held constant at 31.6^{o}C, which was considered to be the local bleaching threshold. Daily observations of coral health were made using coral colour charts and Pulse-Amplitude Modulated Fluorometry (PAM) measurements, recording the time (in days) until each colony bleached. *Post hoc* measurements of zooxanthellae densities were also used to confirm when bleaching occurred. PAM fluorometry measurements, and specifically quenching analyses, were pertinent to define bleaching. The colony experienced different branches being in various stages of bleaching, therefore, the holobiont response to stress was observed by comparing averages of control colonies to that of a colony in question of bleaching. Marked decreases in photosynthetic parameters over a period of three days were followed by an acute rise in heat dissipation (NPQ), or zooxanthellae expulsion (confirmed with zooxanthellae population density analyses), and it was this that was used to establish when bleaching

occurred. There was variation in the time to bleaching both within (3-16 days) and among the two common coral species (*A. nasuta* ranged 12 days while *P. damicornis* ranged 17 days). Following bleaching, coral colonies were returned to ambient temperatures to explore relationships between the time to bleaching and recovery capacity of individual colonies. Those colonies that bleached first tended to be more likely to experience partial mortality or death. In contrast, corals that were resistant to bleaching also recovered quickly and completely. This study shows that there is significant inter- and intra-specific variation in the bleaching susceptibility of different coral colonies, providing some scope for the persistence of species with moderate increases in ocean temperatures.

4.2 Introduction

Bleaching is a generalised stress response common to all zooxanthellate organisms (Williams and Bunkley-Williams 1990), but recent increases in the geographic scale, taxonomic extent, and severity of distinct bleaching events is raising considerable concern about the fate of corals (Wilkinson 1999, Hughes et al. 2003, Carpenter et al. 2008). Bleaching is usually identified as a reduction in visual pigmentation as corals appear pale or white due to the skeleton showing through the transparency of the tissue without pigmentation and/or dinoflagellates (Glynn 1991). Bleaching does not always result in host coral mortality, as the corals may recover, but severe or prolonged episodes of bleaching can lead to very extensive mortality of corals, resulting in a fundamental shift in the dominant habitat-forming organisms on corals reefs (e.g., Riegl 2001). Of greatest concern is the increasing incidence of phase shifts, where reef habitats formerly dominated by reef-building corals have become dominated by macroalgae (Hughes et al. 1987). A reduction in the abundance and diversity of scleractinian corals, and corresponding declines in the topographic complexity of reef habitats, has negative effects for most reef associated species (Munday 2004, Wilson et al. 2006), reducing productivity and biodiversity of these ecosystems. As such, if increasing

incidence of mass bleaching leads to widespread coral loss and reef degradation this will have direct ramifications for the hundreds of millions of people worldwide that directly rely upon coral reefs for food and resources (e.g., Moberg and Folke 1999).

There is clear evidence that the incidence of regional-scale bleaching events due to thermal and photic stress is increasing (Hoegh-Guldberg 1999). However, even during the most severe mass-bleaching episodes that actual incidence of bleaching is very patchy, often affecting only a portion of colonies and/ or species (e.g., Baird and Marshall 2002). This may be interpreted as evidence for marked inter- and intra-specific variation in the susceptibility of corals to ocean warming (Hughes et al. 2003). The sources of this differential susceptibility are poorly understood, and variously attributed to both extrinsic (e.g., fine-scale differences in temperature and light regimes) and intrinsic (phenotypic and genotypic differences in the bleaching susceptibility of closely positioned coral colonies) factors (Hughes et al. 2003). This study attempts to control extrinsic factors and measure the variability in the timing of the bleaching response due to intrinsic variables, such as the zooxanthellae clade (which has been shown to affect the thermal tolerance of a coral, e.g. Berkelmans and van Oppen 2006). Importantly, intrinsic differences in bleaching susceptibility among corals from the same population provide opportunities for evolution and adaptation to changing environmental regimes. However, there are few studies that have explicitly quantified variation in bleaching susceptibility within or among different coral populations, partly due to the lack of a rigorous, quantitative metric for bleaching susceptibility that can be used at the level of individual coral colonies.

Various methods are employed to determine if and when coral bleaching occurs (e.g. Siebeck et al. 2006, Maynard et al. 2008b, Chapter 2). When observing coral bleaching *in situ*, colour charts (Siebeck et al. 2006) are used as a rapid and reliable method to diagnose the status of individual colonies (Baird and Marshall 2002). However, visual techniques

poorly resolve the extent to which individual colonies have actually bleached, and cannot therefore, infer much about the subsequent potential for recovery. It is also difficult to precisely quantify the level of phenotypic or genotypic variation in bleaching susceptibility within and among coral populations (Suggett and Smith 2010), which is critical to understand whether corals may ultimately adapt to changing environmental regimes. Quantification of changes in zooxanthellae densities provides a more reliable method for determining when bleaching has occurred (Fitt et al. 2001, Chapter 3), but requires protracted laboratory processing and cannot provide instantaneous indications of when bleaching has occurred. One alternative real-time measure of bleaching incidence is non-invasive Pulse-Amplitude Modulated (PAM) fluorometry (Schreiber and Bilger 1986, Jones et al. 1998, Hoogenboom et al. 2006), which measures the photosynthetic capacity of the endosymbiont population and has been shown to significantly decrease as bleaching occurs.

Since the introduction of PAM fluorometry in 1985, it has proved vital as the only tool to monitor photosynthetic activity during bleaching stress (Schreiber and Bilger 1987, Lesser 1996, Warner et al. 1996, Jones et al. 1998, Warner et al. 1999, Ralph et al. 2001). Photosystem II (PSII) is often used to quantify photosynthetic efficiency (Genty et al. 1989). One measurement provides data on minimal (F) and maximal (Fm) fluorescence values of Photosystem II (PSII). The ratio between the F and Fm values is the quantum yield, which summarises the efficiency of PSII. When PSII is under stress, two opposing processes are displayed, photoprotection and photoinhibition (Schreiber and Bilger 1986). Reversible damage, or photoprotection, is displayed as a sudden increase in minimal fluorescence (F) and a decrease in maximal fluorescence (Fm), which regulates the quantum yield, while a decrease in minimal fluorescence (F) implies photodamage. Photoinhibitors, which inhibit or quench the quantum yield, play a major role in the repair and damage of PSII. Two photoinhibitory pathways are in direct competition for de-excitation processes 1)

photochemical energy conversion at the PSII reaction centres (photochemical quenching qP) and 2) non-photochemical loss of energy through heat dissipation at antennae (antennae are chlorophyll or xanthophyll) (NPQ) and reaction centres (qN) (Demmig-Adams and Adams 1992, Walz 1998).

The propensity of corals to actually bleach depends upon both the absolute temperatures to which they are exposed and the period of time at which elevated temperatures persist (e.g., Berkelmans 2002). Initial predictions of when mass bleaching was expected to occur was based on 1 degree heating week (Goreau and Hayes 1994), where corals subject to at least one week of temperatures in excess of 1^{o}C above the local summer maxima, or established bleaching threshold, were expected to bleach (Jokiel and Coles 1990, Goreau and Hayes 1994). It has since been realized that coral bleaching does not always occur at 1 Degree heating week; therefore, the concept of a single bleaching threshold based on the maximum temperature and duration of elevated temperatures is being increasingly challenged (e.g. Maynard et al. 2008b, Berkelmans 2009). Moreover, it is clear that not all colonies (even for the most susceptible coral taxa) will bleach at the same level of exposure to elevated temperatures (Hueerkamp et al. 2001, Chapter 4). Phenotypic variation in bleaching susceptibility is important in establishing the likelihood for adaptation among coral populations (Csaszer et al. 2010).

The purpose of this study was to quantify inter- and intra-specific variation in the bleaching susceptibility among two common, susceptible, scleractinian corals on the Great Barrier Reef, *Acropora nasuta* and *Pocillopora damicornis*, which also presented the opportunity to compare spawning and brooding corals, respectively. This required an instantaneous measure of bleaching incidence, and so we used both colour charts and PAM fluorometry to establish the exact day when bleaching occurred for each individual coral colony. To confirm bleaching had occurred, *post hoc* analyses of changes in zooxanthellae

densities were used. This experiment was set up so that corals were exposed to water heated (0.5^{o}C/3rd day) to 31.6^{o}C and held constant (which simulates the 1998 bleaching event at Orpheus Island). Light levels were relatively low (approximately 200 µmol) and were held constant throughout the days of experiments and recovery. This study shows that PAM fluorometry can be used *in situ* to determine the bleaching response of coral colonies, and that quenching analyses, as suggested by Suggett and Smith (2010), are vital to establishing whether corals are chronically photoinhibited or bleached.

4.3 Methods

4.3.1 Experimental setup

These studies were conducted at Orpheus Island Research Station (OIRS) central Great Barrier Reef, Australia, to observe the bleaching response and test for plasticity in the timing (days at a constant 31.6^{o}C before chronic photoinhibition occurred in the zooxanthellae population) of the bleaching response. Two separate, successive experiments were undertaken. For both experiments, 18 colonies of *A. nasuta* (family Acroporidae) and 18 colonies of *P. damicornis* (family Pocilloporidae) were utilised as both are considered susceptible species (e.g. Marshall and Baird 2000), and they have contrasting reproductive modes, spawners vs. brooders, respectively. The sizes of the coral colonies were similar and, for the first experiment, *A. nasuta* averaged 4701±511.4 cm^2 (n = 10) and 4774±558.7 cm^2 (n = 8) for control and experimental colonies respectively. *P. damicornis* (Family: Pocilloporidae) utilised in experiment 1 had an average area of 4,678 cm^2 (± 689.7 SE, n =10) and 4,909 cm^2 (±618.7 SE, n = 8) for control and experimental colonies, respectively. These colonies were collected from the reef crest (≤50m of each other, 3m mean tidal level) in Little Pioneer Bay, Orpheus Island in February 2010.

Experiment 2 was identical to all aspects of treatment to experiment 1, except for two important details. Firstly, the experiment 2 corals were allowed longer acclimation times (15 days as opposed to 5 days) to get consistency amongst beginning measurements. Secondly, collections for experiment 2 were completed in March, while experiments began in April and continued into May. Berkelmans and Willis (1999) has shown that season can have an effect on bleaching susceptibility, specifically at Orpheus Island; therefore, the results of the experiments are reported separately (where significant differences arose). Colonies for use in experiment 2 were collected from the crest (≤50m of each other, 3m mean tidal level) of the neighbouring Pioneer Bay reef. Corals were tagged consecutively on location and were assigned to tanks randomly. Sizes of experiment 2 *A. nasuta* colonies averaged 4,722 cm^2 (±854.9 SE, n = 10) and 4,900 cm^2 (±467.5 SE, n = 8) for control and experimental corals, respectively. 18 more colonies of *P. damicornis* were also collected, with a mean area (cm^2) of 4,733 cm^2 (±764.9 SE, n = 10) and 4,929cm^2 (±384.8 SE, n = 8) for control and experimental corals, respectively.

The thermal regime for both aquaria experiments mirrored the timing, the average maximum temperatures and the rate of change observed during the 1998 Mass Bleaching Event at OIRS (AIMS temperature data), with the exception that experiment 2 had almost two more weeks of acclimation time compared to the first experiment. At the outset of the experiment the temperature was raised from an ambient temperature (28-29^oC) by 0.5^oC every third day, until reaching a temperature of 31.6^oC, which is 1^oC over the mean of the summer monthly maxima temperatures at Orpheus Island (Berkelmans 2009). Temperatures were then held constant at 31.6^oC in experimental aquaria, while temperatures in control tanks and recovery tanks ranged from 28.6-30.1^oC. All tanks experienced similar water flow and exchange rates; air stones were used to oxygenate the water.

During the experiments, aquaria were lit using metal halide lights, providing constant light levels throughout daylight hours. Light levels ranged from 160-280µmol over each coral colony. The average value taken over the centre of each coral colony was 231.8 µmol (±3.1 SE), with a range of 169.6 µmol (±1.7 SE) up to 256.7 µmol (±2.8 SE), with no significant difference among tanks or treatments. During the recovery period, corals were placed in outside raceways covered with shade netting to minimise the light availability (ranged from 100-250µmol). Relatively low light levels were used in this study in comparison to the approximate average of 450 µmol found by Berkelmans and Willis (1999).

4.3.2 PAM Fluorometry

Measurements of the photosynthetic capacity (Walz, diving PAM) for each colony were taken twice daily at 0800 (dark-adapted) and 1400 (light-adapted) for corals in experimental conditions. During experiments corals were held within a very consistent and electronic controlled light regime with lights switched on 0900 and off at 2100. After corals had bleached they were placed in outdoor recovery tanks with ambient light and constant flow through of water pumped directly from the environment (mean temperature = 29.4^{o}C). Measurements of photosynthetic capacity of these corals were conducted at 0330 (dark-adapted) and 1530 (light-adapted) every other day. Times were chosen based around the corals' schedule; therefore, dark-adapted values were taken at the end of the night, before the lights came on or before the sun came up. Each replicate PAM fluorometer reading provided data on minimal fluorescence (**Fo**), maximal fluorescence (**Fm**), photosynthetic **yield** (—), and photochemical (**qP**, ———) and non-photochemical (**qN**, ——— and **NPQ**,———) quenching. The PAM Handbook of Operation (Walz, 1998) was used for the experiment. High replication was used (from 5 initially up to 10-20 per colony during bleaching and

recovery) to observe photosynthetic capacity and graphs (n=72) of multiple variables were updated daily to understand PAM fluorometry readings.

Replication of PAM fluorometry was randomised within the colony, but standardised to the area of branches that zooxanthellae densities and symbiont genotypes were measured, 1-2cm from the tip of the branch. To ensure the accuracy of the distance of the probe to the coral surface area, the tip included in the Walz, Diving PAM was screwed to the probe at about 1-2mm (fixed for duration). Diving-PAM settings: MI: 12, GAIN: 6 (8 for E2), DAMP: 3, SI: 12, Satwidth: 0.6s. Quenching analyses were standardised to a dark-adapted control *A. nasuta*, where F=198, Fm=652, Yield=0.696, qP=1, qN=0 and NPQ=0. Standardised quenching analyses enabled much quicker collection and analysis of data (daily).

4.3.3 Colour charts

Pigmentation of coral colonies was observed via use of coral colour cards, based on a 6-point scale, following Siebeck et al. (2006). Data for shade and colour (e.g. medium brown B350) was collected every third day (0.5^{o}C temperature increase) until at the stable bleaching temperature, when data was collected and analysed (comparison of means) daily. During recovery, experimental corals were paired with control colonies, and photographs were taken every fourth day with colour cards to monitor recovery.

4.3.4 Zooxanthellae densities

Branch samples (≤5cm) representative of the mean colour chart value of the individual colonies of *A. nasuta* and *P. damicornis* were taken immediately upon collection from the field, after acclimation, at each 1.0^{o}C temperature increase (every 6th day), on the day the colony bleached (days are variable, but set to bleached density by PAM, both experiment and

controls sampled) and once during recovery. Therefore, samples were available to compare natural variation in initial zooxanthellae densities, acclimation to experimental conditions, response to stress and recovery from bleaching. Samples for bleached densities were taken before the coral was moved to recovery. All samples were stored (refreshed after 3-5 days) in phosphate-buffered 10% formaldehyde. To process samples for zooxanthellae densities, a 5% HCL solution was used to decalcify coral skeletons and tissue samples were stored in 70% ethanol. Two replicate tissue samples 2.5cm^2 (1-2 cm from the tip, on opposite sides of the tissue sample, reflective of light- or dark-adapted sections and it was all tissue to half the depth of the branch) were individually placed in 1mL of 70% ethanol, homogenised (quick rev (<5 sec) on high first, which puts a stop to the tissue getting tangled in the homogeniser) for 2 minutes for Pocilloporids and 3 minutes for Acroporids. A portion of the solute was placed on a haemocytometer slide for average counts; there were 4 replicates per subsample; 8 per branch. Zooxanthellae density was determined as completed in McCowan et al. (2011).

4.3.5 Genotyping of symbionts

To test for intraspecific variation in the types of zooxanthellae hosted by each coral species (which might affect bleaching susceptibility, *sensu* Berkelmans and van Oppen 2006) zooxanthellae were isolated from tissue samples of each coral colony collected throughout the experiment. Recurrent sampling of the same coral colonies also enabled tests of symbiont shuffling, considered being important in allowing corals to adapt to changing temperature regimes, *sensu* Baker (2003), Fitt et al. (2009). Branch samples (≤3cm, middle-colony) of *A. nasuta* and *P. damicornis* were collected upon arrival (D0), when colonies bleached, and if and when colonies retained their normal pigmentation in the aftermath of the bleaching experiment. Samples were immediately fixed in 100% ethanol, and refreshed (after 3 days) for storage. Genotyping of symbionts was based on a small section of tissue taken 2cm from

the tip of the branch to match with the area from which PAM fluorometer measurements and zooxanthellae densities were determined. Genotyping of symbionts followed van Oppen et al. (2001). PCR amplification was carried out with primers and was run on a 0.8% TAE-agarose gel, excised and purified using the Jetsorb kit. DNA concentration was measured spectrophotometrically at 260nm and products were cloned in pGEM-T. Clones were sequenced, and the final types were determined.

4.3.6 Data analyses

PAM fluorometry measurements were fixed where samples for invasive methods were taken (coined a branch site); however, randomness within colony branch sites caused high variability in mean values of all variables (F, Fm, Yield, qP, qN, and NPQ) since not all branch sites were in the same stage of bleaching at the same time (e.g. stages of bleaching, Table 4.1). For instance, at the DHW (for Acroporids in experiment 1) 5/10 colonies had bleached, but 9.4% of branch sites were healthy (no change), 11.3% were in photoprotection (increased F, decreased Fm), 26.4% were in photorepair (quenching efforts), 32.1% were photodamaged (decrease in variables, dramatic increase in quenching), and 20.8% were photoinhibited (all values low and high quenching efforts) (definitions taken from Chapter 12 of the PAM Handbook of Operation, Walz 1998) (see Table 4.1 for examples). Therefore, to attain a colony-level definition of bleaching, I had to compare the means of healthy control and experimental corals with that of a particular colony in question. Natural variations occurred in the yield of control corals, while minor fluctuations occurred in healthy experimental corals, but if a colony was bleached, the variance in the mean photosynthetic yield was apparent.

The mean (for all PAM variables and colour values) of the control and experimental corals were graphed daily, n= 80 for controls; and n=10 for each experimental coral (n=10),

or n=20 per colony when bleaching. Differential calculus was used to relate photosynthetic capacity of bleached versus control colonies because the mean values of photosynthetic parameters were reflective of all of the different stages of bleaching within individual branch sites, which were highly variable. The slopes of the tangent lines were compared, as was the variance from the mean (noticeable spike). Common trends related to first bleached corals were applied, e.g. 50% drop in average colony yield, consecutive decrease in average colony yield over 6 sampling times, and a rapid increase in NPQ and other forms of quenching. Analysis consisted of comparing the slope of the tangent for the coral in question to other fluorescent measurements (e.g. the yield could be quenched due to a greater per cent of the colony using chemical quenching or fluorescence rather than heat dissipation) and colour. Colony dysfunction (chronic photoinhibition, or bleaching) was observed when multiple parameters showed significant variation from the mean healthy control and experimental corals (Table 4.2). ANOVA was used to compare proportional loss of zooxanthellae densities, photosynthetic parameters and colour chart values between healthy and bleached corals. Regression analyses were used to compare among photosynthetic parameters, zooxanthellae densities and colour chart values.

4.4 Results

4.4.1 Natural variation in health parameters

Before colonies were acclimated to experimental conditions, there was inter- and intra-specific variation in photosynthetic yield (Figure 4.1), colour chart values (Figure 4.3) and zooxanthellae densities (Figure 4.4). The average colony photosynthetic yield (F_v/F_m) for healthy experimental *A. nasuta* was 0.665 (±0.004 SE), while controls averaged 0.667 (±0.005 SE); values ranged from 0.628 F_v/F_m (*A. nasuta* 1; first experiment) up to 0.709 F_v/F_m (*A. nasuta* 28; second experiment). Experimental colonies of *P. damicornis* averaged

0.653 (±0.004 SE), while controls averaged 0.660 (±0.003 SE); these values ranged from 0.626 F_v/F_m (*P. damicornis* 14; first experiment) up to 0.680 F_v/F_m (*P. damicornis* 28; second experiment). Quenching values were essentially non-existent when corals were healthy (e.g. Table 4.1 and 4.2, Figure 4.2). Healthy colour chart values (Figure 4.3) averaged 4.49 (±0.09 SE) for *A. nasuta* experimental corals, while controls averaged 4.36 (±0.11 SE); mean colour chart values ranged from 3.43 (*A. nasuta* 32; E2 experimental coral) to 5.22 (*A. nasuta* 6; first experiment). Colour chart values for *P. damicornis* experimental corals averaged 4.45 (±0.06 SE), ranging from 3.63 up to 4.83. Healthy zooxanthellae densities (Figure 4.4) averaged $6.1x10^6$ cells/cm^2 (± $9.2x10^4$ SE) for *A. nasuta* experimental corals, while controls averaged $6.2x10^6$ cells/cm^2 (±$5.5x10^4$ SE) values were as low as $5.3x10^6$ cells/cm^2 (*A. nasuta* 34; E2 experimental coral) and as high as $7.0x10^6$ cells/cm^2 (*A. nasuta* 32; E2 experimental coral). For *P. damicornis*, healthy zooxanthellae densities averaged $5.3x10^6$ cells/cm^2 for both experiments (±$6.8x10^4$ SE) and controls (±$5.4x10^4$ SE); values ranged from $4.9x10^6$ cells/cm^2 (*P. damicornis* 23 and 31; E2 experimental and control, respectively) up to $6.0x10^6$ cells/cm^2 (*P. damicornis* 22; E2 experimental coral).

4.4.2 Photoinhibition versus chronic photoinhibition

When different stages of bleaching were observed within colonies (e.g. Table 4.1), values were averaged and colonies were compared for their response to stress. On average, fluorescence measurements of colonies began to deviate from the control values 11 days before bleaching was readily apparent based on changes in colour. Initially, this quenching represented photoprotection as Fo values increased, while Fm values decreased (Walz, 1998). Non-photochemical (qN, NPQ) quenching values began to deviate from the mean at 10 to 8 days before bleached, perhaps illustrating repair (Walz, 1998). Seven to six days before

bleaching F', Fm', and yield values began to steadily decline, representative of photodamage. Five to three days before bleaching, photochemical quenching (qP) was high, until the pathway of de-excitation changed back to predominantly non-photochemical means, qN and NPQ. These marked changes in photoinhibition were in concordance with highly variable quenched quantum yields of colonies, which showed continual decreases for six consecutive sampling times (AM and PM, <0.45), and non-photochemical quenching (heat dissipation, Figure 4.2). At this point, colonies were determined chronically photoinhibited, or bleached, and removed from treatment (Table 4.2).

4.4.3 Contrasting measures of bleaching

Photosynthetic yields of bleached corals did not vary significantly by species (ANOVA, $F_{(1, 38)} = 0.224$, p= 0.638) and were significantly lower than that of healthy corals (ANOVA, $F_{(1, 63)} = 116.26$, p <0.001), including both control colonies and all experimental colonies prior to the day of bleaching. However, corals in the second experiment had significantly lower yields than those in the first experiment (ANOVA, $F_{(1, 38)} = 172.22$, $p < 0.001$). Within experimental runs, there was no significant variation in healthy (pre-bleaching) values (ANOVA, $F_{(1, 34)} = 2.545$, p = 0.88); E2 (ANOVA, $F_{(1, 32)} = 4.1033$, p = 0.94), and bleached values were clearly distinguished from healthy values for the first experiment (ANOVA, $F_{(1, 14)} = 127.72$, $p < 0.001$) and second experiment (ANOVA, $F_{(1, 17)} = 140.11$, $p < 0.001$). In the first experimental run, bleached photosynthetic yields averaged 0.509 (± 0.022 F_v/F_m) and ranged from 0.362 F_v/F_m (*P. damicornis*, n = 16) to 0.628 F_v/F_m (*A. nasuta*, n = 7). For the second experimental run, bleached photosynthetic yields averaged 0.396 (± 0.015 F_v/F_m) and ranged from 0.271 F_v/F_m (*A. nasuta* 31) to 0.519 F_v/F_m (*A. nasuta* 35).

The proportion of colonies exhibiting photochemical quenching at PSII reaction centres (qP) did not differ significantly between study species (ANOVA, $F_{(1,38)} = 1.74$, p =

0.80) or experimental runs (ANOVA, $F_{(1,38)} = 1.871$, $p > 0.82$), but bleached corals exhibited significantly higher qP values than healthy corals (ANOVA, $F_{(1, 64)} = 91.36$, $p < 0.001$) (Figure 4.2). The average bleached qP values were 1.87 and ranged from 0 (*A. nasuta* 23 and 31) to 5.36 (*A. nasuta* 33). Non-photochemical quenching at PSII reaction centres (qN) did not vary significantly between coral species (ANOVA, $F_{(1, 38)} = 1.505$, $p = 0.77$), or experimental runs (ANOVA, $F_{(1, 38)} = 1.377$, $p = 0.75$), but qN was significantly higher for bleached corals (ANOVA, $F_{(1, 64)} = 89.73$, $p < 0.001$). The average bleached value of qN was 1.05, which ranged from 0.412 (*P. damicornis* 10) to 1.30 (*A. nasuta* 31).

Non-photochemical quenching in the form of heat dissipation at the antennae varied significantly between treatments (ANOVA, $F_{(1, 38)} = 52.617$, $p < 0.001$), experimental runs (ANOVA, $F_{(1, 38)} = 21.663$, $p < 0.001$) and species (ANOVA, $F_{(1, 38)} = 6.142$, $p = 0.02$) (Figure 4.2). In the first experiment, there was not significant variation between species (ANOVA, $F_{(1, 34)} = 1.9731$, $p = 0.83$), and bleached corals were clearly distinguished from healthy corals (ANOVA, $F_{(1, 14)} = 17.970$, $p < 0.001$). The average bleached value of NPQ was 1.20 (±0.07 SE) and ranged from 0.743 (*P. damicornis*) to 4.42 (*A. nasuta*). Within the second experimental run, there was still significant variation between species (ANOVA, $F_{(1, 38)} = 8.665$, $p < 0.001$), so analyses were run for both species to find that bleached and healthy corals were significantly different for both *A. nasuta* (ANOVA, $F_{(1, 7)} = 43.163$, $p < 0.001$) and *P. damicornis* (ANOVA, $F_{(1,7)} = 54.0093$, $p < 0.001$). For *A. nasuta* in the second experiment, the average bleached NPQ was 5.51 (±1.03 SE), which ranged from 1.49 (*A. nasuta* 32) to 13.400 (*A. nasuta* 31). For *P. damicornis* in the second experiment, the average bleached NPQ was 2.23 (±1.15) and ranged from 1.28 (*P. damicornis* 23) to 3.900 (*P. damicornis* 24).

Colour chart values were significantly different between treatments (ANOVA, $F_{(1, 71)} = 1012.645$, $p < 0.001$), but not runs (ANOVA, $F_{(1, 71)} = 4.545$, $p = 0.96$) or species

(ANOVA, $F_{(1, 71)} = 5.247$, $p = 0.97$) (Figure 4.3). For, *P. damicornis*, the mean colour hue (scored from 0 to 5) of bleached values were significantly different (and much lower) from values for healthy (control) colonies (ANOVA, $F_{(1, 15)} = 567.88$, $p < 0.001$). Similarly, for *A. nasuta* bleached values were clearly distinguishable from healthy values; E1 (ANOVA, $F_{(1, 7)} = 496.696$, $p < 0.001$) and E2 (ANOVA, $F_{(1, 7)} = 298.153$, $p < 0.001$). For *P. damicornis*, average colour chart values of bleached corals were 1.77 (±0.07 SE), which ranged from 1.30 (*P. damicornis* 16, 18, and 24) to 2.25 (*P. damicornis* 22). For *A. nasuta* colonies in the first experiment, average bleached colour chart values were 1.86 (±0.09 SE), which ranged from 1.20 (*A. nasuta* 12) to 2.20 (*A. nasuta* 7). For *A. nasuta* colonies in E2, average bleached colour chart values were 1.12 (±0.10 SE) and ranged from 0.85 (*A. nasuta* 24 and 33) to 1.65 (*A. nasuta* 32).

The zooxanthellae densities of bleached corals were determined *a posteriori*, but clearly showed that zooxanthellae densities in corals presumed to have bleached (based on PAM quenching measurements and colour loss) were 55-77% lower than values recorded among control colonies and experimental colonies on the day of bleaching (Figure 4.4). Changes to the zooxanthellae population density did not vary significantly by species (ANOVA, $F_{(1, 70)} = 0.241$, $p = 0.34$) or experimental run (ANOVA, $F_{(1, 70)} = 0.380$, $p = 0.46$), and bleached corals had significantly lower zooxanthellae densities (ANOVA, $F_{(1, 34)} = 1196.783$, $p < 0.001$) than healthy corals. Bleached zooxanthellae densities averaged 1.97×10^6 cells/cm^2 ($\pm 6.85 \times 10^4$ SE) and ranged from 1.24×10^6 cells/cm^2 (E1, *P. damicornis* 18) up to 2.58×10^6 cells/cm^2 (E1 *P. damicornis* 13). Overall changes in colour for bleached corals were not significantly related to changes in zooxanthellae densities (Figure 4.5) or photosynthetic yield (Figure 4.6).

4.4.4 Variation in the time to bleach

The time taken to bleach varied greatly among colonies and among experiments, ranging from 22-30 days for *A. nasuta* and 21-34 days for *P. damicornis* (Figure 4.7). Between species, *A. nasuta* colonies bleached sooner (mean = 2.4 days ±2.9SE) and had a smaller range -1 to 11 days compared to *P. damicornis* colonies (mean = 4.1 days ±2.7SE), which ranged from -2 to 15 days. The experimental runs showed different timing in the responses, but both experiments showed a range of 5 days for *A. nasuta*, even though the first experiment ranged from 22-27 days while the second experiment ranged from 25-30 days. For *P. damicornis*, the range of bleaching days (13) was higher in the first experiment (9), from day 21-30 days than it was in the second experiment (6), which showed more of a delay in bleaching response from day 28-34 (Figure 4.7).

Aside from differences in the time to bleach, there were also apparent differences among colonies in the photosynthetic response of bleaching. Most of the corals subjected to increased temperatures exhibited decreased, or quenched, photosynthetic yields due to a dramatic peak in NPQ, or heat dissipation at the antennae. In these colonies, both values returned to normal, following bleaching (and a return to ambient temperatures), and these colonies stabilised (expelled harmful zooxanthellae) and recovered. The remainder of colonies exhibited little to no photochemical or non-photochemical quenching (see Table 4.2); therefore these corals did not exhibit quenched photosynthetic yield. In these *A. nasuta* the first evidence of stress was tissue sloughing rather than apparent bleaching.

4.4.6 Intra-specific variation in bleaching susceptibility

4.4.6.1 Initial health parameters

Zooxanthellae density, colour and PAM Fluorometry were used to consider health status in the experiment. The time it took colonies to bleach was not related to any of variations in initial health parameters (Figure 4.8). Intraspecific variation in the timing of bleaching did not relate to the initial zooxanthellae densities for *A. nasuta* ($r^2 = 0.0634$, $p > 0.05$) or *P. damicornis* ($r^2 = 5x10^{-5}$, $p > 0.05$), nor did the time to bleach relate to initial colour for either *A. nasuta* ($r^2 = 0.0010$, $p > 0.05$) or *P. damicornis* ($r^2 = 0.0004$, $p > 0.05$). For PAM Fluorometry, there were a few initial values that were compared, and. The time to bleach best related to F (minimal fluorescence) for *A. nasuta* ($r^2 = 0.35$, $p < 0.05$), but not *P. damicornis* ($r^2 = 0.20$, $p > 0.05$). For maximal fluorescence (Fm), there was a significant relationship to time to bleach for *A. nasuta* ($r^2 = 0.20$, $p < 0.001$), but not for *P. damicornis* ($r^2 = 0.06$, $p > 0.05$). The quantum yield did not have a significant relationship with time to bleach for either *A. nasuta* ($r^2 = 0.04$, $p > 0.05$) or *P. damicornis* ($r^2 = 0.014$, $p > 0.05$) colonies. The amount of zooxanthellae loss was not affected by the time to bleach for *A. nasuta* ($r^2 = 3x10^{-5}$, $p > 0.05$); however there was a significant relationship for *P. damicornis* ($r^2 = 0.22$, $p < 0.05$).

4.4.6.2 Colour

Colour (measured through coral colour charts) appeared to affect the susceptibility of *P. damicornis* colonies. Yellow-brown bleached earlier (days 21-25 or -2 to 2) than green (days 28-30 or 5-7) or pink colonies (days 28-34 or 5 to 11). Colour (blue versus cream tips) had no pronounced effect on the time for *A. nasuta* colonies to bleach.

4.4.6.3 Zooxanthellae Clades

Intraspecific variation in bleaching may be related to differences in the specific zooxanthellae hosted by individual coral colonies (Tables 4.3 & 4.4, Figure 4.9), as colonies with more than

one type of zooxanthellae bleached earlier than those with only one type. No *P. damicornis* colonies had more than one type of zooxanthellae; however, two of the colonies harboured a different type of zooxanthellae and were among the first colonies to bleach (Table 4.4). In contrast, four *A. nasuta* colonies began the experiment with more than one type of zooxanthellae (Table 4.3); and, they bleached first (Figure 4.9), expelling one clade of zooxanthellae, often leaving the more common clade within the colony (which was most often C2). The exception to this was A17 which lost C2 type zooxanthellae and maintained C3. All of the colonies with more than one dominant symbiont type bleached at or before one degree heating week (Table 4.3 and 4.4).

4.4.7 Fate of bleached corals

4.4.7.1 Colony recovery

Recovery of corals took many pathways. Both species did experience partial mortality (often at the tips or base of the colony where they bleached first). The only whole colony mortality of a *P. damicornis* was the first colony to bleach; all other colonies experienced some degree of partial mortality. Forty per cent of the *A. nasuta* sloughed tissue after bleaching zooxanthellae, and of that forty per cent, forty per cent recovered tissue within 2-4 weeks after sloughing tissue. Recovery was likely to be higher, since the second experiment corals were only observed for up to one week. Uptake of new algae was monitored and the branch samples that were collected for this showed that it was consistently clade C2. Corals re-browned from 3 weeks onward of bleaching though some healthy algae (F values of 200-300) were observed within 1 week of bleaching for *A. nasuta*. Only one branch of one *P. damicornis* colony had a greenish tint, and that was the first colony to bleach and subsequently died. Every colony of *A. nasuta* had at least some greenish tinted branches (even controls had greenish tinted branches at their bases). The time to bleach was inversely

correlated with the time for recovery, whereby corals that took longer to bleach also recovered fastest. This was most obvious in *P. damicornis* ($r^2 = 0.918$, $p > 0.001$), but was also apparent for *A. nasuta* ($r^2 = 0.4738$, $p > 0.001$).

4.4.7.2 Post-bleaching mortality

Following the experiment, 14 colonies of *A. nasuta* died within 6 months, including 4 control colonies, and 8 experimental colonies. Importantly, 40% of corals that tissue sloughed experienced the phoenix effect (e.g. Riegl 2002), whereby corals maintained some tissue in their perforated skeletons, and upon return to ambient conditions, allowed the tissue to come back out. After 1 year, overall survival occurred for 47% of *A. nasuta* colonies, but 12/19 colonies had exhibited significant levels of partial mortality. For *P. damicornis*, only 5 colonies died (2 controls, 3 experimental) during the first six months, and after a year 46% of colonies were found alive; partial mortality among these colonies was negligible.

4.5 Discussion

4.5.1 Quantifying variation in bleaching susceptibility

This study has revealed, and indeed quantified, significant intra-specific variation in the bleaching susceptibility of two common reef-building corals, based on the time taken for individual colonies to bleach when exposed to experimentally controlled and consistent temperature stress. Based on daily PAM-fluorometer measurements and analysis, and later confirmed by pronounced declines in zooxanthellae densities, the time taken for colonies to bleach ranged from 22-30 days for *A. nasuta* and 21-34 days for *P. damicornis*. All colonies did eventually bleach at 31.6^{o}C (which is 1^{o}C above the mean of the summer monthly maxima temperatures for Orpheus Island), but only after at least 3 weeks exposure. Mass-bleaching is expected to occur when corals are exposed to temperatures of $\geq$1^{o}C above the

long-term summer weekly maxima for ≥ 1 week, or 1 Degree Heating Week (DHW) (Coles and Jokiel 1977, Goreau and Hayes 1994). The DHW concept is increasingly being challenged (e.g. Maynard et al. 2008a, Berkelmans 2009), and the results of this study show that if the experiment had stopped at 1DHW fewer than one third of *A. nasuta* and *P. damicornis* colonies (which are commonly regarded amongst most susceptible taxa, Marshall and Baird 2000) would have been bleached. These results do however correspond well with the latest bleaching thresholds used by the NOAA National Environmental Satellite, Data and Information Service (NESDIS) which predict that significant coral bleaching occurs at >4 DHW, with widespread bleaching and significant mortality expected at >8 DHW (www.coralreefwatch.noaa.gov/satellite/dhw.php). It is generally assumed however, that variation in bleaching susceptibility occurs among, rather than within coral species, such that a greater range of species (rather than a greater number of colonies) will be affected by severe or prolonged exposure to high temperatures (e.g., Berkelmans 2002).

Quantifying variation in the responses of individual colonies is critically important for establishing the differential susceptibility and adaptive capacity of coral populations, which further informs the likely fate of corals under changing environmental regimes (e.g., Pandolfi et al. 2011). Ultimately, this may be best achieved in controlled experimental studies (which help to minimise possible confounding effects of different micro-climates), but it is also theoretically possible to quantify differential bleaching susceptibility in the field during the course of mass-bleaching events, whereby the extent of bleaching within individual coral colonies is measured based on the timing and extent of zooxanthellae loss (see also Chapters 2 and 3). However, precise estimates of zooxanthellae loss require extensive laboratory processing (Chapter 2), and in this study the PAM fluorometer measurements were critical in providing instantaneous measurements of bleaching responses among individual colonies. Importantly, marked changes in the rate of change in the photosynthetic yield alongside rapid

increases in quenching efforts provided an effective indication of the incidence of bleaching. However, given different bleaching responses, overall changes in photosynthetic yield poorly reflected the proportional loss of zooxanthellae in bleached colonies (Figure 4.6). Similarly, loss of colour is an effective and reliable way to establish when bleaching has occurred (e.g. Marshall and Baird 2002) but the extent of colour loss (e.g., Siebeck et al. 2006) did not correspond closely with the extent of zooxanthellae loss (Figure 4.5), which suggests that observations attempting to quantify zooxanthellae densities based upon colour will be biased due to pigmentation loss, rather than a loss of zooxanthellae population density. This requires considerable caution when attempting to quantify phenotypic variation in bleaching responses or estimating recovery times based on colour change (Suggett and Smith 2010).

Aside from establishing exactly when corals bleach, fluorometry measurements also reveal differences in the responses of corals to environmental stress (e.g., temperature), though there are some complexities in interpreting changes in resulting measurements. The photoinhibition model of coral bleaching presented by Jones et al. (1998) recognises several distinct phases of chronic photoinhibition (or bleaching); i) photoprotection, ii) photorepair, iii) photodamage, and iv) photoinhibition. During this sequence F and Fm values remain constant during photoprotection and photorepair, but photosynthetic yields become highly variable during photodamage and photoinhibition. In the formative stages, quenching analyses are important in revealing photoprotection (i.e. xanthophyll cycling or heat dissipation) and photorepair occurring simultaneously, but with competing roles. Once colonies bleach, the photosynthetic yield again stabilises. Increases in photosynthetic yields among bleached corals are counter-intuitive, but reflect selective expulsion of defective zooxanthellae (Hoegh-Guldberg and Smith 1988, Okamoto et al. 2005). The specific physiological processes that lead to photoinhibition remain unclear (e.g., Smith et al. 2005),

but are likely to vary depending upon the relative contribution of high light, high temperature and/ or other adverse environmental conditions to the bleaching response.

4.5.2 Phenotypic variation in bleaching susceptibility

Most studies observe variation in bleaching susceptibility based on the extent of bleaching apparent within individual colonies after a set period of time, usually at the end of an experiment (e.g. Berkelmans and Willis 1999, Hueerkamp et al. 2001). These studies almost invariably show that there are some corals that do not bleach, reflective of marked intra-specific variation in bleaching susceptibility, but these studies do not effectively capture the full range of variation in bleaching susceptibility. Intra-specific variation in the time to bleach provided an important measure of phenotypic variation in bleaching susceptibility, which was also ecologically relevant. *P. damicornis* colonies that took the longest time to bleach also recovered first, and with minimal partial mortality.

Variation in the time to bleach clearly reflects different bleaching responses, though it is unknown to what extent these alternative responses are determined by the physiological condition (which, will then vary temporally) or inherent differences in genotypic responses. Bleaching (opposed to tissue sloughing) occurred due to expulsion of photosynthetically inhibited zooxanthellae, which is evident based on non-photochemical quenching, or heat dissipation at the antennae immediately prior to observed bleaching (Figure 4.2). This is contrary to previous findings of release of photosynthetically intact zooxanthellae (e.g. Ralph et al. 2001) and suggests that for short-term (hours) thermal stress corals chose a flight response and bleach competent zooxanthellae as a pre-emptive strike to minimise damage caused by excessive free oxygen radicals (Jones et al. 1998). In contrast, this experiment (which simulated a natural bleaching event) showed that colonies that resisted bleaching, or chose a fight response, and only bleached compromised zooxanthellae when they became a

burden. These differential responses are likely to vary in their effectiveness depending on the extent and duration of the thermal stress. If conditions return to normal relatively quickly, then those corals that have resisted bleaching and managed to maintain high densities of zooxanthellae will recover very quickly. If however, adverse conditions persist for a protracted period, then excessive retention of zooxanthellae and cumulative effects of excess free radicals may lead to permanent tissue damage (Hueerkamp et al. 2001, Jones 2008, but see Toller et al. 2001), in which case it would have been better to rapidly expel zooxanthellae as soon as temperatures began to increase.

For corals with low levels of colony integration (e.g., many massive corals, where connectivity among polyps is limited to thin tissue connections over the surface of the skeleton) decisions to expel or retain zooxanthellae may be made at the level of individual polyps. Differential responses (along with differences in energy reserves) may then lead to localised tissue loss, though low levels of partial mortality are much better than whole colony mortality. This appears to be more of an obstacle for corals with high levels of connectivity that cannot partition disturbances, such that most branching corals tend to exhibit whole colony bleaching and mortality, rather than differential responses among parts of the colony (Marshall and Baird 2000). This is the first study, to my knowledge, to document partial mortality caused by sublethal bleaching in an *Acropora* coral. However, this serves to reinforce the marked intra-specific differences in the bleaching responses and bleaching susceptibility among sympatric colonies.

4.5.3 Causes of intra-specific variation in bleaching susceptibility

Intraspecific differences in bleaching susceptibility are often attributed to differences in either the type (Rowan et al. 1997, Sampayo et al. 2008) or density of zooxanthellae (Fitt et al. 2001, Li et al. 2008) within host coral tissues. There are also significant intra-specific

differences in concentrations and types of fluorescent pigments (Salih et al. 2000), which have been related to differences in resistance to temperature and UV exposure. Alternatively, the size or physiological condition of individual colonies may also influence their susceptibility to bleach (e.g., van Woesik et al. 2012). In this study, differences in the diversity of zooxanthellae types (rather than the specific clade) appeared to influence the timing to bleach in *A. nausta*, whereby colonies that initially hosted more than one type of zooxanthellae were amongst the first to bleach, and rapidly lost one (often the least abundant) type of zooxanthellae. This may simply reflect differences in the thermal tolerances of different zooxanthellae, which are compromised and lost at different levels of thermal stress. Furthermore, colonies of *A. nasuta* were specifically tested for the acquisition of a secondary type of zooxanthellae (e.g., C2) during rapid recovery of photosynthetic yields with acquisition of green wire-like algae in branch samples. However, no evidence of symbiont shuffling was reported, at least not in response to bleaching.

For *P. damicornis*, intra-specific variation in bleaching susceptibility appeared to be strongly linked to differences in colour, which may reflect differences in concentrations or types of fluorescent pigments (*sensu* Salih et al. 2000). Colonies that had a yellow-brown hue bleached earlier (days 21-25) than colonies that were predominantly green (days 28-30) or pink (days 28-34). These differences in the colour of *P. damicornis* have been linked to differences in the amount of the pigment pocilloporin, whereby pink colonies had an overall pigment (pocilloporin) concentration that was significantly higher than that of brown colonies (Takabayashi and Hoegh-Guldberg 1995), which is consistent with increased resistance to bleaching. Although there was variation in *A. nasuta*, with some branches having blue tips and other cream tips, there was no pronounced effect on the susceptibility to bleaching, as both colours ranged the span of the bleaching days; however, corals with blue-tipped branches were more likely to use extended fluorescence and tissue slough.

Although there were marked intra-specific differences in zooxanthellae densities at the start of the experiment, this did not appear to influence the time to bleach. This is consistent with the results of Chapter 3, which suggests that initial zooxanthellae densities have limited influence on bleaching susceptibility and mortality (but see Li et al. 2008). However, this contradicts the results of Cunning and Baker (2013), who show that excessive symbionts increases susceptibility to bleaching. It could be that none of the corals here were experiencing excessive amounts of symbiotic algae. However, the range found for mean zooxanthellae densities ($4.9 – 7.0x10^6$ cells/cm^2) is well above average, although the method used, decalcification (Chapter 2), has been shown to give higher estimates of mean zooxanthellae density (Chapter 3), presumably because it captures the tissue that perforates the skeleton (Chapter 2).

Size also, did not have an effect on the timing of the bleaching response for either of the study species (see also Chapter 5). It has been shown that smaller corals can show less susceptibility to extreme temperatures compared to larger conspecifics (Arthur et al. 2006, Shenkar et al. 2005), but this appears to be tied with morphology and/or life history characteristics. For instance, Fong and Glynn (2001) found that partial mortality in massive *Gardinoseris planulata* (complex spawner) decreased as size increased. However, Shenkar et al. (2005) found that smaller sizes were beneficial to encrusting *Oculina patagonica* (robust spawner). Furthermore, size frequencies are often reduced due to partial mortality during bleaching events (McClanahan et al. 2009). Maturity may play a role, as juvenile corals have more energy to invest compared to their adult counterparts, and their smaller size allows for higher mass transfer of free radicals (Nakamura and Van Woesik 2001). There was no account of differences in physiological condition among colonies used in this experiments, but it is likely that this will have a major influence on the capacity to withstand bleaching (at least over relatively short time frames) and this could vary independent of colony size.

4.5.4 Conclusions

This study has revealed significant intraspecific variation, not only in bleaching susceptibility among colonies of common coral species, but also in the bleaching response. For A. *nasuta*, differences in bleaching susceptibility were partially explained by the diversity of symbionts, whereby colonies lost secondary symbiont types when subject to increased temperature. For *P. damicornis*, variation in bleaching susceptibility was strongly linked to colour, reflective of differences in the concentration of fluorescent pigments (Takabayashi and Hoegh-Guldberg 1995). It is not clear whether individual corals can evolve to acquire greater bleaching resistance, or whether increased exposure to adverse environmental conditions will selectively remove highly sensitive phenotypes, thereby naturally increasing the proportion of individuals that possess these important adaptations for global climate change (e.g., Sampayo et al. 2008). It is however, important to assess how differences in the bleaching response influence the ultimate fate of these coral colonies. Clearly, those colonies with the greatest bleaching resistance will be unaffected by all but the most extreme environmental conditions, but importantly, this study suggests that when these colonies do bleach, they also have greatest capacity to recover and persist. Ultimately, significant interspecific variation in bleaching susceptibility and bleaching responses will ensure the persistence of coral populations during all but the most extreme, changes in global climate (Hughes et al. 2003). There is therefore, an urgent need to constrain greenhouse gas emissions and prevent devastating effects of extreme climate change, but also more work is needed to assess the level of intraspecific variation in bleaching susceptibility within and among different coral species.

Table 4.1: Comparison of actual examples of PAM Fluorometry readings for stages of bleaching, from healthy to photoinhibited

Variable	F’	Fm’	Yield	qP	qN	NPQ
Healthy	185	658	0.715	1	0	0
Photoprotection	343	836	0.589	1	0	0
Photorepair	221	472	0.531	1.398	0.729	1.031
Photodamaged	128	242	0.471	0	1.342	14.209
Photoinhibited	31	41	0.243	0	1.375	22.357

Table 4.2: Comparison of average healthy and bleached PAM Fluorometry values with quenching analysis

Variable	F’	Fm’	Yield	qP	qN	NPQ
Control	130-300; mean 185	400 – 700, mean 600	0.61-0.70, mean 0.66	1	0	0
Bleached	Significant increase followed by a rapid decline, for 3 days	3-day mean of 200 (within SE)	3 consecutive decreasing days: 3-day mean <0.45 (within SE)	3-day mean of 0 or >2 (within SE)	3-day mean of 0 or >2 (within SE)	3-day mean of 0 or >2 (within SE)

Table 4.3: Bleaching variables for *Acropora nasuta* (TTB=time to bleach, CO(i)=initial colour, %ΔCOΔ(b)=change in colour due to bleaching, ZD(h)=mean healthy zooxanthellae density (cells/cm2, in millions), %ΔZD(b)=per cent changed due to bleaching, ZC=zooxanthellae clade, MY(b)=mean bleached photosynthetic yield, %ΔMY(b)=per cent change in mean photosynthetic yield, NPQ(b), qN(b), qP(b)= bleached values for quenching

	Tag	TTB	CO(i)	%ΔCO(b)	%ΔCO(b+7)	ZD(h)	%ΔZD(b)	ZC(h)	ZC(b)	ZC(r)	MY(b)	%ΔMY(b)	NPQ(b)	qN(b)	qP(b)
Exp't 1	4	22	400	60	79	6.3	77	C3 > C2	C3	C3	359	43	1.41	0.96	0.96
	3	22	475	61	81	5.8	66	C3	C3	C3, C2	425	35	0.97	0.87	0.87
	1	23	408	53	71	6.5	66	Unk D, C1			339	47	1.28	0.91	0.91
	9	23	445	62	80	6.2	55	C3	C3	C3	468	26	0.69	0.8	0.78
	12	24	447	73	63	6	68	C2	C2	C2	521	17	1.1	0.92	0.92
	8	24	484	57	82	5.9	73	C3	C3		467	30	1.22	0.94	0.94
	17	24	490	62	67	6.4	61	C2 > C3	C3	C3	468	27	1.57	1	1
	7	25	490	57	82	6.4	67	C3			565	14	2.58	1	1
	6	26	523	61	79	5.9	59	C3	C3	C3	524	20	1.7	0.99	0.99
	18	29	458	57	80	5.7		C3			510	19	1.19	0.93	0.93
Exp't 2	32	25	343	49	49	7	68	C2	C2	C2	424	35	2.2	0.95	4
	29	25	467	68	76	6.6	68	C2>C3		C2	414	40	6.54	1.22	0
	33	28	375	73	72	6.3	66	C2	C2	C2	289	56	2	0.95	8.36
	35	28	493	76	73	6.1	68	C3	C3	C3	436		2.01	0.78	1.58
	24	28	425	72	64	5.3	60	C3			344	47	4.4	1.13	0.42
	23A	28	450	73	67	6.1	64	C3		C3	359	46	4.56	1.12	2.38
	23B	30	450	73	79	6	71	C3		C3	403	39	2.9	0.99	1.42
	22	30	483	77	77	6.6	72	C3		C3	384	44	2.3	0.93	1.92
	34	30	457	67	68	5.3	67	C3		C3	373	45	1.98	0.95	2.82
	31	30	446	64	73	6.1	67	C3	C3	C3					
	30	30	430	79	73	6.4	70	C2	C2	C2	414	39	2.26	0.96	1.29

Table 4.4: Bleaching variables for *Pocillopora damicornis* (TTB=time to bleach, $CO_{(i)}$=initial colour, %ΔCOΔ(b)=change in colour due to bleaching, ZD(h)=mean healthy zooxanthellae density (cells/cm2, in millions), %ΔZD(b)=per cent changed due to bleaching, ZC=zooxanthellae clade, MY(b)=mean bleached photosynthetic yield, %ΔMY(b)=per cent change in mean photosynthetic yield, NPQ(b), qN(b), qP(b)= bleached values for quenching

	Tag	TTB	CO(i)	%ΔCO(b)	ZD(h)	%ΔZD(b)	ZC	MY(b)	%ΔMY(b)	NPQ(b)	qN(b)	qP(b)
Exp't 1	3	21	419	52	5	74	C1	359	43	1.41	0.96	0.96
	8	23	452	55	5.7	72	C1+	425	35	0.97	0.87	0.87
	16	23	420	68	5.4	61	C1+	339	47	1.28	0.91	0.91
	10	24	443	64	5.8	59	C1	468	26	0.69	0.8	0.78
	6	24	462	61	5.1	66	C1	521	17	1.1	0.92	0.92
	13	24	464	66	5.6	54	C1	467	30	1.22	0.94	0.94
	5	25	483	56	5.3	62	C1	468	27	1.57	1	1
	7	28	404	61	5.1	69	C1	565	14	2.58	1	1
	18	28	448	71	5	75	C1	524	20	1.7	0.99	0.99
	14	30	434	54	5.4	65	C1	510	19	1.19	0.93	0.93
Exp't 2	19	28	450	62	5.6	65	C1	424	35	2.2	0.95	4
	24	28	480	73	5.8	69	C1	414	40	6.54	1.22	0
	36	30	434	63	5	65	C1	289	56	2	0.95	8.36
	23	30	476	58	4.9	63	C1	436		2.01	0.78	1.58
	29	32	432	72	5.3	71	C1	344	47	4.4	1.13	0.42
	30	32	387	48	5.4	71	C1	359	46	4.56	1.12	2.38
	25	32	428	53	5.1	62	C1	403	39	2.9	0.99	1.42
	22	34	482	53	6	39	C1	384	44	2.3	0.93	1.92
	26	34	456	52	5.4	69	C1	373	45	1.98	0.95	2.82
	28	34	439	57	5.2	72	C1	414	39	2.26	0.96	1.29

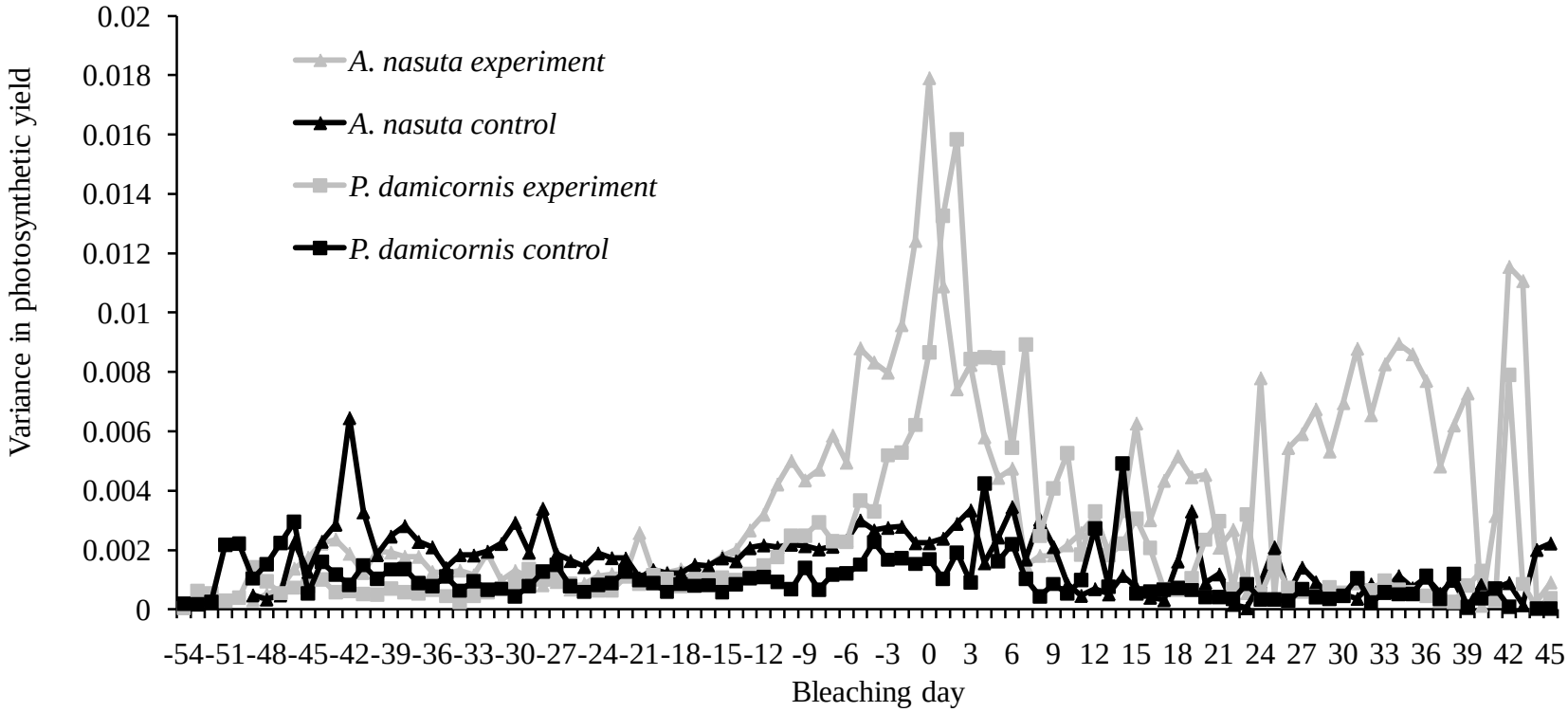

Figure 4.1: Variance in the photosynthetic yield for the duration of the experiment. The x-axis is re-scaled for each colony to standardise changes relative to the day of bleaching, which is set as time 0, regardless of the time taken for colonies to bleach

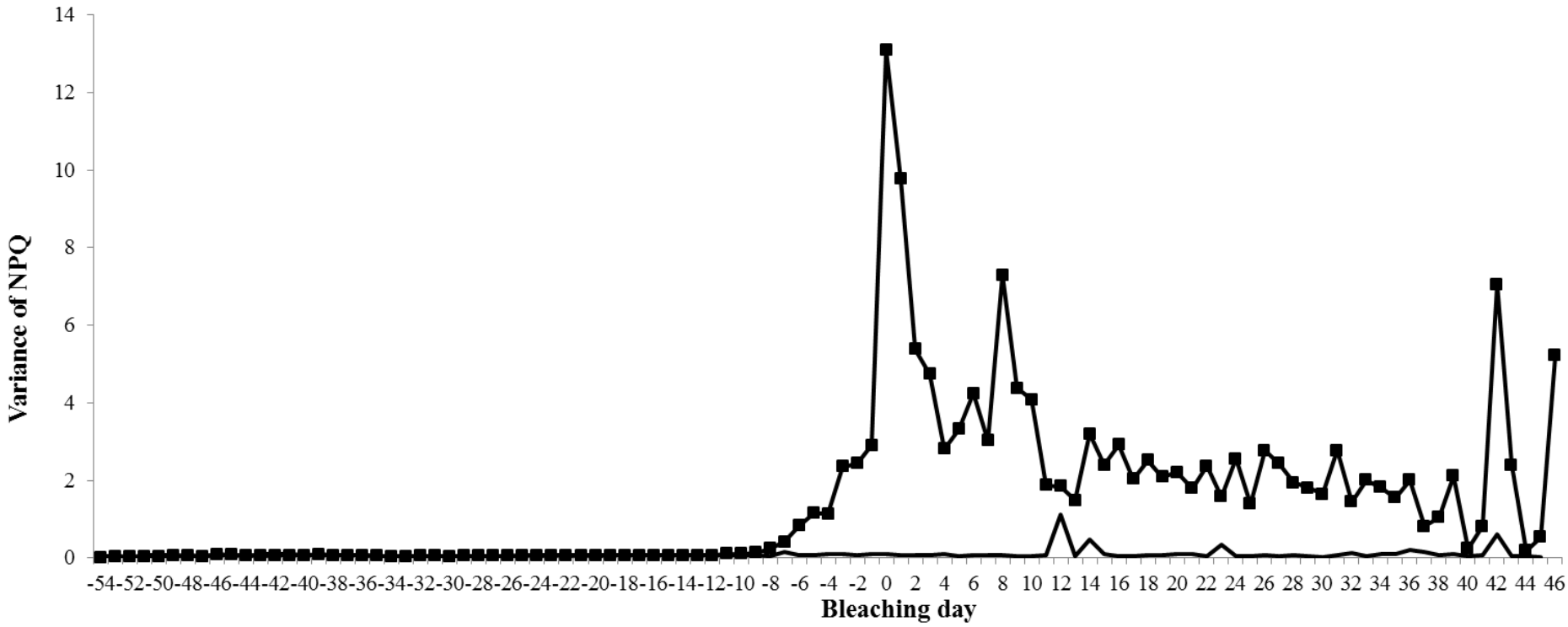

Figure 4.2: Variance in the non-photochemical quenching (heat dissipation at the antennae) of coral colonies. Data is pooled for the two study species (*A. nasuta* and *P. damicornis*), given no significant difference). The control colonies are the solid black line, while the experimental corals are the squared line. The x-axis is re-scaled for each colony to standardise changes relative to the day of bleaching, which is set as time 0, regardless of the time taken for colonies to bleach.

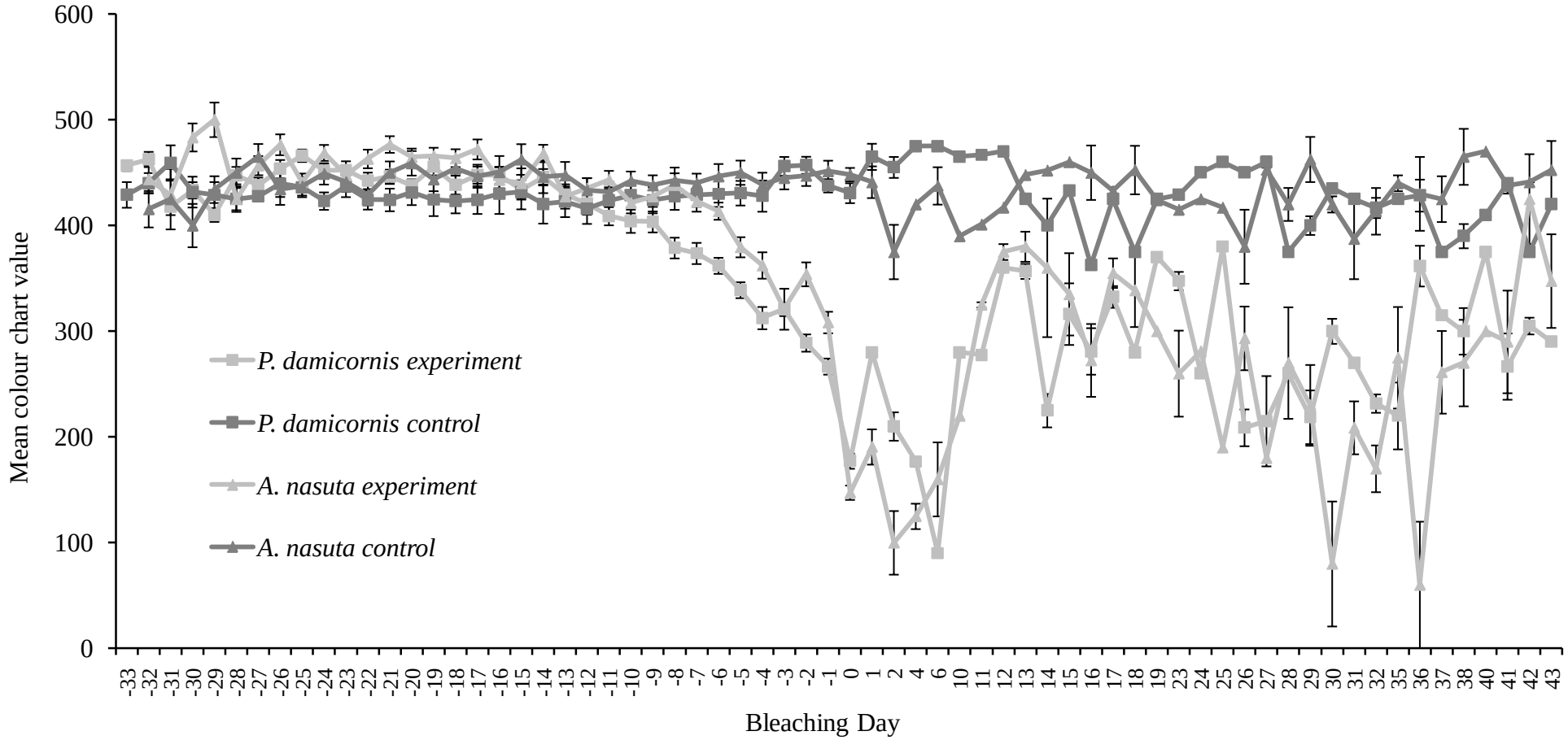

Figure 4.3: Coral colour card scores for *A. nasuta* and *P. damicornis* for the duration of the experiment. The x-axis is re-scaled for each colony to standardise changes relative to the day of bleaching, which is set as time 0, regardless of the time taken for colonies to bleach.

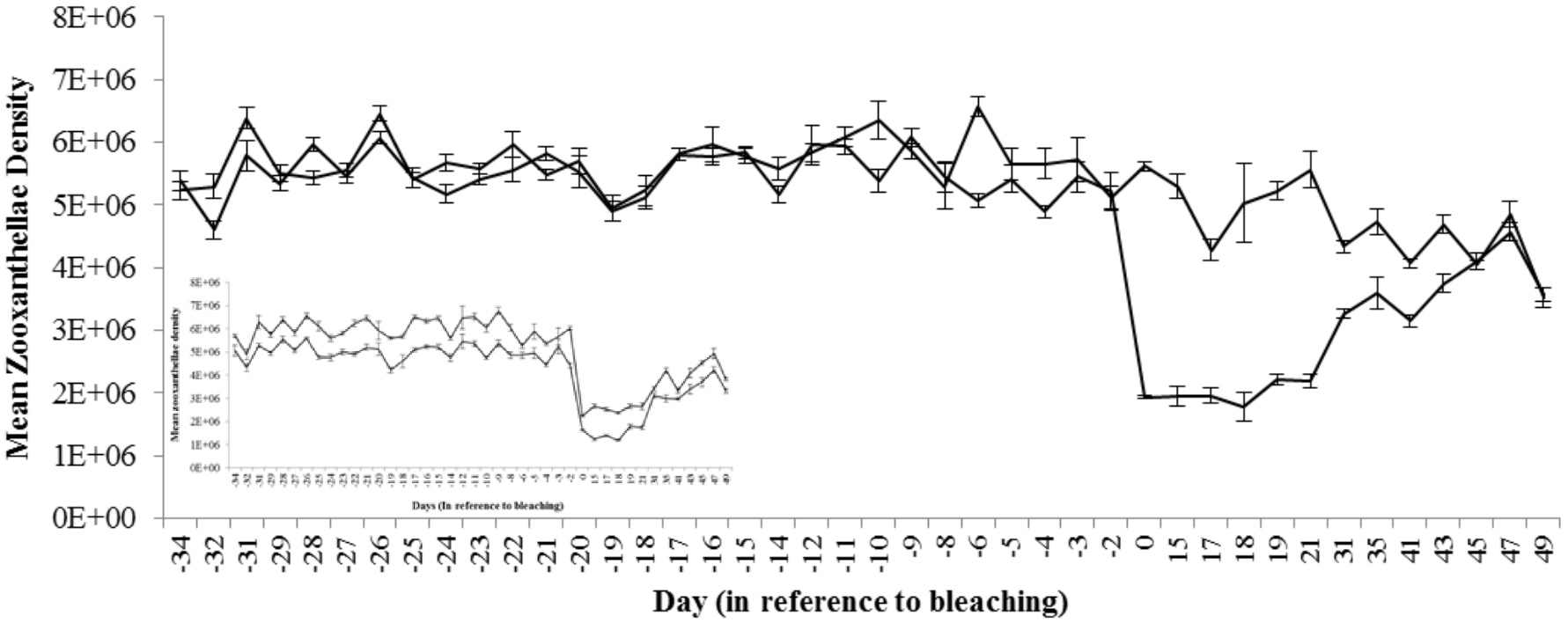

Figure 4.4: Mean (±SE) zooxanthellae densities for control (no significant change through time) and experimental colonies of *A. nasuta* and *P. damicornis* (NB. Data pooled among species, given no significant difference). The x-axis is re-scaled for each colony to standardise changes relative to the day of bleaching, which is set as time 0, regardless of the time taken for colonies to bleach. Inset shows that zooxanthellae loss is similar for both light- and dark-adapted sections of experimental branches.

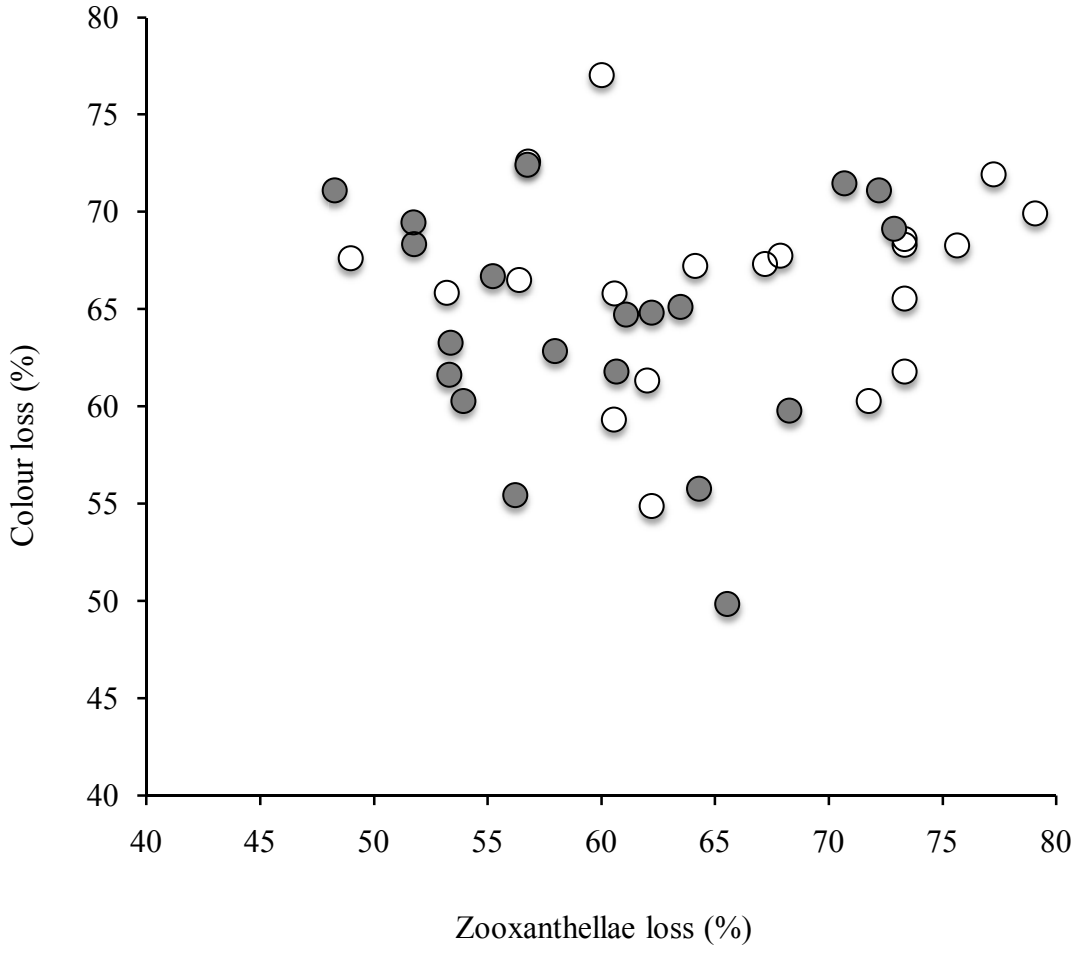

Figure 4.5: Scatterplot showing relationship between proportional colour loss and proportional declines in zooxanthellae for *Acropora nasuta* (white circles) and *Pocillopora damicornis* (grey circles). There was no significant relationship for either coral species ($r^2 < 0.05$).

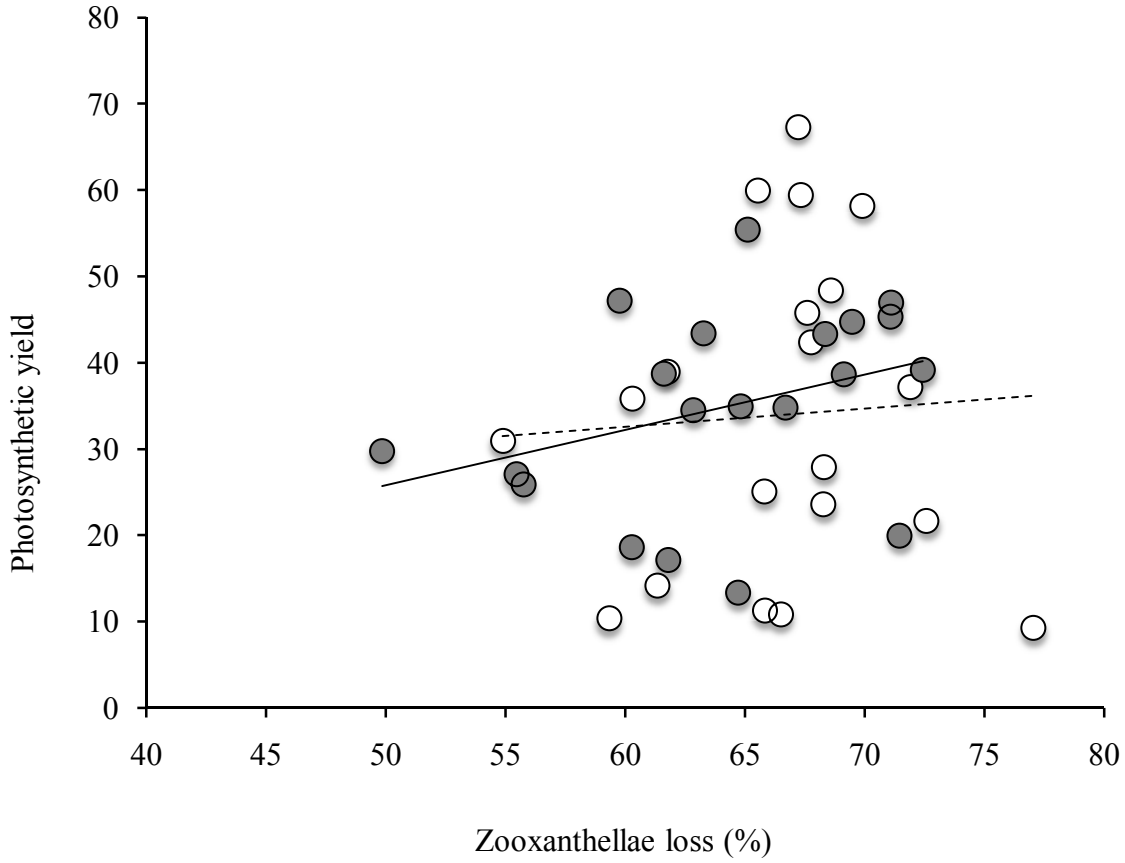

Figure 4.6: Scatterplot showing relationship between photosynthetic yield and proportional declines in zooxanthellae for *Acropora nasuta* (white circles) and *Pocillopora damicornis* (grey circles). There was no significant relationship for either *P. damicornis* or *A. nasuta*.

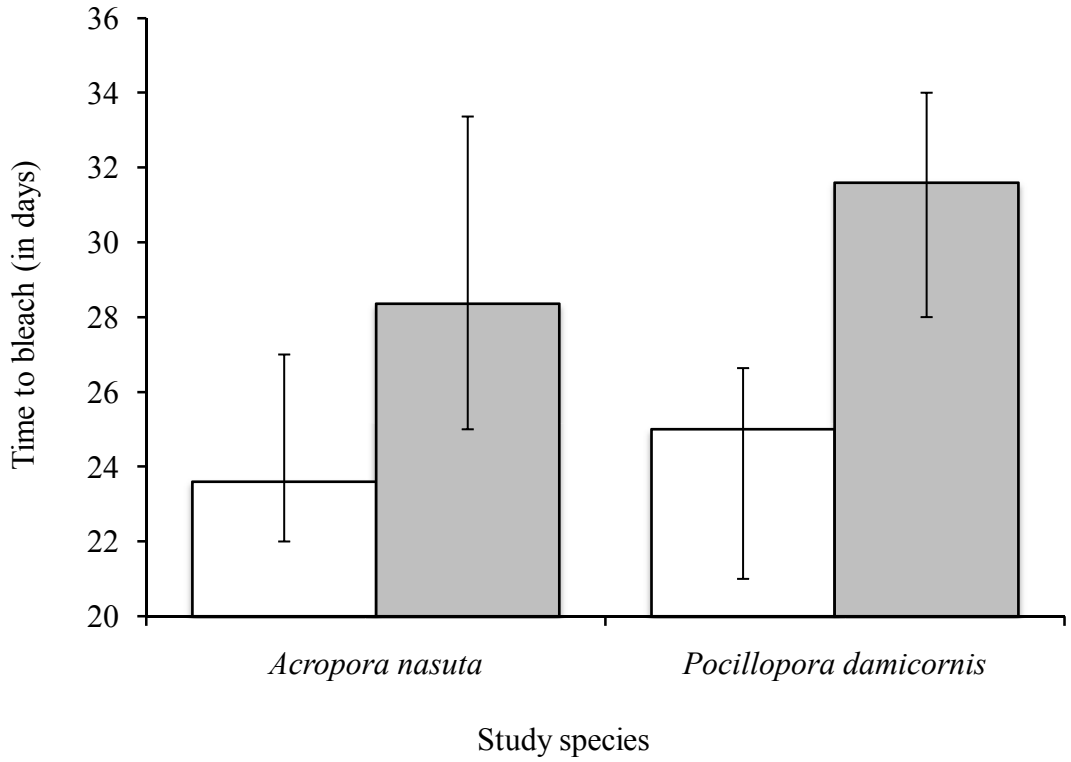

Figure 4.7: Mean and overall range (as indicated by error bars) in time to bleach (in days) for two different study species (*Acropora nasuta* and *Pocillopora damicornis*) subject to warming under experimental conditions in two successive experiments (Experiment 1 – white bars, Experiment 2 – grey bars).

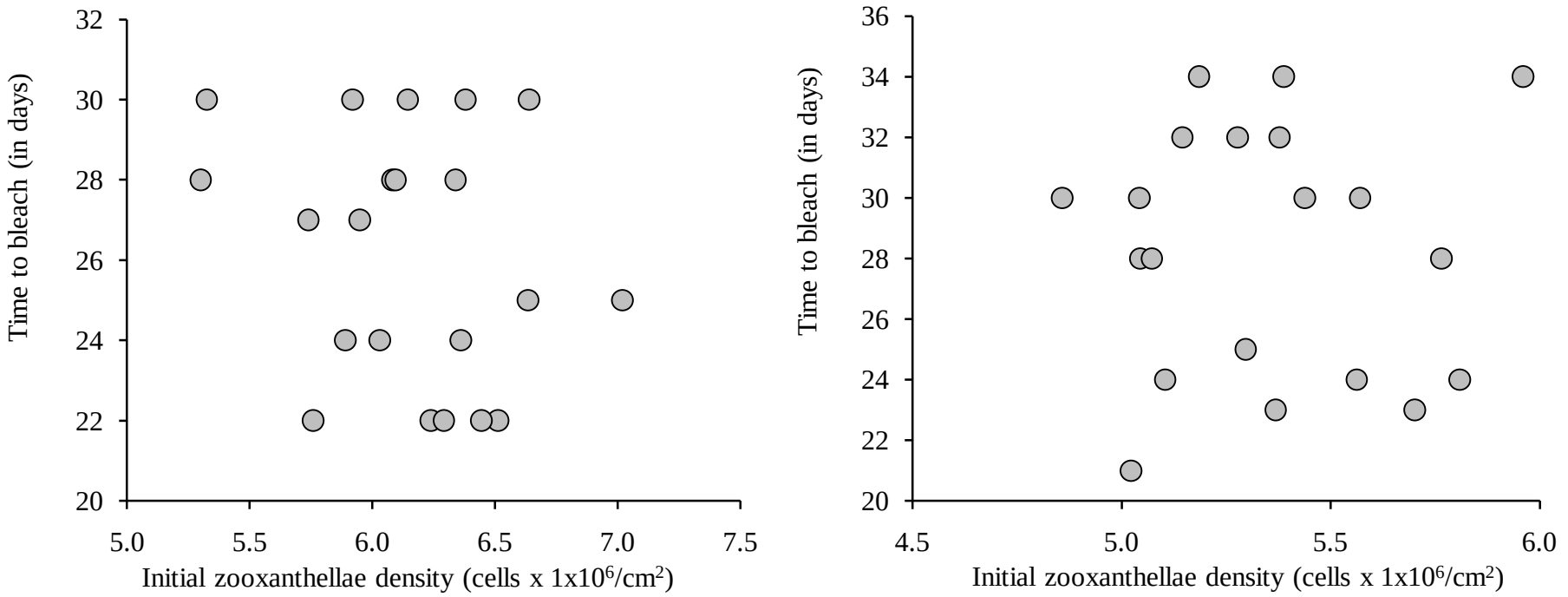

Figure 4.8: Scatterplots for *A. nasuta* (left) and *P. damicornis* (right) of initial zooxanthellae density compared to the time it took colonies to bleach at a constant 31.6°C. There was no significant relationship for either species.

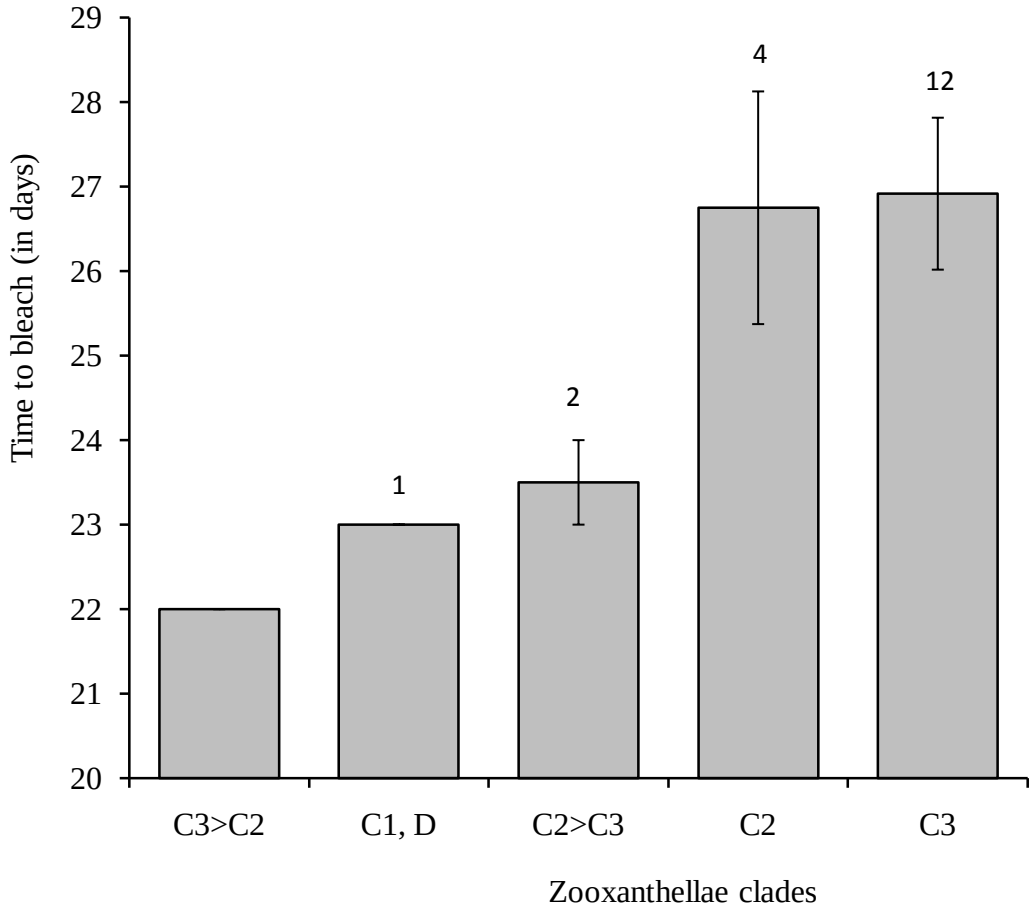

Figure 4.9: Comparison of the time to bleach for colonies of *Acropora nasuta* with different intial assemablages of zooxanthellae. The occurrence and relative abundance of different *Symbiodinium* clades was established using PCR with individual primers developed for all known clades that occur in *Acropora*.

Chapter 5: Changes in bleaching susceptibility among corals subject to ocean warming and recurrent bleaching in Moorea, French Polynesia[2]

5.1 Abstract

Climate-induced coral bleaching poses a major threat to coral reef ecosystems, mostly because of the sensitivities of key habitat-forming corals to increasing temperature. However, susceptibility to bleaching varies greatly among coral genera and there are likely to be major changes in the relative abundance of different corals, even if the wholesale loss of corals does not occur for several decades. Here we document variation in bleaching susceptibility among key genera of reef-building corals in Moorea, French Polynesia, and compare bleaching incidence during mass-bleaching events documented in 1991, 1994, 2002 and 2007. This study compared the proportion of colonies that bleached for four major genera of reef-building corals (*Acropora, Montipora, Pocillopora and Porites*), during each of four well-documented bleaching events from 1991 to 2007. *Acropora* and *Montipora* consistently bleached in far greater proportions (up to 98%) than *Pocillopora* and *Porites*. However, there was an apparent and sustained decline in the proportion of colonies that bleached during successive bleaching events, especially for *Acropora* and *Montipora*. In 2007, only 77% of *Acropora* colonies bleached compared with 98% in 1991. Temporal variation in the proportion of coral colonies bleached may be attributable to differences in environmental conditions among years. Alternately, the sustained declines in bleaching incidence among highly susceptible corals may be indicative of acclimation or adaptation. Coral genera that are highly susceptible to coral bleaching, and especially *Acropora* and *Montipora*, exhibit

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temporal declines in their susceptibility to thermal anomalies at Moorea, French Polynesia. One possible explanation for these findings is that gradual removal of highly susceptible genotypes (through selective mortality of individuals, populations, and/ or species) is producing a coral assemblage that is more resistant to sustained and ongoing ocean warming.

5.2 Introduction

Disturbances have an important influence on the structure and dynamics of shallow marine environments (Hughes et al. 2003), and especially for coral reef ecosystems (Karlson and Hurd 1993, Nystrom and Folke 2001). The frequency and severity of episodic disturbances has increased greatly in recent years due to emerging effects of global climate change combined with increasing anthropogenic pressures (Hughes et al. 2003, Gardner et al. 2003). Increases in disturbance frequency, severity and diversity are leading to widespread degradation of coral reef ecosystems. Wilkinson (2004) estimated that 20% of the world's coral reefs have already been severely degraded, whereby coral cover has declined by >90% and there is limited prospect of recovery. Coral-reef degradation is mostly concentrated in eastern Africa, South-East Asia, and the central and southern Caribbean (Wilkinson 2004) in areas with large human populations and a long history of exploitation (Pandolfi et al. 2003). Coral reef degradation is therefore caused, or at least precipitated, by direct anthropogenic disturbances. Climate change is also contributing to recent coral reef degradation (Hoegh-Guldberg 1999, Wilkinson 2004), and is expected to become the major cause of habitat degradation on reefs toward the latter part of this century (Hoegh-Guldberg et al. 2007).

The most apparent effects of climate change in natural ecosystems are changes in phenology (e.g., altered timing of reproductive activities (Walther et al. 2002, Parmesan and Yohe 2003) and shifts in species ranges (Thomas et al. 2004, Perry et al. 2005, Poloczanska

et al. 2007). Climate change has also caused dramatic shifts in the relative abundance of species (e.g., arctic, arid, tropical rainforests and coral reef ecosystems), and has contributed to species extinctions (Walther et al. 2002, Thomas et al. 2004). Thomas et al. (2004) predicted that 15–37% of species in terrestrial ecosystems will become extinct given a 2.0°C increase in mean global atmospheric temperatures. Significant species losses will not only reduce biological diversity but also potentially undermine ecosystem function, increasing the likelihood of ecosystem collapse (Tilman 2000, Myers and Knoll 2001).

On coral reefs, climate change (specifically, anomalously high sea temperatures) has been linked to large-scale coral bleaching (Jokiel and Coles 1990, Glynn 1991, Goeau et al. 2000). Many reef-building corals function close to their upper thermal limits, such that small increases in maximum temperatures (as little as 1.0°C; Jokiel and Coles 1990) lead to a breakdown in the relationship between the coral host and the critical photosynthesizing symbiotic zooxanthellae that give corals their colour. Declines in the densities of zooxanthellae leave corals 'bleached'. The increasing occurrence and severity of climate-induced coral bleaching reflects gradual increases in global sea-surface temperatures (SST). On average, global SST has increased by 0.7°C in the last century (Hoegh-Guldberg et al. 2007), bringing baseline ocean temperatures much closer to the maximum thermal tolerances for reef-building corals. As a consequence, naturally occurring temperature anomalies (e.g., linked to El Niño) increasingly cause thermal tolerances of corals to be exceeded, resulting in more frequent and severe episodes of coral bleaching (Hoegh-Guldberg 1999, Stone et al. 1999).

Climate-change models predict a further 1.8–4°C increase in temperatures for tropical regions over the next century (Hoegh-Guldberg et al. 2007). By 2050, most coral reefs are expected to be subject to annual thermal anomalies equivalent to the conditions in 1998 (Sheppard 2003, Hoegh-Guldberg et al. 2007), which caused extensive coral bleaching in

every ocean, and killed up to 90% of coral on individual reefs. Unless corals can adapt, ocean warming could cause wholesale loss of corals within the 21st century (Hoegh-Guldberg 1999), leading to fundamental shifts in the structure and function of coral reef ecosystems (Hughes et al. 2007). While it is likely that corals can adapt to changing environmental conditions, it is unclear whether adaptation can occur fast enough to ensure long-term persistence of coral-dominated ecosystems (Donner 2009). However, mass bleaching (especially when accompanied by highly selective mortality) is likely to impose strong selective pressures on coral populations and communities (Baird and Marshall 2002), leading to rapid increases in the prevalence of corals that are resistant or resilient to high temperatures (Glynn et al. 2001, Guest et al. 2012).

If corals can adapt or acclimate to ocean warming, we would expect a temporal decline in the proportion of coral colonies that bleach, or die, at a given temperature. In Moorea, French Polynesia, in the central Pacific, major episodes of coral bleaching have occurred every 2–5 years since 1991 (Trapon et al 2011), corresponding with significant positive temperature anomalies (Hoegh-Guldberg 1999, Penin et al. 2007). This study quantified the proportion of coral colonies that bleached and/or died during a mass-bleaching event in late summer 2007. Our findings were then compared with well-documented and comparable bleaching events in 1991, 1994, and 2002, testing for declines in bleaching susceptibility among key genera of reef-building corals. There was evidence of temporal declines in bleaching susceptibility in some (but not all) coral genera, which is suggestive of population acclimation or community-level adaptation. While climate-change will undoubtedly cause, and is already having, major impacts on coral reef ecosystems (Hoegh-Guldberg et al. 2007, Pratchett et al. 2008), variation in the current and future capacity of corals to resist bleaching will lead to marked changes in community composition, prior to wholesale loss of these important habitat-forming organisms.

5.3 Methods

This study was conducted on the northern coast of Moorea (17°30' S, 149°50' W), Society Islands, French Polynesia. Sampling was conducted at two locations, Vaipahu and Tiahura, separated by approximately 2 kilometres on the north coast of Moorea (Pratchett et al. 2011a). Sampling was conducted in six distinct reef zones: 1) the inner reef flat (1–2 m depth); 2) the outer reef flat (1–3 m depth), 3) the reef crest (3–5 m depth), 4) the shallow reef slope (7–9 m depth), 5) the mid-slope (10–12 m depth), and 6) the deep slope (18–20 m depth). Corals were sampled using five replicate (50 × 4-m) belt transects, classifying all corals to one of four different bleaching categories; 1) no bleaching (0%), 2) moderately (<50%) bleached, 3) mostly (>50%, but <100%) bleached, and 4) completely (100%) bleached, based on the proportion of the tissue area that was conspicuously pale or white, but not dead. All corals were also identified to genera and categorized to one of three different size classes; 1) <10cm maximum diameter, 2) 10-50cm maximum diameter, and 3) >50 cm maximum diameter. Bleaching susceptibility was calculated using the bleaching index (BI) that weights the proportion of colonies that bleached (i.e. $0B_1 + 1B_2 + 2B_3 + 3B_4 / 6$) by the severity of bleaching, following Guest et al. (2012). A non-parametric Friedman test was carried out to compare bleaching and mortality index (BMI) values among size classes for each of the four major coral genera (*Acropora*, *Montipora*, *Pocillopora* and *Porites*).

Taxonomic differences in bleaching susceptibility, as well as absolute rates of coral bleaching, recorded in 2007 were compared with well-documented bleaching events over the past 2 decades. Changes in coral cover and composition (mostly to genus) have been reported in Moorea since the 1970s, focusing on Tiahura reef (and to a lesser extent at Vaipahu) on the north-west corner of Moorea (Trapon et al. 2011). During this period, coral assemblages have been subject to many acute (pulse) disturbances, which have caused major changes in the community structure. Most notably, multispecific coral bleaching has been reported every 3-4

years since 1983 (1984, 1987, 1991, 1994, 2002, 2003, and 2007), corresponding with periods when sea surface temperature increased above 29.0 °C (Hoegh-Guldberg and Salvat 1995, Trapon et al. 2011, Adjeroud et al 2009). However, the best documented instances of bleaching, including estimates of proportional bleaching in each of the major coral genera, occurred in 1991 (Salvat 1992), 1994 (Hoegh-Guldberg and Salvat 1995) 2002 (Penin et al. 2007) and 2007 (Pratchett et al. 2011a). For each of these studies, we used data on the proportion of colonies within each of four major genera that had exhibited bleaching. All four studies report on proportional bleaching for major coral genera on the outer reef slope. Of the available data we have restricted attention to sites (i.e., Tiahura and Viapahu) on the north coast, with sampling conducted at depths between 6 and 18m. A non-parametric Friedman test was carried out to compare bleaching susceptibility among four major coral genera (*Acropora, Montipora, Pocillopora* and *Porites*) across years (1991, 1994, 2002, and 2007).

Sea surface temperature data for the summer periods (Feb-Apr) between 1991 and 2007 were acquired from the NOAA Pathfinder v5.0 dataset and weekly night-only composites were gap filled following Heron et al. (2010). The temperature time-series for the 4×4 km pixel closest to the centre of the two study sites was extracted to determine the summer average temperature. The threshold for thermal stress was defined as the maximum monthly mean (MMM = 29.0 °C; Liu et al. 2003) of the dataset and accumulated heat stress (in °C-weeks) was calculated for all temperatures exceeding the MMM value. Accumulated heat stress is similar to a 'degree heating week' (which accumulates only when temperature is MMM +1 °C or above; Liu et al. 2003) in that one week at 1 °C above the MMM results in 1 °C-week of accumulated heat stress. Comparison of annual summer temperature and accumulated heat stress during bleaching years and from non-bleaching years was undertaken using Student's t-test.

Cloud data for the summer periods (Feb-Apr) between 1991 and 2007 were acquired from the International Satellite Cloud Climatology Project (ISCCP; isccp.giss.nasa.gov; Rossow and Schiffer 1991) for the ~250×250 km pixel that included the study sites. Cloud cover values in the early-afternoon (local time 12:00 and 15:00) were analysed following Mumby et al. (2001). The median of these summer cloud cover observations during the 17-year study period was determined as 27.7%. To evaluate the impact of light exposure on the observed levels of bleaching, the proportion of early-afternoon summer cloud cover observations less than 25% (i.e., higher than median insolation) was determined for Feb-Apr of each year (Figure 5.1). Comparison of cloud cover proportion during bleaching years and from non-bleaching years was undertaken using Student's t-test.

5.4 Results

From February 2007, coral assemblages on the north coast of Moorea were subjected to a prolonged period (11 of 12 consecutive weeks) of water temperatures in excess of 29.0°C. These conditions caused accumulated heat stress (in °C-weeks) of 4.63; less than the 7.96 experienced in 2002, but comparable with the 5.25 and 6.18 experienced in 1994 and 1991, respectively (Figure 5.1a). In 2007, 32.8% (n = 2180) of colonies were bleached. There were also low levels of recent coral mortality. However, this mortality cannot be unequivocally attributed to temperature stress and bleaching because there were also moderate densities of the coral feeding starfish *Acanthaster planci*, in areas where bleaching was observed (Pratchett et al. 2011a).

Taxonomic differences in bleaching susceptibility were very pronounced (Figure 5.2): 64.9% (n = 548) of colonies of *Acropora* were bleached, whilst only 2.44% (n = 286) of *Porites* colonies exhibited any evidence of bleaching. Moreover, most *Acropora* were completely (100%) bleached, whereas most bleached colonies of *Pocillopora* and *Porites*

were only partially bleached. Bleaching susceptibility was greatest for *Acropora* (BI = 51.87), then *Montipora* (BI = 19.30), *Pocillopora* (BI = 13.81), and *Porites* (BI = 0.84). Analysis of the bleaching and mortality response (BMI) across these four major coral genera revealed significant differences in size-specific bleaching susceptibility (Friedman test = 40.38, df = 3, $p < 0.01$). For *Acropora* and *Montipora*, bleaching susceptibility was fairly consistent across small, medium and large colonies (Figure 5.3). For *Pocillopora* and *Porites* however, there was a marked effect of colony size on bleaching susceptibility. In *Pocillopora*, large colonies (>50 cm diameter) had a much higher susceptibility (BI = 47.62) compared with smaller colonies (Figure 5.3). For *Porites*, bleaching was restricted to small and medium sized corals (<50 cm diameter), with no bleaching recorded amongst colonies >50cm in diameter (Figure 5.3).

Since 1979, coral reefs on the north coast of Moorea have been subject to several major disturbances, including seven bleaching events, two cyclones and two major outbreaks of *Acanthaster planci* (Adjeroud et al. 2009, Trapon et al. 2011). The best-documented bleaching events occurred in 1991 (Salvat 1992), 1994 (Hoegh-Guldberg and Salvat 1995) 2002 (Penin et al. 2007) and 2007 (Pratchett et al. 2011a). On each of these occasions, the proportion of colonies that bleached tended to be recorded for each of the major coral genera (e.g., *Acropora, Montipora, Pocillopora* and *Porites*). Analysis of the bleaching incidence for these four major coral genera revealed significant change in the relative susceptibility of taxa among years in which bleaching was documented (Friedman test = 39.67, df = 3, $p < 0.01$). Importantly, the proportion of colonies that bleached during each bleaching episode was very different (Figure 5.4). The proportion of *Acropora* (Figure 5.4A) and *Montipora* colonies (Figure 5.4B) that bleached in 2002 was lower than in previous bleaching events and lower again in 2007. Indeed, bleaching susceptibility in these genera appears to be decreasing

through time. There also appears a similar trend for *Porites* (Figure 5.4C), but not for *Pocillopora* (Figure 5.4D).

Higher maximum temperatures, and/ or prolonged exposure to unusually high temperatures typically results in much higher incidence of bleaching and mortality (Hoegh-Guldberg 1999). Accordingly, bleaching has been observed at Moorea in years (1991, 1994, 2002, 2003, and 2007) when accumulated heat stress is highest and at least above 4 (Figure 5.1a). The accumulated heat stress in these five years was significantly higher (Student's t = 61.65, df = 15, p = 1.08e-6) than for all other years when no bleaching was recorded. Average annual temperatures have increased gradually (0.16 °C per decade) since 1991 (Figure 5.1b). The heat stress experienced in 2003 and 2007 is comparable with or higher than in 1991 and 1994 but the severity of mass bleaching (the proportion of colonies bleached) has declined from 1991 to 2007.

Temporal declines in bleaching incidence reported for *Acropora* and *Pocillopora* during the four well-documented bleaching events in Moorea (Figure 5.4) are not clearly linked to successive declines in the local levels of average temperature or higher cloud cover (Figure 5.5). There was also no significant differences in annual summer temperature for the five years when bleaching was observed and the remaining non-bleaching years (Student's t = 2.887, df = 15, p = 0.11). The synergistic effects of high light levels and high temperature on coral bleaching suggest that cloud incidence could influence the extent and severity of observed bleaching. However, the percentage of summer cloud cover observations less than the median value was similar between 1991 and 2007; and the difference between the five analysed bleaching years (including 2003) and the remaining (non-bleaching) years was not significant (Student's T-test, t = 1.61, df = 15, p = 0.22).

5.5 Discussion

Bleaching incidence recorded in each of four major coral genera (*Acropora*, *Montipora*, *Pocillopora* and *Porites*) in 2007 was much lower compared with previously documented mass bleaching at Moorea. The overall proportion of corals bleached in 2007 (25% of colonies across all species) was approximately half that recorded in 1991 (51%). For *Acropora*, which is typically the genus most susceptible to coral bleaching (Baird and Marshall 2002), there was a 30% decline in bleaching incidence from 1991 to 2007 and a relatively consistent decline in the proportion of colonies that bleached over the four well-documented bleaching events. There are a number of environmental factors that may moderate bleaching responses during periods of high temperatures, particularly high cloud cover (Done 1999, Mumby et al. 2001, Loya et al. 2001). We failed to find any systematic trend in local environmental conditions (e.g., temperature or cloud cover) that might account for the sustained declines in bleaching susceptibility observed for highly susceptible genera, *Acropora* and *Montipora*. It is possible therefore, that recurrent bleaching has resulted in increased resistance to bleaching especially among the most susceptible genera (e.g., *Acropora*), as has been reported elsewhere (Glynn et al. 2001, Bena and van Woesik 2004, Guest et al. 2012).

Bleaching susceptibility varied greatly within and among coral genera on reefs in Moorea, with the potential to alter both population and community structure (Loya et al. 2001). With increasing environmental stress, it is expected that coral populations will have faster turnover and become increasingly dominated by small corals (Done 1999). Changes in population structure may be further reinforced by size-based differences in bleaching susceptibility (Loya et al. 2001), as was evident among *Pocillopora* corals at Moorea (Figure 5.3). However, size-selectivity in bleaching susceptibility is not always apparent (Baker et al. 2008) and was not observed for *Acropora*. It is suggested that smaller and flatter corals have

a greater mass-transfer capacity, corresponding with greater resistance to bleaching (Nakamura and van Woesik 2001). However, this does not effectively account for observed inter- or intra-generic differences in bleaching susceptibility (see also van Woesik et al. 2012). Many such generalities in patterns of bleaching susceptibility lack empirical support (Baird and Marshall 2002), emphasizing the need for much greater research on individual susceptibilities of different corals.

Variation in bleaching susceptibility may also be attributable to differences in the predominant type of zooxanthellae hosted by corals (Glynn et al. 2001, Baker et al. 2008). In the eastern Pacific, for example, increasing thermal tolerance of *Pocillopora* was linked to increased prevalence of colonies that host a thermally tolerant clade D symbiont (Glynn et al. 2001). Similarly, *Pocillopora* in French Polynesia host a diversity of symbionts, including clade D (Magalon et al. 2007), which may explain their low level of bleaching susceptibility compared with many other geographic locations (McClanahan et al. 2004). For other coral genera, which may be incapable of switching symbionts (Goulet 2006), prior exposure to environmental extremes may have stimulated photo-protective mechanisms (e.g., increased concentrations of certain pigments) that reduce bleaching susceptibility (Dunne and Brown 2001, Brown et al. 2002b).

Distinguishing between individual acclimation versus selective mortality and directional changes in the structure of coral assemblages requires detailed information on the long-term bleaching susceptibility and subsequent fate of individually tagged corals (Baird and Marshall 2002). However, recent changes in the community structure of coral assemblages in Moorea (Berumen and Pratchett 2006, Adjeroud et al. 2009, Pratchett et al. 2011a, Trapon et al. 2011) may reflect selective removal of susceptible phenotypes. Notably, Pichon (1985) recorded 39 species of *Acropora* from French Polynesia, during surveys conducted prior to 1981, when *Acropora* was the dominant coral genera. In comparison,

biodiversity assessments conducted in the late 1990's recorded only 22 *Acropora* species in French Polynesia (Karlson et al. 2004). Contrasting estimates of species richness between these two studies are probably due partly to differences in methodologies, including sampling intensity and range of habitats actually sampled. However, local abundance of *Acropora* has declined substantially (>80%) since 1979 (Trapon et al. 2011), and it is likely that this has reduced coral diversity. Rapid adaptation through selective removal of susceptible genotypes is also likely to be most pronounced in populations or taxa that typically experience high levels of bleaching-related mortality, like *Acropora*.

Coral reef ecosystems are widely regarded to be among the most threatened ecosystems, due to increases in ocean temperatures and extreme temperature sensitivities of most reef-building corals (Hoegh-Guldberg et al. 2007). However, observed declines in bleaching susceptibility among reef-building corals suggest that there is some capacity for adaptation, which will delay devastating effects of global climate change. The critical question is how far can the adaptive capacity of scleractinian corals extend? Inherent limits to the rate or extent of acclimation and adaptation may simply delay local and global extinctions of coral species subject to ever increasing ocean temperatures (Baker et al. 2008). Further, bleaching events coincident with periods of anomalously high temperatures are one of many selective forces on reefs. Gradual acclimation and adaptation to increased temperatures by coral assemblages in Moorea and elsewhere can easily be undone by other natural and anthropogenic stressors and disturbances. Outbreaks of crown-of-thorns starfish, *Acanthaster planci*, for example, may have altogether different selective forcing on coral populations and communities.

The most pronounced declines in coral cover observed in Moorea since 1979 have been associated with outbreaks of *A. planci* (Trapon et al. 2011). Most recently, outbreaks of *A. planci* occurred in 2006, reducing total coral cover by >50% and further reducing coral

diversity (Pratchett et al. 2011a, Kayal et al. 2012). Moreover, outbreaks of *A. planci* have had disproportionate effects on *Acropora* corals, leading to marked changes in the coral communities since 1979. Mortality caused by these disturbances is likely to have eliminated some or even all of the increased thermal tolerance gained between successive bleaching events. The capacity for scleractinian corals to adjust to, and cope with, ongoing increases in ocean temperatures may be appreciable (Glynn 1991, Maynard et al. 2008a, Guest et al 2012). In order to maximize adaptive capacity to climate change it will continue to be important to minimize the diversity, frequency and severity of other anthropogenic disturbances that also effect coral reef organisms.

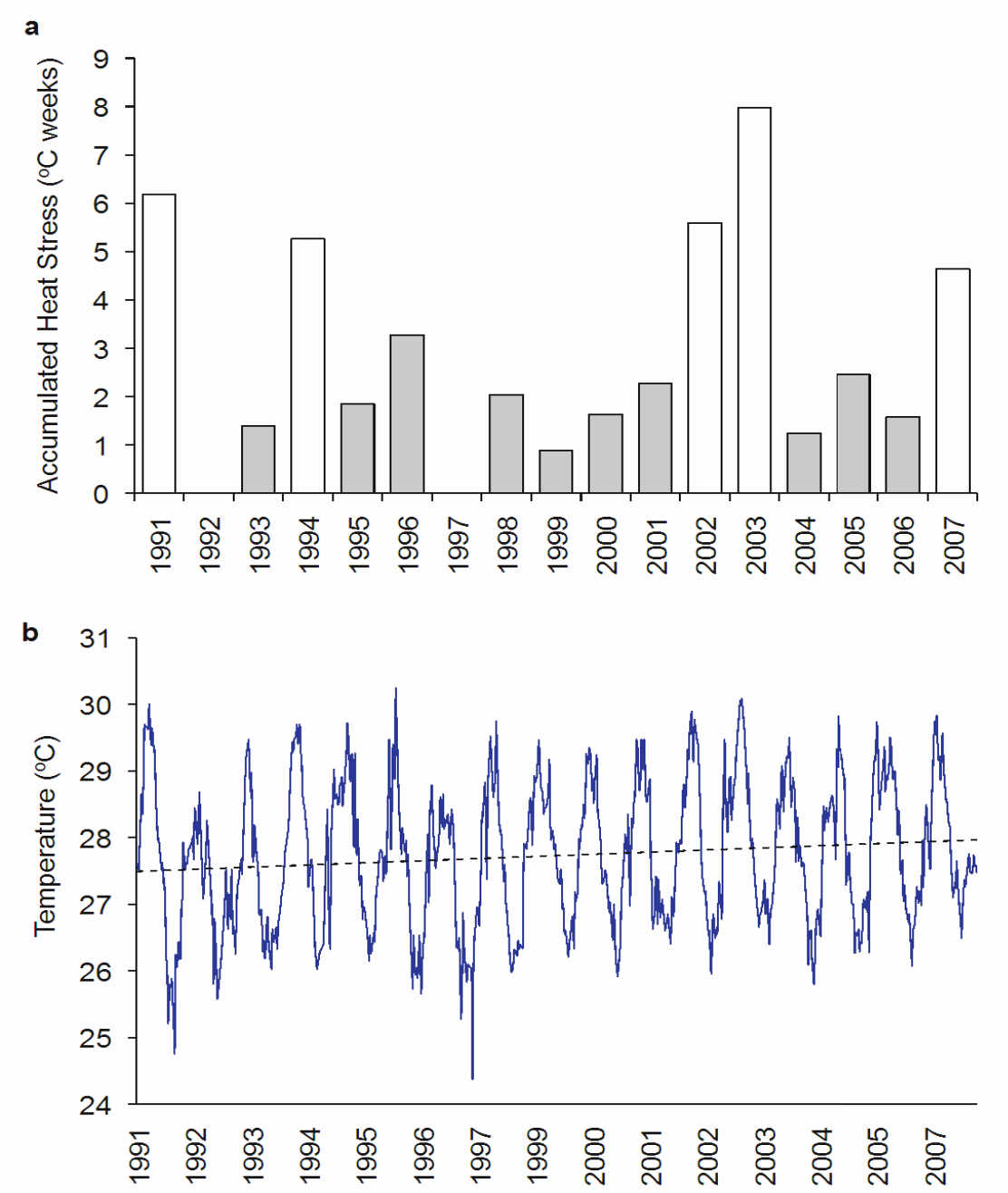

Figure 5.1: Annual thermal conditions in Moorea from 1991 to 2007. Data were derived from the NOAA Pathfinder v5.0 dataset. a) Annual accumulated heating stress (in °C-weeks) was calculated by summing positive anomalies above the maximum monthly mean of 29.0 °C. Years where bleaching was observed are shown in white. b) The long-term trend in daily temperature reveals an increase during the study period at a rate of 0.16 °C per decade.

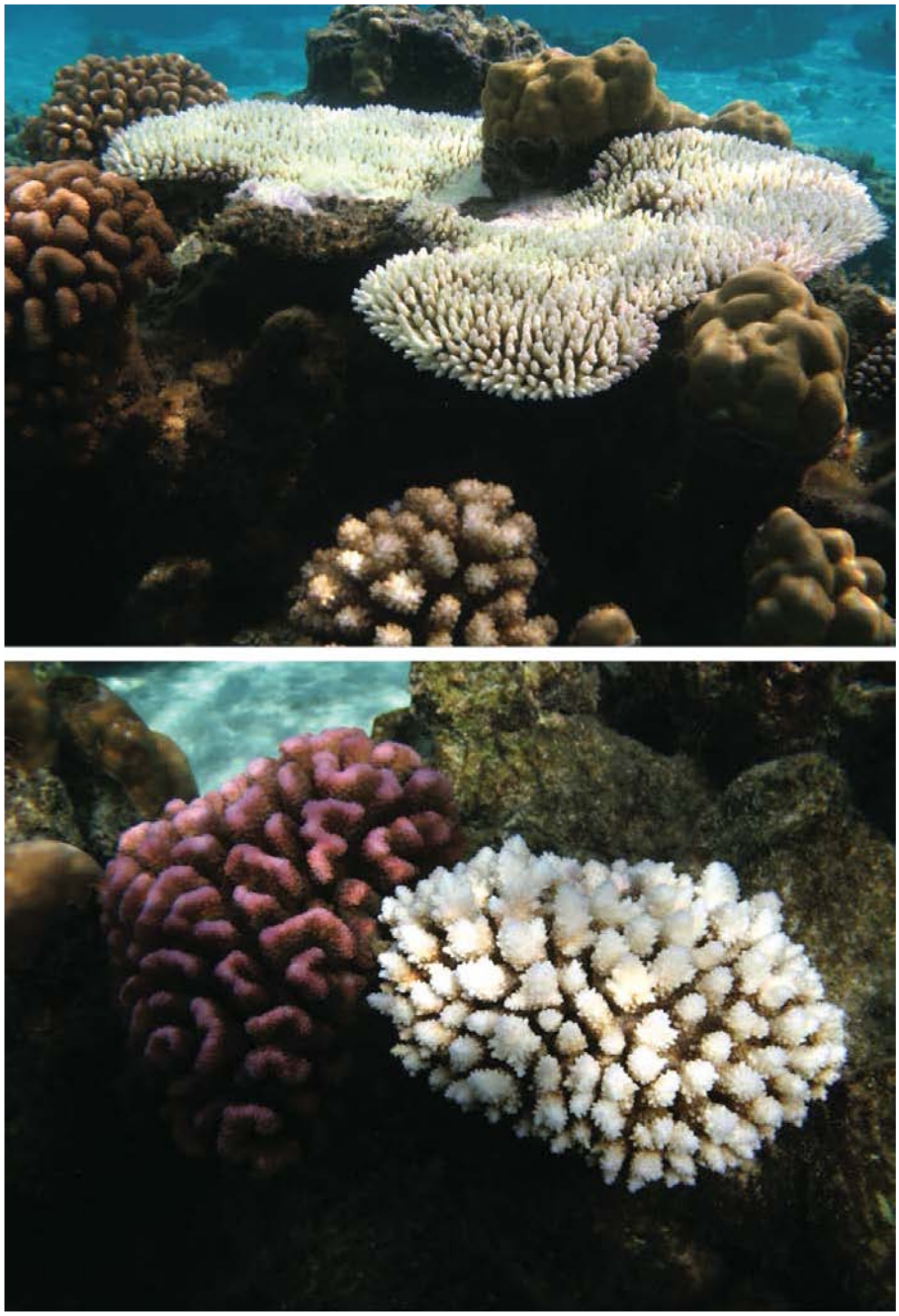

Figure 5.2: Taxonomic differences in bleaching susceptibility in Moorea, French Polynesia. Taken in late summer 2007, these photographs show bleached *Acropora* adjacent to colonies of *Pocillopora* and *Porites*, which are seemingly unaffected. *Pocillopora* are generally considered to be amongst the most susceptible genera to climate-induced coral bleaching (e.g., McClanahan et al. 2004), but *Pocillopora* corals exhibit unusually high resistance to high temperatures at Moorea.

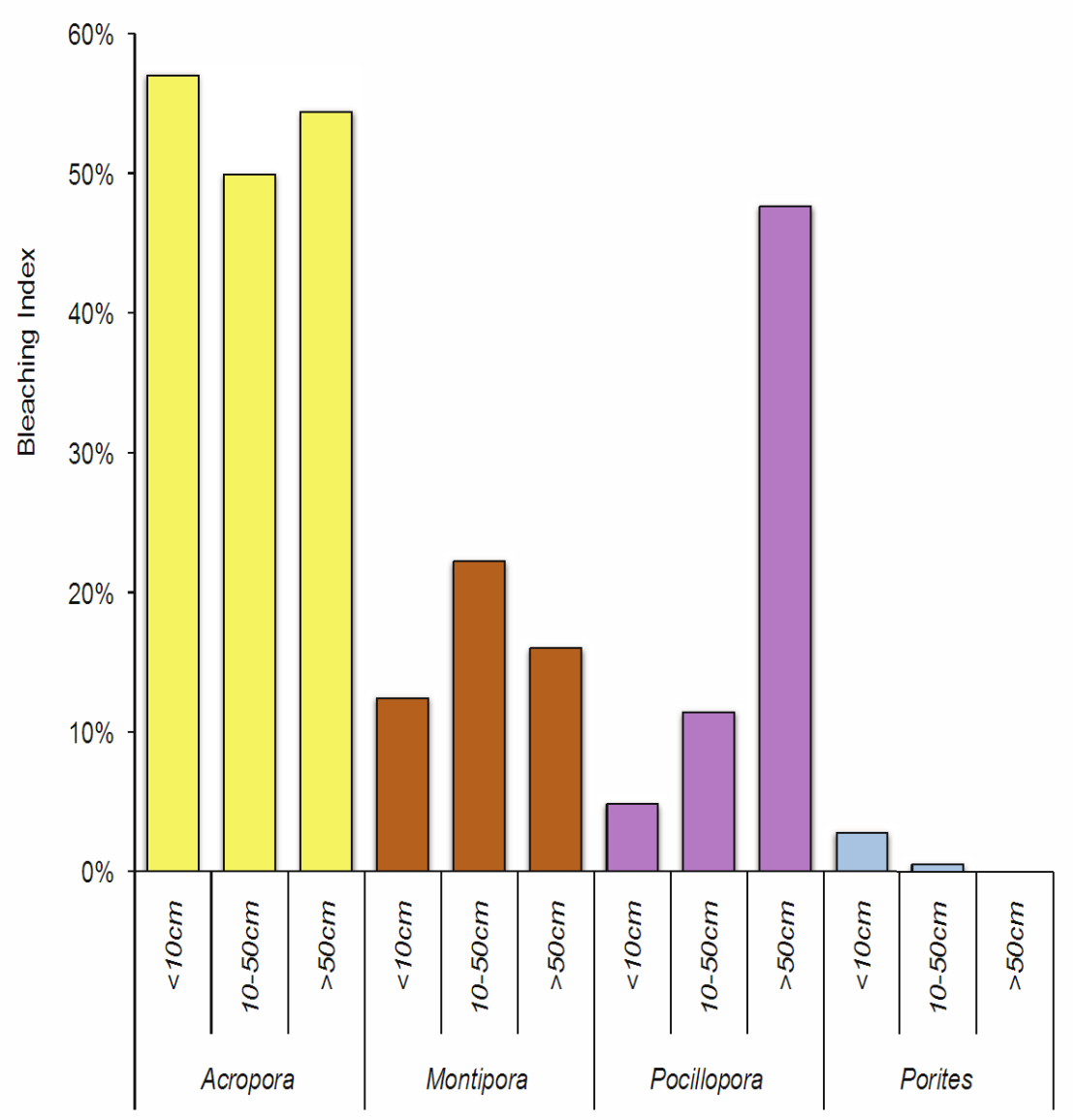

Figure 5.3: Size-specific bleaching susceptibility for four key genera (*Acropora*, *Montipora*, *Pocillopora*, and *Porites*) in 2007. Bleaching susceptibility was calculated using a bleaching index (BI) that weights the proportion of colonies that bleached by the severity of bleaching, following Guest et al. (2012).

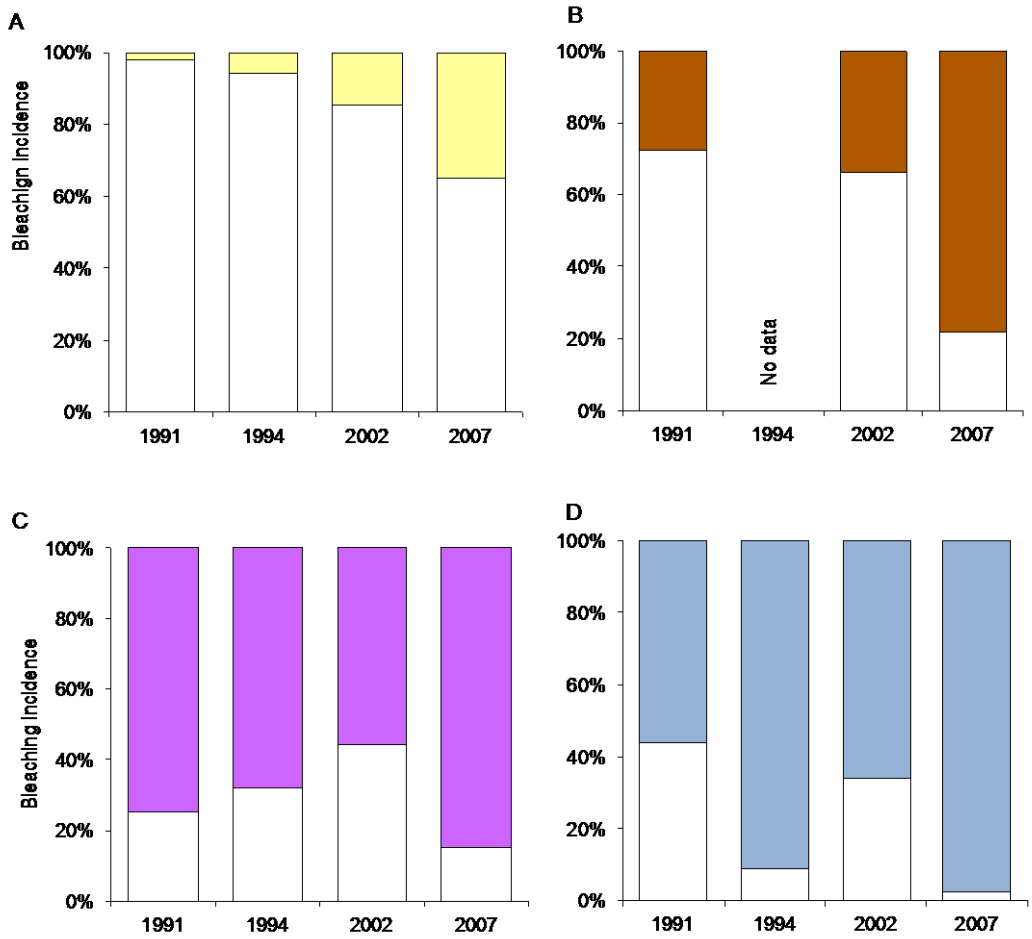

Figure 5.4: Proportional bleaching in A) *Acropora*, B) *Montipora*, C) *Pocillopora*, and D) *Porites* during well documented bleaching events in Moorea, French Polynesia. Graphs distinguish the proportion of colonies for each genus that had any evidence of bleaching (in white) from those that did not bleach (coloured) in 1991, 1994, 2002 and 2007. All surveys were conducted on the outer reef slope (6-18m depth) along the northern coast of Moorea.

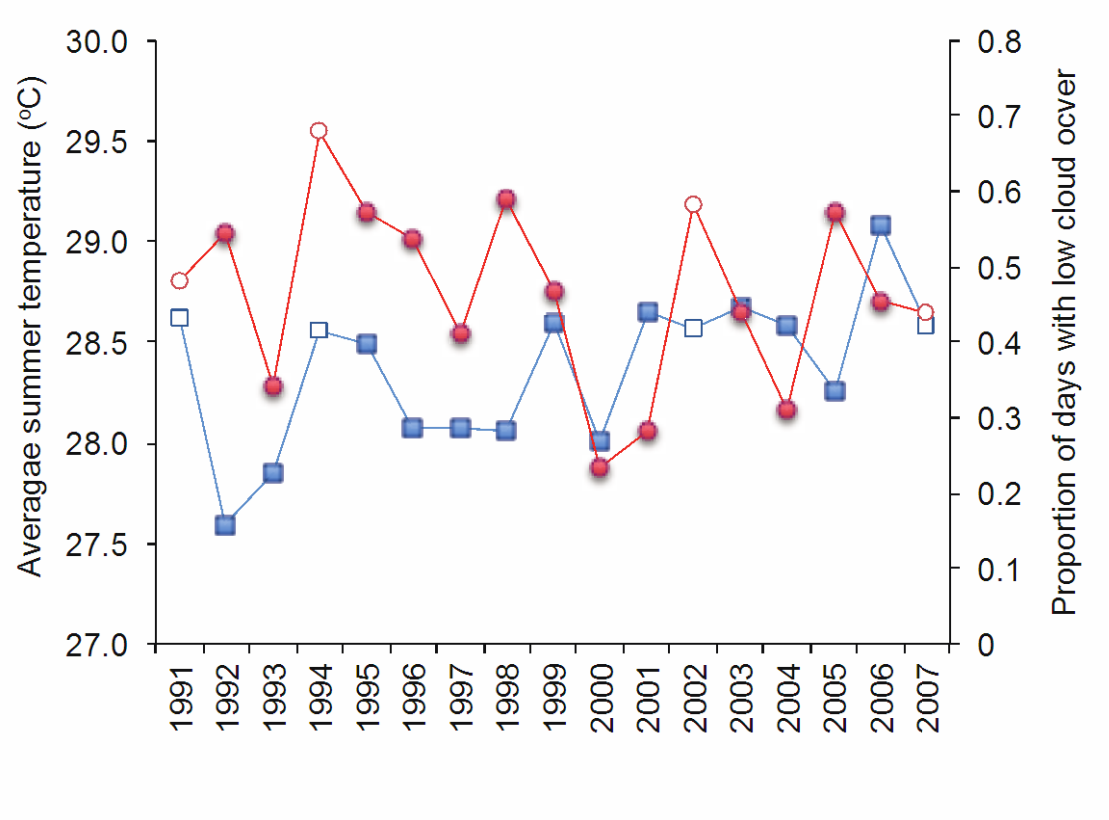

Figure 5.5: Summer temperature and cloud conditions at Tiahura Reef, Moorea, from 1991 to 2007. Average sea surface temperature (red circles) and the proportion of early-afternoon summer cloud cover observations less than the summer median (blue squares). Open symbols represent the four bleaching events considered in 1991, 1994, 2002 and, 2007.

Chapter 6: Temporal and spatial variation in bleaching susceptibility and mortality.

6.1 Abstract

Mass bleaching events do not affect all corals equally, often leading to strong selective forcing on community structure of coral assemblages. Several studies have compiled data on the differential susceptibility of corals to climate induced coral bleaching, but there has been little effort to establish whether the hierarchy of bleaching susceptibility is spatially or temporally consistent; most studies compare a maximum of two distinct geographic locations. The purpose of this study is to test whether taxonomic (generic) variation in bleaching susceptibility and mortality is consistent among broad geographic regions (ocean basins), and through time (decades). Data was compiled from 105 distinct studies, spanning the Pacific, Indian and Atlantic Oceans, and from 1982 to 2013. Differences in bleaching susceptibility and mortality were apparent among different coral genera, but the hierarchy of bleaching susceptibility differs on geographic scales, among ocean basins. These large-scale differences may be attributable to inherent differences in biology (e.g., geographic variation in associations between corals and their symbionts), but may reflect taxonomic differences in the capacity of corals to acclimate or adapt when facing extreme environmental changes. Among decades, it is apparent that bleaching susceptibility and mortality have generally declined over time, possibly reflecting increased bleaching resistance due to selective removal of highly susceptible phenotypes. Despite spatial and temporal variation in bleaching susceptibility, there are overarching patterns linking bleaching susceptibility to basic growth form, whereby a much higher proportion of columnar, foliose, tabular and branching corals bleached and died relative to submassive, encrusting, massive, laminar and free-living corals.. Although coral morphology is highly confounded with taxonomy, growth form accounted for a much greater proportion of variation in bleaching susceptibility than taxonomy. However,

differences in bleaching susceptibility and mortality were not consistent among growth forms within families. For Faviidae, sub-massive and massive species (e.g. *Favites* spp.) had higher bleaching susceptibility than branching species, but the reverse was true among Acroporidae and Poritidae. The data suggest that generalities about the susceptibility of branching versus massive corals (and among other major growth forms) arise at least in part because certain growth forms are over-represented by highly susceptible coral taxa (e.g., *Acropora*) or perhaps because branching corals generally maintain higher dominance than massive corals. Variation in bleaching susceptibility is expected to lead to marked shifts in the structure of coral assemblages, but complexity in responses of different corals suggest that it is not altogether clear which corals will dominate future coral assemblages.

6.2 Introduction

Large-scale mass bleaching of scleractinian corals has occurred at many locations throughout the last few decades (Williams and Bunkley-Williams 1990, Huppert and Stone 1998) and is generally indicative of large-scale environmental stress (Williams et al. 1987, Glynn 1991). These major bleaching episodes are tightly linked with ENSO cycles (Glynn and D'Croz 1990, Toth et al. 2012) and sustained increases in the mean temperature of shallow ocean environments (Goreau and Hayes 1994). Moreover, increases in the spatial and temporal incidence of mass bleaching events over the last two decades are unequivocally linked to global warming, and are predicted to continue to increase in both scale and intensity for at least the next 50-100 years (Hoegh-Guldberg 1999, Sheppard 2003, Donner et al. 2005, McWilliams et al. 2005). Hoegh-Guldberg (1999) predicted that coral bleaching would become an annual event on the Great Barrier Reef by 2020, and likely therefore to cause extensive coral loss (see also Sheppard 2003). This assumes however, that corals lack the capacity to adapt or acclimate to changed environmental conditions (Hughes et al 2003).

Moreover, increased incidence of climate-induced coral bleaching is likely to cause changes in the relative abundance of different corals, rather than killing all corals at similar time frames (Hughes et al. 2003, Pratchett et al. 2013).

Since the earliest mass-bleaching events, it was apparent that corals vary in their susceptibility to bleaching (Williams and Bunkley-Williams 1990), whereby the proportion of colonies that are affected varies among species, genera and families. In general, families of corals that are mostly characterised by branching growth forms (e.g., Acroporidae and Pocilloporidae) are considered to be most susceptible to bleaching and experience highest rates of mortality once bleached (Baird and Marshall 2002, Jones 2008). In contrast, families of corals that typically have massive morphologies (e.g., Faviidae, Mussidae, and Poritidae) appear fairly resistant to increasing temperature, being among the last to bleach and more frequently experience partial, rather than whole colony mortality (Brown and Suharsono 1990, McClanahan 2000, Baird and Marshall 2002, Riegl 2002). These patterns of differential bleaching susceptibility and mortality have led authors to group corals into either branching or massive growth forms without consideration for taxonomy (Arthur 2000, Iluz et al. 2008, Spencer et al. 2000). However, coral morphology is highly confounded with taxonomy (Veron 2000) and the role of differential morphology within (rather than between) coral families in explaining bleaching susceptibility has not been tested.

Different genotypes vary in their thermal thresholds, such that moderate changes in environmental conditions may kill highly sensitive colonies within local populations, but some colonies are likely to resist these disturbances (Jokiel 2004, Hughes et al. 2012). On large scales (e.g., among locations at different latitudes) the bleaching threshold for a particular coral species vary by several degrees, which is generally attributed to local acclimation/ adaptation (Jokiel and Coles 1990, Coles and Brown 2003). It is likely therefore, that even more pronounced genetic plasticity in responses to thermal stresses may exist for

genera and morphologies throughout their extensive geographic ranges, often encompassing one or more entire ocean basins (Veron 2000), providing significant capacity for adaptation to changing environmental conditions. McClanahan et al. (2004) show that bleaching hierarchies are fairly consistent between the Great Barrier Reef and Kenya; however, there were inconsistencies in taxonomic susceptibility for *Acropora*, branching *Porites* and *Pavona*. The generic hierarchy of McClanahan et al. (2004) was also fairly consistent with Brown and Suharsono (1990) and the specific hierarchy of Loya et al. (2001). There has not, however, been a global assessment of generic susceptibility or mortality, taking advantage of the plethora of published information regarding bleaching susceptibility and/ or mortality.

Bleaching susceptibility and bleaching-related mortality are not always equivalent (Baird and Marshall 2002, McCowan et al. 2012). Bleaching susceptibility is generally calculated based on the proportion of colonies that exhibit any evidence of bleaching (including those who experienced bleaching-related mortality), while mortality is only the numbers that have died following bleaching. Mortality is important when considering selective filtering and shifts in community composition. For instance, in Baird and Marshall (2002), mortality rates and reproductive rates were used to determine shifts in community assemblages.

Recent increases in the incidence and severity of climate-induced coral bleaching has generated significant information on comparative rates of bleaching susceptibility and mortality. There are at least 105 studies (Table 6.1), which have specifically quantified variation in temperature-related bleaching susceptibility within and among scleractinian corals. The intensity of research on this area stresses the importance of coral bleaching in potentially structuring future coral assemblages. The purpose of this study was to analyse meta-data on proportional bleaching susceptibility across different coral genera to test whether there i) there are geographic differences in the hierarchy of bleaching susceptibility

and ii) whether there is any evidence (if there is sufficient data) that this hierarchy is changing through time since initial observations conducted in the 1980's. Additionally, this study used the extensive database of bleaching observations to test whether bleaching susceptibility and mortality is related to coral morphology, independent of taxonomic (family-level) affinities among coral species.

6.3 Methods

A total of 2,272 distinct records of bleaching susceptibility among different coral general and/ or subsequent rates of mortality were obtained by extracting data from 105 scientific papers published from 1982 to 2013. Most records (45.5%) were from the Pacific Ocean, thought there were still a substantial number of observations from both the Indian and Atlantic Oceans (680 and 559, respectively). There have been a variety of methods used to document bleaching susceptibility and/ or mortality, such as qualitative observations of relative bleaching susceptibility, quantifications (using colour cards), and relative zooxanthellae densities. To standardize across studies, we used the most commonly reported metric based on proportion of colonies that are affected, with no discrimination between the specific level of bleaching exhibited by each colony; however, per cent affected was used (e.g. for method comparisons, McCowan et al. Chapters 2 and 3). Also, few studies have explicitly followed the fate of bleached colonies, but mortality rates may be inferred based on relative changes in the abundance (colony density or percentage cover) of different corals when bleaching is known to have occurred.

From each study, data was extracted on the mean level of bleaching susceptibility and/ or associated mortality for each distinct taxa (genus where possible) at each distinct geographic location. Bleaching susceptibility was recorded as the per cent of colonies

((#bleached/#observed)*100) within a given genera that exhibited any sign of bleaching, inclusive of slight or partial paling through to recent whole colony mortality. Wherever explicit information on the fate of bleached corals (e.g. long-term survival or mortality) was also recorded, proportional mortality was calculated as a proportion of colonies that experienced either whole colony mortality = ((#died/#observed)*100) or partial mortality (included in mortality) = ((mean % of colony mortality/# of colonies with mortality)/#observed)*100). In many instances, mortality could not be unequivocally attributed to the recent bleaching, although some followed closely enough to observe the difference (e.g. Baird and Marshall 2002, Obura 2001), and there were rarely if ever any measures of mortality rates from control or reference locations. However, it is very likely that recent bleaching episodes, especially where bleaching was extremely severe, contributed to subsequent high rates of whole colony mortality and/ partial mortality. There are also likely to be a wide range of variables that are likely to confound estimates of bleaching susceptibility and subsequent mortality rates (e.g., depth, habitat, the severity of the temperature stress, and the history of prior disturbances), but very few studies provide this level of detail and there were insufficient data records to explore fine-scale patterns (e.g., among locations or habitats). There was also no attempt to explicitly quantify the degree of thermal or environmental stress experienced during each of the independent studies; it is recognized that bleaching susceptibility and mortality vary depending on the magnitude and extent of the stress event (i.e. maximum SST and degree heating weeks), but the goal of this study was test for changes in the relative susceptibility and mortality among different coral taxa.

Variation in bleaching susceptibility and mortality was analysed using a three-way ANOVA, testing for differences among genera, among oceans (Indian, Pacific and Atlantic), and among decades (1980s, 1990s, 2000s and 2010s). All data were arcsine-square root

transformed and only genera for which there were >10 records were included (Table 6.2). Given that regional comparisons are likely to be confounded by the differences in the species pool, analyses were also repeated using only those genera (e.g., *Acropora*, *Millepora, Favia*, and *Porites*) for which there are extensive records of bleaching susceptibility and mortality across all three regions (Table 6.2). The relationship between bleaching susceptibility and bleaching mortality was also examined, using a linear regression to test whether genera with the highest proportion of colonies that exhibited any signs of bleaching were also the same genera that had experienced the highest bleaching-related mortality. As for the previous analyses, this was tested using only those genera (n = 37), for which they were 10 or more records (Table 6.2).

Variation in bleaching susceptibility and mortality among different growth forms was also analysed using ANOVA, comparing among each of the nine major growth forms (branching, columnar, encrusting, foliose, free-living, laminar, massive, submassive, and tabular). To test the effect of colony morphology on bleaching susceptibility, the database was substantially restricted to include only data where the morphology was explicitly categorised in the study (n=65), or morphology could be clearly assigned for the taxonomic groupings used. For example, where bleaching rates were reported for specific study species with very consistent morphologies (e.g., *Acropora hyacinthus* = tabular), these data were included. The database had 1,434 records for bleaching susceptibility for corals with a specifically reported or inferred growth forms, and 1,181 entries for bleaching-related mortality. To test whether these differences are confounded by taxonomy, a nested ANOVA was also run, comparing among growth forms within each of three most commonly studied families (Acroporidae, Faviidae and Poritidae), which exhibit a range of different growth forms. Tukey's *post hoc* tests were used to explore significant results.

6.4 Results

6.4.1 Overall bleaching susceptibility and mortality

A total of 1,933 records of bleaching susceptibility were extracted from the 105 scientific papers considered in this study, that report that proportion of colonies within a given genera that exhibit any signs of bleaching (Table 6.2). There were markedly fewer records (1,374 records) for the proportion of colonies that actually died after bleaching. Not unexpectedly, bleaching susceptibility varied significantly among coral genera (ANOVA, $F_{(36, 1716)} = 3.95$, $p<0.001$). Tukey's *post hoc* test revealed three homogeneous subsets for bleaching susceptibility; there were six genera (*Echinopora*, *Diploasterea*, *Ctenactis*, *Cyphastrea*, *Echinophyllia* and *Madracis*) that bleached significantly less than other extensively studied coral genera (Figure 6.3). The genera that consistently experienced the highest incidence of bleaching (>50% of colonies) were *Seriatopora*, *Agaraicia*, *Stylophora*, *Montastrea*, *Psammacora*, *Sideastrea*, *Acropora*, *Millepora*, *Colpophyllia* and *Diploraia* (Figure 6.1).

As for bleaching susceptibility, the proportion of colonies that died following bleaching was significantly different among genera (ANOVA, $F_{(36, 1344)} = 3.99$, $p<0.001$). However, there were striking differences in the hierarchy of bleaching susceptibility versus bleaching mortality (Figure 6.1, 6.2). The relationship between bleaching susceptibility and bleaching mortality was positive, but not significant (Figure 6.2). Importantly, some genera that experienced very high incidence of bleaching (>50% of colonies bleached) exhibited very low levels (<10%) of post-bleaching mortality (e.g., *Montastrea*, *Psammacora*, and *Sideastrea*), while others (e.g., *Acropora* and *Millepora*) tended to have very high levels of bleaching and subsequent mortality (Figure 6.1). This may be due to differences in the biomass of species, such that high biomass species have low incidence of bleaching-related

mortality and low biomass species have high incidence of bleaching-related mortality (Loya et al. 2001).

6.4.2 Spatial and temporal differences in bleaching susceptibility

Analyses of large-scale patterns in bleaching susceptibility revealed significant temporal and spatial variation. Most notably, there was a significant interaction between coral genera, ocean basin and decade (Table 6.3), though the interaction was clearly driven by limited observations of some genera from just one location. Considering only widespread genera (e.g., *Acropora*, *Millepora*, *Favia* and *Porites*) there was still significant large-scale variation in both susceptibility and mortality (Table 6.4), but the only significant interaction was between ocean and decade. In general, bleaching susceptibility declined through time (Table 6.5, Figure 6.3), declining from a mean of 64.47% (±4.17 SE) in the 1980s down to 37.37% (±1.81 SE) in the 2010s. However, this trend was most apparent in the Atlantic, whereas there was no reported bleaching in the Indian Ocean in the 1980's, after which time there has been a sustained decline in bleaching susceptibility (Table 6.5). In the Pacific, bleaching susceptibility declined from the 1980s to the 2000s, but is so far, much higher in the 2010s compares to the 2000s (Table 6.5). The overall incidence of bleaching in the Indian Ocean 41.67% (±1.35 SE), was lower compared to the Atlantic Ocean (mean of 49.55% ±1.52 SE) or Pacific Ocean (mean of 45.22% ±1.345 SE), but this was not significant (Table 6.4).

Geographic patterns of bleaching susceptibility were very different among the major coral genera (*Acropora*, *Millepora, Favia,* and *Porites*) that have been extensively studied in all three ocean basins. For both *Millepora* and *Acropora*, the mean proportion of colonies exhibiting bleaching has been much higher in the Pacific compared to the Indian or Atlantic (Figure 6.4). In contrast, the average proportion of colonies that bleach for *Porites* has been lower in the Pacific compared to other ocean basins (Figure 6.4). For *Favia*, average

bleaching susceptibility has been significantly lower in the Indian Ocean compared to the other ocean basins, but similarly high (40-50%) in the Pacific and Atlantic (Figure 6.4). Direct comparisons of bleaching susceptibility between the Indian and Pacific Ocean (where there is greatest overlap in the species assemblages), revealed no significant relationship (Pearsons correlation = 0.21, n = 26, p = 0.31). Some coral genera (*Coeloseris, Goniatsrea* and *Symphyllia*) bleached disproportionately more in the Indian Ocean compared to the Pacific, while others (*Seriatopora* and *Millepora*) exhibited much higher bleaching susceptibility in the Pacific compared to the Indian Ocean (Figure 6.5).

6.4.4 Morphology

Bleaching susceptibility varied significantly among growth forms (ANOVA, $F_{(6, 815)} = 717.7$, $p < 0.001$), and Tukey's *post-hoc* tests revealed two very distinct groups; tabular, columnar, foliose and branching corals bleached significantly more than massive, submassive, free-living, laminar and encrusting corals. Variation in subsequent rates of mortality were also significant (ANOVA, $F_{(6,699)} = 114.9$, $p < 0.001$) and were generally higher in the same growth forms that had the highest bleaching susceptibility (Figure 6.6).

When considering growth forms nested within families, there were significant differences among growth forms that masked any differences among families (Table 6.6). Growth form accounted for a much greater proportion of variation in bleaching susceptibility (64%) than did taxonomy (22%), but differences among growth forms were not consistent across families (Figure 6.7). For Faviidae, sub-massive and massive species (e.g. *Favites* spp.) had higher bleaching susceptibility than branching species, but the reverse was true among Acroporidae and Poritidae. Significant differences in bleaching susceptibility among growth forms nested within families (Table 6.6) swamped any differences among families.

The proportion of colonies that died following bleaching differed significantly among growth forms within families (Figure 6.7), but also among the three key families (Table 6.6). More than half (51%) of the variation in proportional mortality was explained by growth form, but family explained a further 41%. *Post-hoc* tests show that Faviidae had lower mortality than either Acroporidae or Poritidae, even though susceptibility was similar for all three families (Table 6.6). However, the overall differences between mortality of growth forms within these three families are such that Faviidae had the highest mean mortality of massive colonies, but the lowest mean mortality of branching colonies. Moreover, encrusting and submassive corals showed minimal mortality due to bleaching. Mortality patterns among Acroporidae growth forms were consistent with bleaching susceptibility (e.g., high susceptibility led to high mortality), but this was not the case among Faviidae growth forms (Figure 6.7).

6.5 Discussion

6.5.1 Bleaching susceptibility and mortality

There has been considerable scientific interest in differential bleaching susceptibility among different types of corals (e.g., Loya et al. 2001, Marshall and Baird 2002, McClanahan et al. 2004), motivated by the idea that there are likely to be "winners" and "losers" among coral assemblages as coral reef ecosystems are increasingly subject to climate-induced increases in sea surface temperatures (Loya et al. 2001). Provisional comparisons among distinct studies have tended to suggest that the general hierarchy of bleaching susceptibility is fairly consistent among locations, habitats and seasons (e.g., Nakamura and van Woesik 2001, McClanahan et al. 2004). McClanahan et al. (2004) assert that there are consistent taxon based responses to climate-induced coral bleaching, based on comparisons of relative

bleaching of 19 coral taxa (combination of genera and growth forms) between Kenya and Australia's Great Barrier Reef. However, exceptions to the normal bleaching hierarchy (e.g., apparent reversals in bleaching susceptibility) are being increasingly reported (e.g. Guest et al. 2012), questioning whether relative bleaching susceptibility is context specific, or varies over large spatial and temporal scales. There is increasing evidence that prior exposure to extreme temperatures and/ or mass-bleaching episodes leads to increased bleaching resistance (Brown et al. 2000, Dunne and Brown 2001, Maynard et al. 2008a, Middlebrook et al. 2008, Pratchett et al. 2013, Thompson and van Woesik 2009). This may be explained by i) loss of more susceptible individuals ii) acclimatization iii) a change to a more tolerant symbiont community, and/ or iv) greater energy reserves in later disturbances (Thompson and van Woesik 2009, Maynard et al. 2008a, Pratchett et al. 2013). It is also clear that bleaching thresholds vary spatially for individual coral taxa (Hughes et al. 2003), though it is not clear whether this leads to geographic changes in relative bleaching susceptibility among taxa, or if the entire assemblage is simply more resistant at locations exposed with generally higher or more frequent exposure to extreme temperatures (Castillo and Helmuth 2005, Oliver and Palumbi 2011).

Formal analyses of published data from around the world did not reveal significant geographic variation in bleaching incidence, though there was significant variation in rates of bleaching mortality across the different ocean basins (Table 6.4). The power of these analyses was highly constrained due to limited overlap in the range of corals that have been studied at each location, associated with geographic variation in the species pool. Even so, there were however, several examples of apparent geographic differences in bleaching susceptibility. For example, *Montastrea* appears relatively resistant to bleaching in the Pacific, but the proportion of colonies that are reported to have bleached during mass-bleaching episodes in the Caribbean are amongst the highest of any coral anywhere in the world. *Millepora* also

stood out as the most susceptible coral in the Pacific Ocean, whereas in the Indian and Atlantic Oceans it had intermediate bleaching susceptibility. Direct comparison of coral genera that have been extensively studied across all ocean basins showed that bleaching hierarchies are not geographically consistent (Figure 6.4); the strong trend in the Pacific Ocean of *Millepora* > *Acropora* > *Favia* > *Porites* was not present in the other ocean basins. In the Atlantic Ocean, for instance, *Acropora* was the least susceptible of the four genera. More striking was the clear lack of consistency in the hierarchy of bleaching susceptibility between the Pacific and Indian, where it was possible to directly compare the average proportion of colonies that bleach for 26 different genera (Figure 6.5). Contrary to McClanahan et al. (2004), who reported a consistent hierarchy of bleaching susceptibility between studies conducted in the Indian and Pacific oceans (albeit with generally higher levels of bleaching reported in the Indian Ocean, specifically Kenya) there was no significant correlation when comparing mean bleaching susceptibility across all multiple studies conducted in each ocean.

Marked taxonomic differences in bleaching susceptibility, as observed in this study, are commonly attributed to inherent differences in the biology and physiology of corals, including differences in colony morphology (as discussed later), tissue thickness and photo-protective mechanisms (Dunne and Brown 2001, Loya et al. 2001), associated symbionts (e.g., Baker 2001, Rowan 2004), colony integration and energetic reserves (Soong and Lang 1992, Baird and Marshall 2002), and differential mass-transfer capabilities (Nakamura and van Woesik 2001). However, these differences among taxa are likely to be highly conserved across locations and habitats. For example, *Acropora* and *Pocillopora* corals have been shown to have lower densities of fluorescent tissue pigment granules (Salih et al., 2000), compared to *Porites*, *Favia* and other slower-growing massive corals, which have relatively high densities of fluorescent tissue pigment granules. These differences contribute to the

higher bleaching susceptibility among *Acropora* and *Pocillopora*, compared to *Porites* and *Favia* (McClanahan et al. 2004). Variability in the timing of observations of bleaching susceptibility and mortality relative to the onset of the stress event may also account for apparent taxonomic differences in susceptibility, whereby branching and tabular *Acropora* colonies do exhibit bleaching much sooner (typically within 2 months; Baird and Marshall 2002, Obura 2001), whereas massive colonies have been observed bleached from 3- 14 months after the onset of the anomalous temperatures (Lang et al. 1992, Moothien-Pillay et al. 2005).

Geographic variation in bleaching susceptibility may be attributable to large-scale differences in the biology of individual corals, such as geographic variation in symbionts with which corals are associated (Glynn et al. 2001, Baker et al. 2008). In the eastern Pacific, for example, increasing thermal tolerance of *Pocillopora* was linked to increased prevalence of colonies that host a thermally tolerant clade D symbiont (Glynn et al. 2001), compared to the Indian Ocean and western Pacific. It is also possible, that spatial variation in bleaching susceptibility reflects taxonomic differences in the capacity of corals to acclimatise and adapt. Bleaching susceptibility may be modified in response to differential exposure to generally higher or more frequent exposure to extreme temperatures, and it likely that there will be strong taxonomic differences in the capacity of corals to adapt and acclimatize to changing environmental conditions (Coles et al. 1976, Berkelmans and Willis 1999, Brown et al. 2000, Hughes et al. 2003, Pandolfi et al. 2011). Importantly, taxa that show the greatest contrast in bleaching susceptibility among locations and habitats (e.g., *Acropora* and *Pocillopora*, Guest et al. 2012), have life history traits (fast growth and early maturation) most likely to lead to rapid adaptation, and can experience very high bleaching-related mortality (e.g., Chapter 5), providing strong selection for adaptation.

6.5.2 Spatial variation in bleaching susceptibility

If corals are adapting to ocean warming, we would expect a temporal decline in the proportion of coral colonies that bleach, or die. Accordingly, this study revealed an inter-decadal trend of declining bleaching susceptibility, apparent at global scales (Figure 6.3). This was less obvious when considering the trends in bleaching-related mortality (Table 6.5), though there was a decline in mortality from the 1980s to 2000s and recent increases in mortality may reflect further increases in baseline temperatures, such that extreme temperatures are beginning to exceed the tolerances of even highly resistant coral taxa. Sustained declines in bleaching susceptibility may be ascribed to individual (colony-level) acclimation (Glynn et al. 2001, Baker 2001) and/ or selective mortality of highly susceptible genotypes (Dunne and Brown 2001, Maynard et al. 2008a, Chapter 5), making populations and communities more resistant to high temperatures. Individual coral colonies or populations may acclimate to changing temperature regimes by switching from thermally sensitive to thermally tolerant *Symbiodinium* (Glynn et al. 2001, Baker 2001) or increasing concentrations of certain pigments that reduce bleaching susceptibility (Dunne and Brown 2001, Brown et al. 2002a). There is not as yet, any clear evidence of sustained physiological changes consistent with the geographic scale and scope of this study. There are also a number of environmental factors that may moderate bleaching responses during periods of high temperatures, including low light (due to depth, shading, turbidity, or cloud cover), wave action and high water flow, and high nutrient availability (Baker et al. 2008). However, it seems unlikely that there would have been sustained changes in these environmental conditions on global scales.

6.5.3 Morphology

It is generally assumed that branching corals are more susceptible to bleaching than massive colonies (e.g., Loya et al. 2001). Our analyses of published literature support this claim whereby branching, columnar and tabular corals have greater susceptibility and mortality than massive, submassive, encrusting and free-living corals. However, growth form is highly confounded by taxonomy and at least some of the observed differences in bleaching susceptibility among growth forms are attributable to variations in taxonomic susceptibility (Figure 6.2). Branching corals, for example, are dominated by Acroporidae and Pocilloporidae, which have generally higher sensitivity to extreme temperatures, compared to branching Faviidae. Also, massive Acroporidae (e.g., *Astreopora*) are relatively uncommon on most reefs, but exhibit the highest bleaching susceptibility and mortality. Establishing generalities in bleaching susceptibility is important to understand potential mechanisms by which corals could adapt, but the role of morphology must be considered in light of marked differences in the taxonomic composition of corals that exhibit these different growth forms.

The rank order of bleaching susceptibility recorded in this study is not entirely consistent with patterns reported elsewhere. Columnar corals, for example, are often grouped with massive species and thought to have relatively low susceptibility to bleaching (e.g., Obura 2001). However, the observed order is consistent with differences in mass-transfer capacity, whereby flatter and smaller corals have a greater capacity to remove potentially deleterious superoxides and other oxygen radicals, compared to more erect and branching forms (Nakamura and van Woesik 2001). This may further explain why branching corals generally experienced higher rates of mortality compared to massive corals (Figure 6.6), especially after very severe bleaching. However, differences in overall shape of coral colonies do not effectively account for all inter- or intra-generic differences in bleaching susceptibility (Chapter 5).

Morphological variations in bleaching susceptibility may also be attributable to inherent differences in growth rates, broad differences in life-history strategies (Baird and Marshall 2002), thermal tolerances of photo-endosymbionts (Berkelmans and van Oppen 2006), tissue thickness, and/ or marked differences in colony size and age (Loya et al. 2001). Perhaps the best explanation for consistent difference in bleaching susceptibility among (but not necessarily within) different coral taxa is the level of physiological integration (Baird and Marshall 2002), which is highly linked with morphology, particularly within taxa. For species with polyps that are physiologically independent, (e.g. massive colonies) only polyps directly affected by both heat and light respond, as predicted by the photoinhibition model of coral bleaching of Jones et al. (1998). The result is that bleaching within the colony is patchy and rates of whole colony mortality are low, as observed for many massive species. In contrast, taxa that are highly integrated cannot contain the damage, such that the entire colony often bleaches and rates of whole colony mortality are therefore high (e.g. *Acropora*).

6.5.4 Conclusions

Documented increases in the frequency and extent of mass coral bleaching are often cited as evidence that climate-related changes in environmental conditions have now exceeded the tolerance of most (if not all) scleractinian corals, and that there is extremely limited capacity for corals to adapt (e.g., Hoegh-Guldberg et al. 2002, 2007). If so, it seems very likely that projected increases in ocean temperatures, and other climatic disturbances (especially ocean acidification), will lead to increasing loss of coral species and accelerated degradation of coral reef environments (Hoegh-Guldberg and Bruno 2010). However, this study adds to a growing body of evidence (reviewed by Pandolfi et al. 2011) that there is substantial variation in the responses of corals to emerging threats, both climatic and non-climatic. This suggests that there is significant adaptive capacity, and individual corals will acclimatize to

changing environmental conditions and/ or there will be selective mortality and associated shifts in assemblage structure. There are likely to be limits to thermal adaptation and acclimatization, and these may incur tradeoffs in the overall fitness of coral populations (Pandolfi et al. 2011), such that sustained increases in greenhouse gas emissions will increasingly challenge the persistence of reef organisms (and especially corals) and the degradation and loss of corals will be very patchy in time and space. It is also clear that effects of climate change on coral reefs are operating against a backdrop of many other more direct anthropogenic disturbances, which either undermine the capacity of corals to acclimate and adapt (e.g., Chapter 5) or increase vulnerability to observed and projected environmental changes.

Significant spatial, temporal and taxonomic variation in bleaching susceptibility among corals makes it unlikely that there will be wholesale loss of coral assemblages (*cf.* Hoegh-Guldberg et al. 2007), at least not in short to medium time scales (years to decades). There will however, be changes in the structure of coral assemblages. It is generally expected that branching *Acropora* and Pocilloporidae, which form much of the habitat complexity of Indo-Pacific reefs, will decline in abundance owing to their increased thermal sensitivity compared to coral with more robust and massive morphologies (e.g., Riegl and Purkis 2009). However, the increasing incidence of climate-induced coral bleaching will not necessarily favour those corals that are resistant to bleaching (Pandolfi et al. 2011, Baker et al. 2008). Bleaching-susceptible species (e.g., *Acropora*) often have faster rates of recovery from disturbances, and could potentially increase in abundance, depending on the specific frequency versus severity of major bleaching events (Hughes et al. 2003, Baker et al. 2008). Accordingly, highly susceptible corals (e.g., *Acropora*) have become even more dominant in the aftermath of severe bleaching at some locations (see Sheppard et al. 2002). However, ongoing research is necessary to test how changing environmental regimes will affect the

underlying population dynamics and demographic rates of key coral taxa. This study also highlights the critical importance of long-term studies to explicitly quantify changes in bleaching susceptibility and associated rates of mortality, which may indicate adaptation to rising temperatures.

Table 6.1: List of published papers on coral bleaching susceptibility and mortality by Ocean basin

Ocean Basin	References
Atlantic	Aronson et al. 2002, Brandt 2009, CARICOMP 1997, Ceneno 2002, Clark et al. 2009, Cowan 2006, Goenega et al. 1989, Goreau 1992, Jeffrey et al. 2006, Kramer and Kramer 2000, Lang et al. 1988, McField 1999, Miller et al. 2011, Mumby 1999, O'Farrell and Day 2005, Oxenford et al. 2008, Porter et al. 1989, Quinn and Kojis 2008, Steiner and Kerr 2008, Whelan et al. 2007, Wilkinson 1998, Wilkinson 2000, Wilkinson and Souter 2005, Williams and Bunkley-Williams 1990, Winter et al. 1998
Indian	Ammar et al. 2011, Arthur 2000, Barid 2008, Brown and Phongsuwan 2012, Floros et al. 2004, Furby et al. 2012, Goorah et al. 1998, Hardman et al. 2004, Hoeksema and Matthews 2011, Klinthong and Yeeemin 2012, McClanahan et al. 2001, McClanahan 2004a and b, Mohammed and Mohammed 2005, Moothien-Pillay et al. 2005, Obura 2001, Phongsuwan 1988, Riegl 2002, Schuhmacher et al. 2005, Sebastian et al. 2009, Spencer et al. 2000, Wilkinson 1998, Wilkinson 2000, Williams and Bunkley-Williams 1990
Pacific	Alling et al. 2008, Ayling and Ayling 1999, Baird and Marshall 1998, Baird and Marshall 2002, Berkelmans et al. 2004, Brown and Suharsono 1990, Bruno et al. 2001, CARICOMP 1997, Carriquiry et al. 2001, Chin and Ayling 2000, Cohen et al. 1997, Cumming et al. 2000, Dalton and Carroll 2011, Davies et al. 1997, Drollet et al. 1994, Drollet et al. 1995, Fagerstrom and Rougerie 1994, Feingold 2001, Fong and Glynn 2001, Gleason 1993, Glynn 1983, Glynn and Colgan 1992, Glynn and de Weerdt 1991, Glynn et al.. 1988, Guest et al. 2012, Guzman and Cortes 2001, Harriott 1985, Harrison et al. 2011, Hoegh-Guldberg and Salvat 1995, Hoeksema 1991, Iluz et al. 2008, Jiminez et al. 2001, Jokiel and Brown 2004, Jones 1997, Jones 2008, Jones et al. 2000, Kayanne et al. 2002, Kenyon and Brainard 2006, Loya et al. 2001, Marshall and Baird 2000, Maynard et al. 2008a, McClanahan et al. 2004, Mumby et al. 2001, Ortiz et al. 2009, Penin et al. 2013, Podesta and Glynn 2001, Reyes-Bonilla 1993, Salvat 1991, Stimson et al. 2002, Thomson et al. 2011, Tuan et al. 2000, van Woesik et al. 2012, Wilkinson 1998, Wilkinson 2002, Williams and Bunkley-Williams 1990, Williams et al. 2010, Wilson et al. 2012, Yamashiro et al. 2005, Yuchareon and Yeemin 2012, Yusuf and Jompa 2012

Table 6.2: Number of records for each genera and geographic location (ocean basin). Genera with <10 records were excluded from the analysis. Only 4 genera were studied extensively in all three regions (shown in bold).

Genera	Indian	Pacific	Atlantic	Grand Total
Acanthastrea	7	8		15
Acropora	**116**	**108**	**41**	**265**
Agaricia			61	61
Alveopora	7			7
Astrangia			1	1
Astreopora	11	7		18
Cladocora			1	1
Coeloseris	5	2		7
Colpophyllia			22	22
Coscinarea	5	1		6
Ctenactis	2	8		10
Cycloseris		1		1
Cyphastrea	6	9		15
Dendrogyra			8	8
Diaseris		2		2
Dichocoenia			8	8
Diploastrea		14		14
Diploria			56	56
Echinophyllia	8	3		11
Echinopora	20	10		30
Eunicia			1	1
Euphyllia		1		1
Eusmilia			6	6
Favia	**28**	**19**	**13**	**60**
Favites	18	12		30
Fungia	14	31		45
Galaxea	19	13		32
Gardinoseris	1	8		9
Goniastrea	21	17		38
Goniopora	7	15		22
Gyrosmilia	2			2
Halomitra		1		1
Heliofungia		5		5
Heliopora		8		8
Helioseris			1	1
Herpolitha		6		6
Heteractis	2			2
Hydnophora	14	13		27
Isophyllastrea			1	1
Isophyllia			8	8

Isopora		1		1
Leptastrea		5		5
Leptoria	5	8		13
Leptoseris		3	3	6
Lobophyllia	3	12		15
Lobophyton	1			1
Lobophytum		1		1
Madracis			22	22
Manicina			4	4
Meandrina			14	14
Merulina		8		8
Millepora	**10**	**22**	**30**	**62**
Montastrea		19	82	101
Montipora	34	67		101
Mussa			3	3
Mussismilia			3	3
Mycedium		1		1
Mycetophyllia			11	11
Oculina			2	2
Oulophyllia	3			3
Oxypora		1		1
Pachyseris		10		10
Palythoa	1		5	6
Pavona	13	38		51
Pectinia		6		6
Physogyra		3		3
Platygyra	27	21		48
Pocillopora	29	131		160
Podabacia		1		1
Porites	**47**	**122**	**72**	**241**
Psammocora		19		19
Sandolitha		1		1
Sarcophyton	1	1		2
Scolymia		1	6	7
Seriatopora	4	9		13
Siderastrea	2		49	51
Sinularia	1	2		3
Solenastrea			3	3
Stephanocoenia			13	13
Stylocoeniella		2		2
Stylophora	12	14		26
Symphyllia	4	10		14
Tubipora		1		1
Turbinaria	2	8		10
Xenia	1			1
Grand Total	513	870	550	1933

Table 6.3: ANOVA on proportional bleaching (A) and proportional mortality (B), comparing testing for differences among genera, among oceans (Indian, Pacific and Atlantic), and among decades (1980s, 1990s, 2000s and 2010s). All data were arcsine-square root transformed and only genera for which there were >10 records were included (see Table 6.2). "*" indicates level of significance: "*" $p<0.05$, "**" $p<0.01$, "***" $p<0.001$.

A) Susceptibility

Source	SS (Type III)	*df*	MS	F	*p*
Genera	28.30	39	0.73	3.85	0.00***
Ocean	0.67	2	0.33	1.77	0.17
Decade	2.49	3	0.83	4.40	0.00***
Genera × Ocean	8.72	27	0.32	1.71	0.01**
Genera × Decade	25.41	74	0.34	1.82	0.00***
Ocean × Decade	5.94	6	0.99	5.25	0.00***
Genera × Ocean × Decade	10.10	34	0.30	1.58	0.02**
Error	296.44	1573	0.19		
Total	1386.63	1762			

B) Mortality

Source	SS (Type III)	*df*	MS	F	*p*
Genera	18.06	39	0.46	2.60	0.00***
Ocean	1.67	2	0.84	4.69	0.01**
Decade	1.31	3	0.44	2.46	0.06
Genera × Ocean	5.70	26	0.22	1.23	0.20
Genera × Decade	13.69	68	0.20	1.13	0.22
Ocean × Decade	2.61	5	0.52	2.93	0.01**
Genera × Ocean × Decade	7.41	22	0.34	1.89	0.01**
Error	217.24	1220	0.18		
Total	443.53	1390			

Table 6.4: ANOVA on proportional bleaching (A) and proportional mortality (B), testing for differences among common and widespread genera (*Acropora*, *Favia*, *Milleopora*, *Porites*; cf. Table 6.3), among oceans (Indian, Pacific and Atlantic), and among decades (1980s, 1990s, 2000s and 2010s). All data were arcsine-square root transformed. "*" indicates level of significance: "*" $p<0.05$, "**" $p<0.01$, "***" $p<0.001$.

A) Susceptibility

Source	SS (Type III)	*df*	MS	F	*p*
Genera	2.08	3	0.69	3.36	0.02*
Ocean	0.72	2	0.36	1.75	0.18
Decade	2.38	3	0.79	3.84	0.01**
Genera × Ocean	2.97	6	0.49	2.39	0.03*
Genera × Decade	3.58	9	0.40	1.93	0.05
Ocean × Decade	6.79	6	1.13	5.47	0.00***
Genera × Ocean × Decade	3.31	10	0.33	1.60	0.10
Error	121.53	588	0.21		
Total	533.63	628			

B) Mortality

Source	SS (Type III)	*df*	MS	F	*p*
Genera	3.70	3	1.23	5.18	0.00***
Ocean	3.20	2	1.60	6.72	0.00***
Decade	0.75	3	0.25	1.04	0.37
Genera × Ocean	1.82	6	0.30	1.27	0.27
Genera × Decade	1.93	9	0.22	0.90	0.52
Ocean × Decade	5.25	5	1.05	4.41	0.00***
Genera × Ocean × Decade	2.83	7	0.40	1.70	0.11
Error	110.50	464	0.24		
Total	239.81	500			

Table 6.5: Mean (±SE) proportional bleaching (A) and proportional mortality (B) among major ocean basins, and among decades. Data was extracted from 105 published studies, recording the proportion of colonies of each of 37 different coral genera that exhibited any sign of bleaching during major mass-bleaching events. Given limited effect of genera, when constraining the analyses to widespread genera (Table 6.4), data is pooled across genera.

A) Susceptibility

Decade	Atlantic	Indian	Pacific	Grand Total
1980's	77.37 (6.72)	0.00 (0.00)	60.37 (5.10)	64.47 (4.17)
1990's	43.82 (2.03)	59.69 (2.61)	57.49 (1.95)	53.15 (1.27)
2000's	52.72 (2.27)	35.42 (2.32)	20.16 (2.32)	37.81 (1.45)
2010's	20.00 (n =1)	32.71 (2.97)	40.26 (2.27)	37.37 (1.81)
Grand Total	49.55 (1.52)	41.69 (1.60)	45.22 (1.34)	45.45 (0.86)

B) Mortality

Decade	Atlantic	Indian	Pacific	Grand Total
1980's	13.24 (5.70)	0.00 (0.00)	37.32 (6.26)	32.18 (5.27)
1990's	4.88 (0.90)	32.45 (3.50)	23.13 (2.20)	17.91 (1.28)
2000's	13.99 (1.78)	4.80 (1.22)	24.67 (8.92)	9.99 (1.12)
2010's		19.66 (2.44)	27.12 (3.12)	23.50 (2.00)
Grand Total	8.91 (0.93)	16.91 (1.41)	25.96 (1.72)	17.73 0(.85)

Table 6.6: Nested ANOVA to test for variation proportional bleaching (A) and proportional mortality (B) among growth forms within three key families (Acroporidae, Poritidae and Faviidae) with highly diverse growth forms. All data were arcsine-square root transformed. "*" indicates level of significance: "*" $p<0.05$, "**" $p<0.01$, "***" $p<0.001$.

A) Susceptibility

Source	SS (Type III)	*df*	MS	F	*p*
Family	0.85	2	0.42	0.89	0.42
Growth form (Family)	15.37	13	1.21	4.64	0.00***
Error	166.06	638	0.26		

B) Mortality

Source	SS (Type III)	*df*	MS	F	*p*
Family	0.94	2	0.69	1.19	0.32
Growth form (Family)	9.67	12	0.36	3.83	0.00***
Error	112.74	536	0.21		

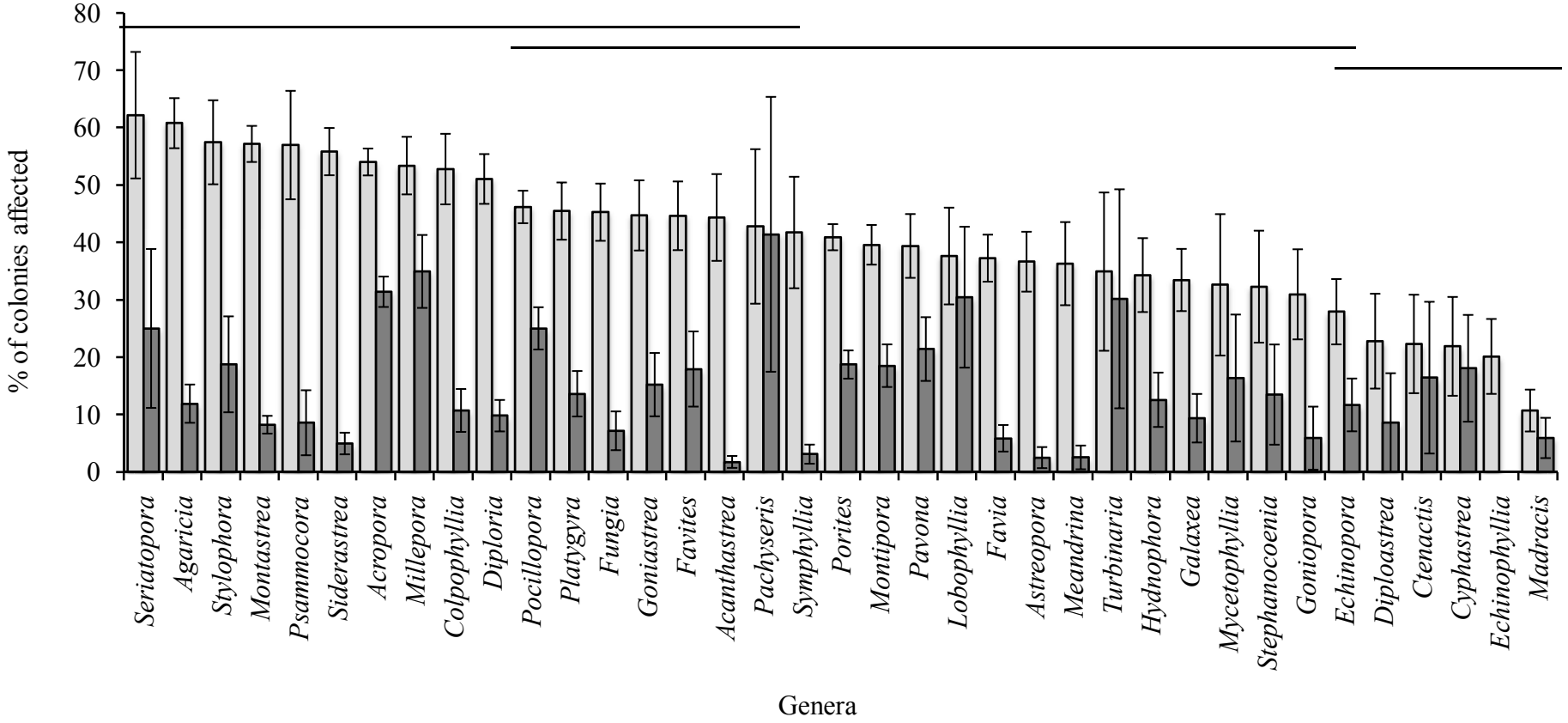

Figure 6.1: Mean (±SE) bleaching susceptibility (light grey bars) and mortality (dark grey bars) by coral genera, arranged in order of declining bleaching susceptibility. Data extracted from 105 published studies, pooling across the three ocean basins (Indian, Pacific and Atlantic). Horizontal lines indicate homogenous subsets from Tukey's *post-hoc* test for bleaching susceptibility.

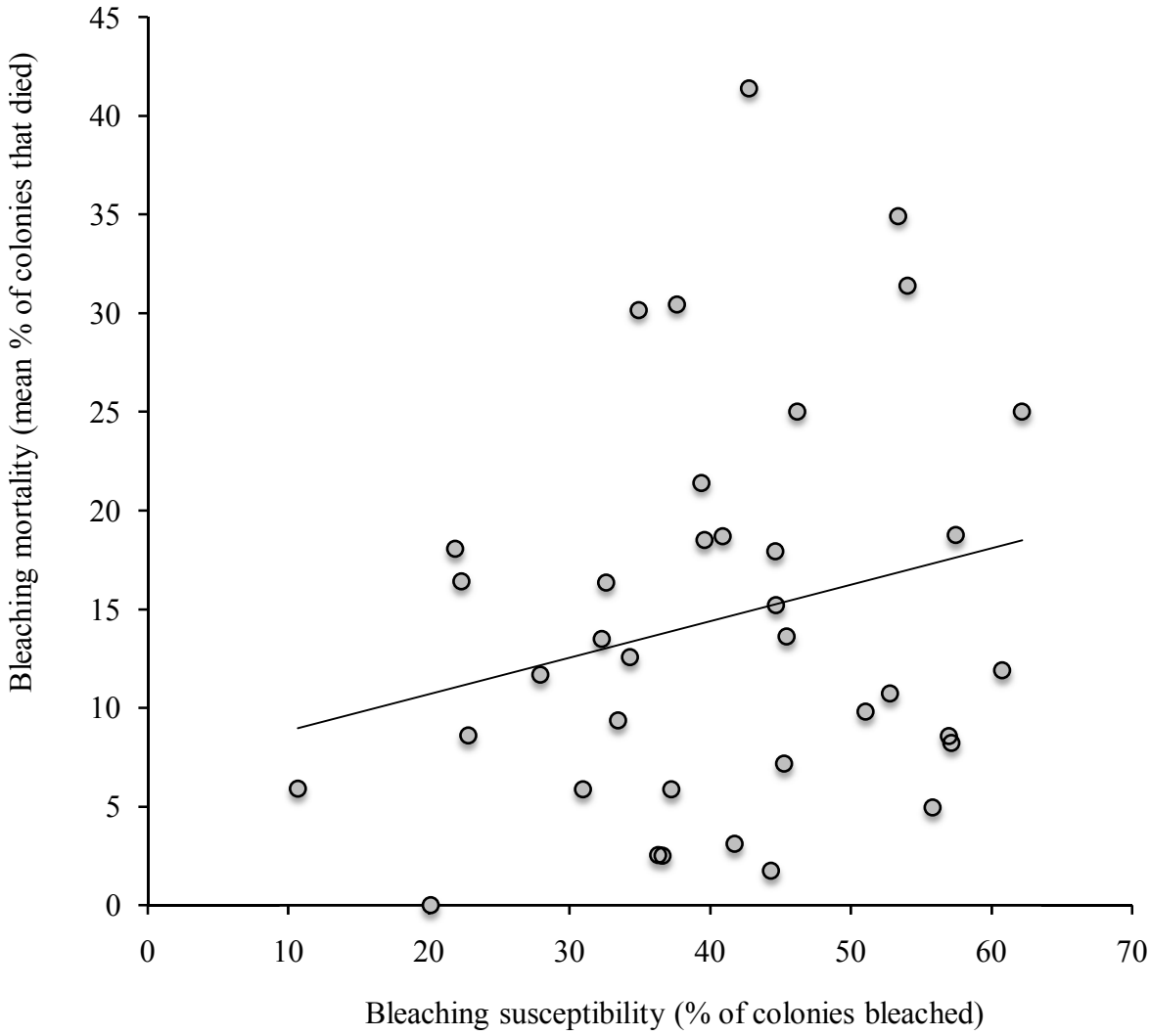

Figure 6.2: Relationship between mean bleaching susceptibility versus mean bleaching mortality for 37 different coral genera. Data extracted from 105 published studies, recording the proportion of colonies of each genus that bleached and or died (actual values for each genus are shown in Figure 6.1). While there is a slight positive relationship between bleaching susceptibility and mortality, this was not significant ($r^2 = 0.05$, n = 37, $p = 0.17$).

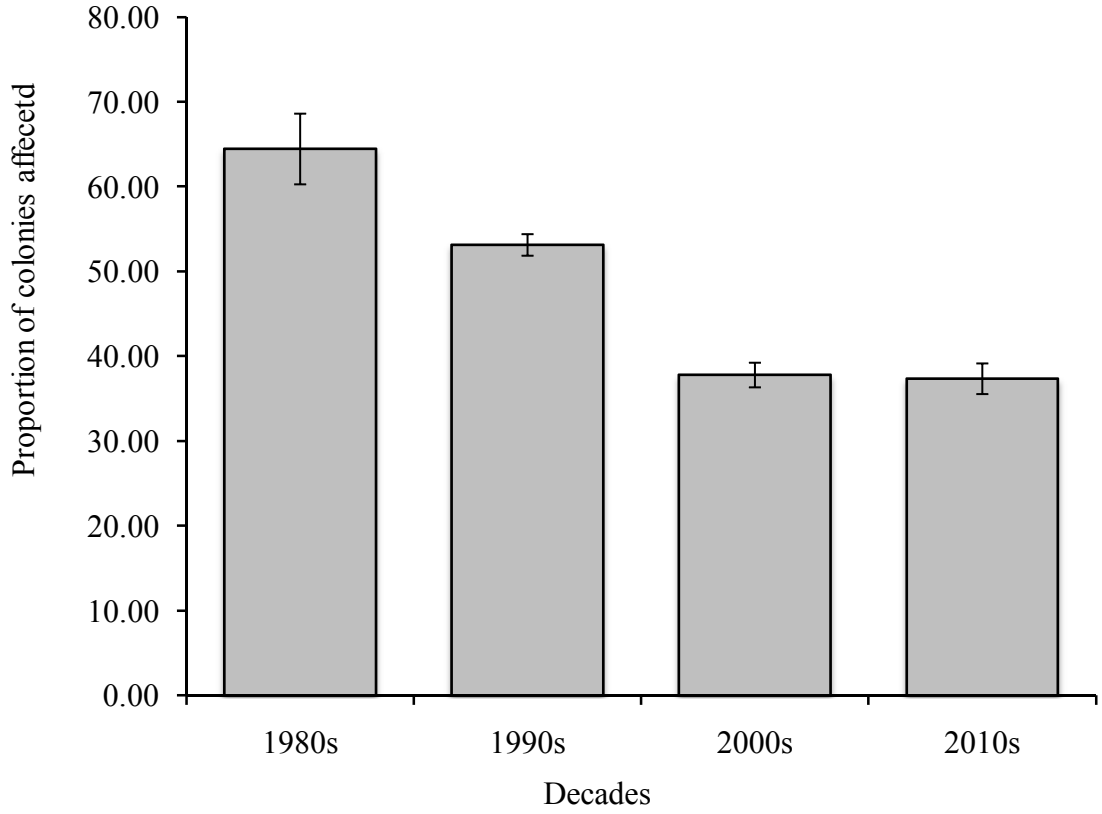

Figure 6.3: Mean (±SE) bleaching susceptibility recorded in each decade from the 1980s to 2010s.

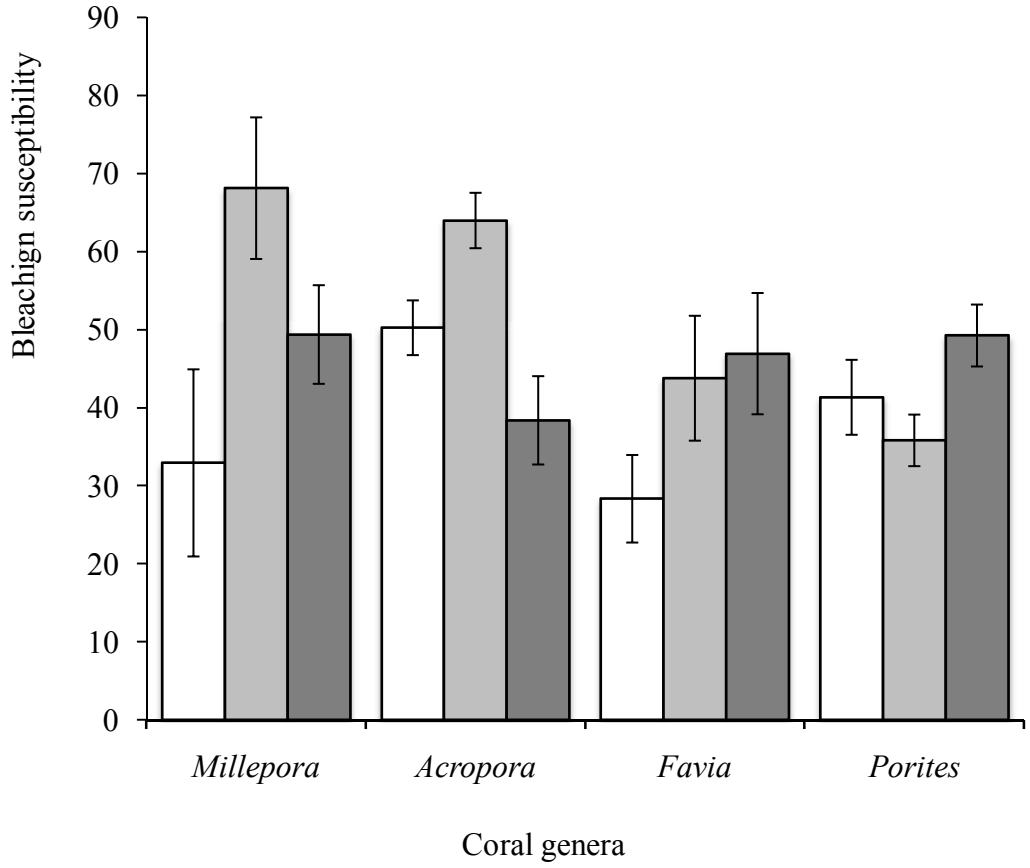

Figure 6.4: Mean (±SE) bleaching susceptibility for widespread coral genera in the Indian Ocean (white bars), Pacific Ocean (light grey bars) and Atlantic (dark grey bars).

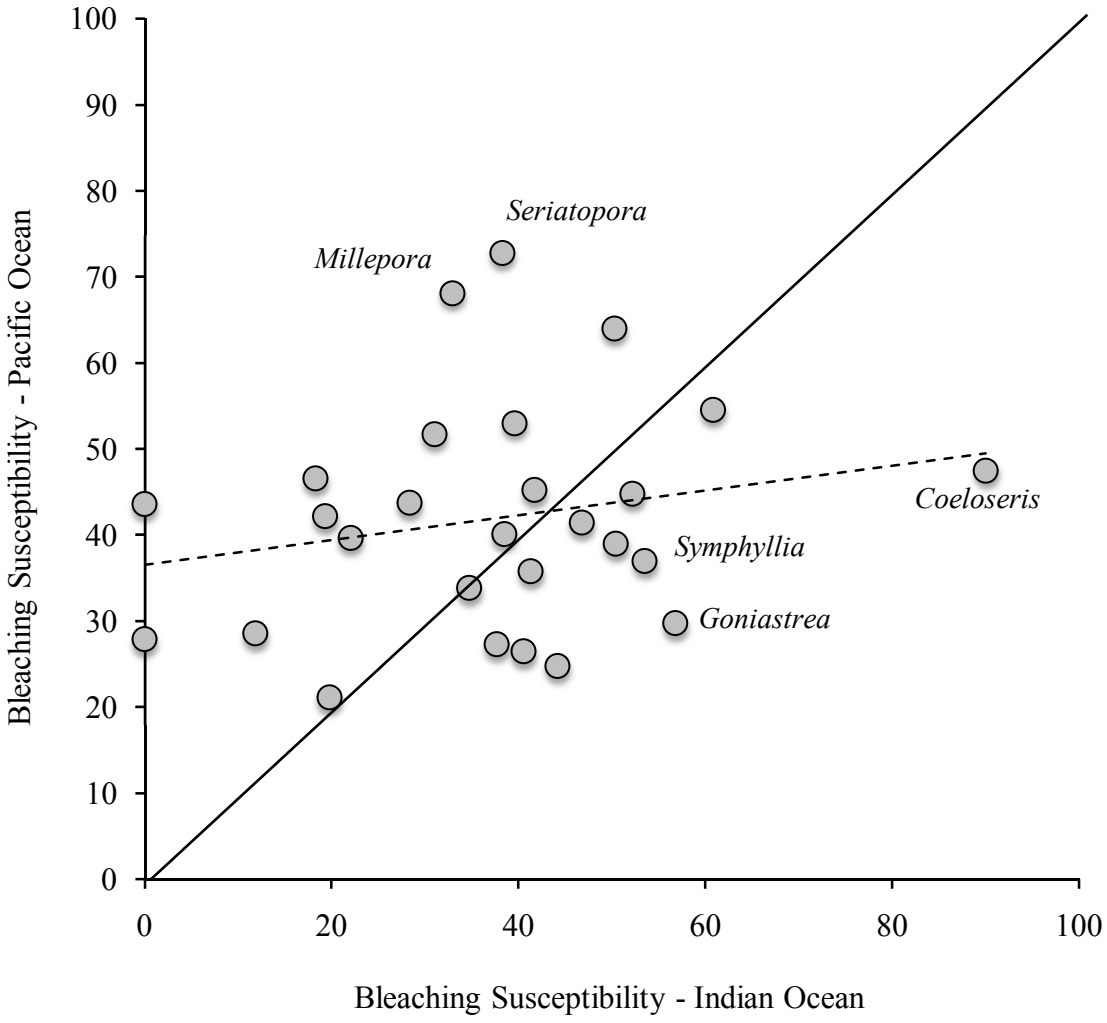

Figure 6.5: Scatterplot of bleaching susceptibility (average proportion of coral colonies that exhibit bleaching during major mass-bleaching events) for coral genera that have been studied in both the Indian Ocean and Pacific Ocean. The diagonal solid line indicates the perfect correlation (slope = 1) assuming there is geographic consistency in bleaching susceptibility. The actual relationship (indicated by the dashed line) is not significant ($R = 0.21$, $n = 26$, $p = 0.31$). Each point represents a distinct genus, but to avoid crowding only key genera (that differ between oceans) are shown.

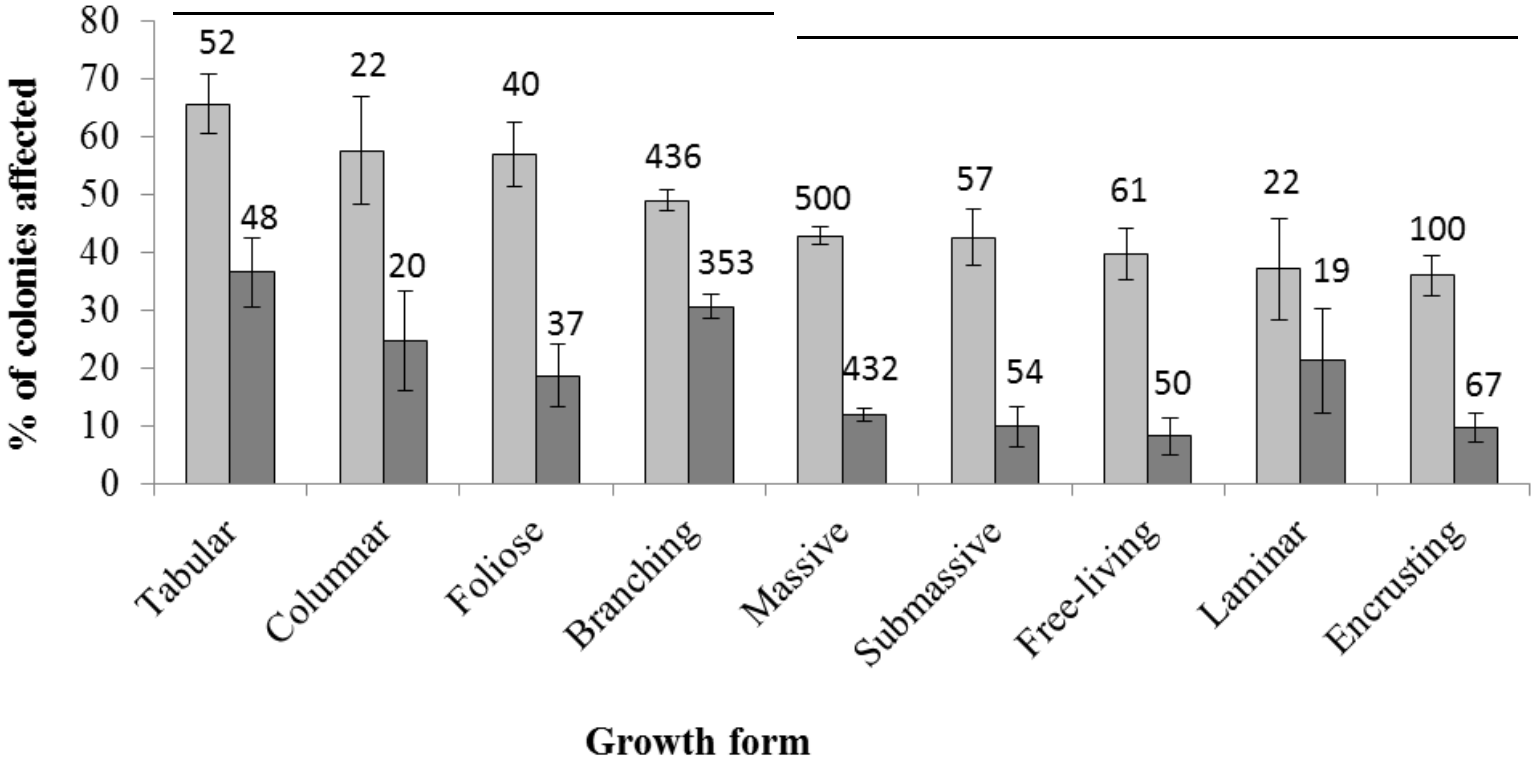

Figure 6.6: Mean (±SE) bleaching susceptibility (white) and mortality (grey) for different growth forms, irrespective of taxonomy. Data are averaged across studies with complete observations of the event. Horizontal lines indicate homogenous subsets from Tukey's *post-hoc* test for bleaching susceptibility.

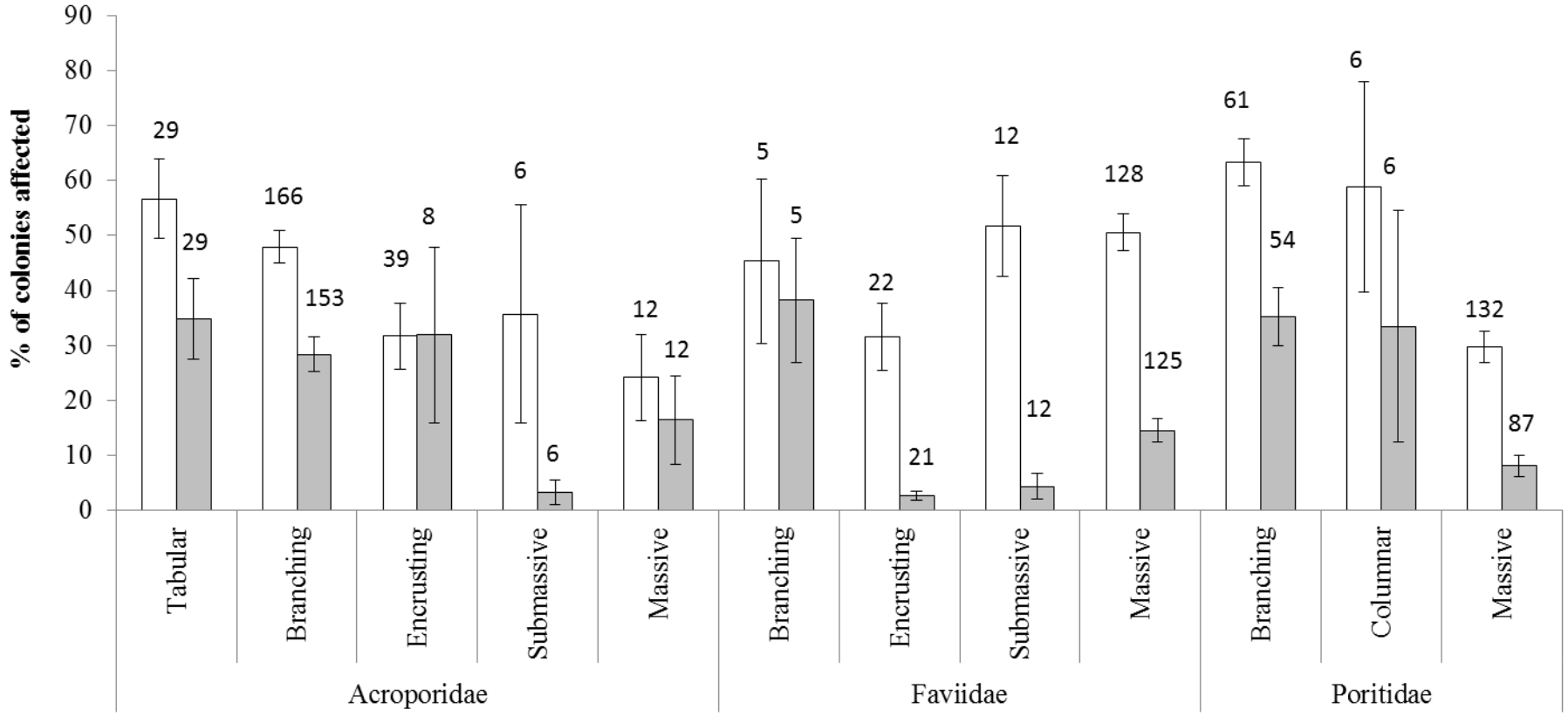

Figure 6.7: Mean (±SE) bleaching susceptibility (white) and mortality (grey) for different growth forms, within three families of scleractinian corals (Acroporidae, Faviidae and Poritidae) that exhibit a range of growth forms (n is shown on graph, error bars are ±SE).

7.0 General Discussion

The overarching aim of the research presented in this thesis was to quantify the inherent variation in bleaching susceptibility within and among different coral taxa, which is fundamental in establishing the capacity of coral populations to cope with rapidly increasing ocean temperatures (Hughes et al. 2003, Pandolfi et al. 2011), as well as explicitly testing for changes in bleaching susceptibility through time (e.g., Guest et al. 2012, Pratchett et al. 2013). Climate change represents a significant and increasing threat to tropical marine ecosystems (Bopp et al. 2013) and especially coral reefs (Hughes et al. 2003, Hoegh-Guldberg 2004, 2005, Hoegh-Guldberg and Bruno 2010, Pandolfi et al. 2011). Critical environmental changes that are occurring in tropical oceans due to global climate change include changes in seawater chemistry (declining pH and reduced aragonite saturation), changes in the position and strength of major ocean currents, and decreases in oxygenation of surface waters (e.g., Bopp et al. 2013). However, it is increasing ocean temperatures that are the most immediate and important climatic effect (e.g. Hoegh-Guldberg 1999). Coral reefs are particularly sensitive to sustained and ongoing increases in ocean temperatures because reef-building corals are already living very close to their upper thermal limits (e.g., Jokiel and Coles 1990). Unpredictable, but increasingly frequent and severe spikes (positive anomalies) in temperatures are exceeding the thermal limits of these important contributors to reef structure and function, leading to either acute stress (e.g., bleaching) and/ or mortality (Hoegh-Guldberg 1999, Stone et al. 1999). Rates of ocean warming are likely to accelerate over the coming century (Bopp et al. 2013) taking baseline temperatures even closer to current thermal limits of coral reef organisms. It is expected therefore, that the frequency and/ or intensity of mass bleaching episodes will increase, potentially causing regional extinctions of important reef-building taxa and widespread loss of coral-dominated ecosystems (Hoegh-Guldberg et al. 2007). However, this assumes that there is limited scope for adaptation, either

because there is limited phenotypic or genotypic plasticity in responses of coral populations to emerging environmental stresses, or the rate of change exceeds the time required for sufficient evolutionary responses (but see Pandolfi et al. 2011).

Disturbance plays an important role on coral reefs, contributing to high levels of biodiversity (e.g., Connell 1978, Dial and Roughgarden 1998). However, the cumulative effects of climate change, more direct anthropogenic disturbances (e.g., overfishing, sedimentation, eutrophication and pollution), and natural disturbances (e.g., severe tropical storms) are exceeding the thresholds of disturbance that have generally positive effects. While it may be premature to be talking about the global extinction of specific coral species (e.g. Baker et al. 2008, Carpenter et al. 2008), it is clear that coral reefs are being rapidly degraded throughout the world (e.g., Wilkinson 2004) and increasing effects of global climate change will only compound the problems (e.g., Wooldridge 2009). Given ongoing climate change, shifts in the communities of scleractinian corals are bound to occur (Connell 1978), reflective of differences in the inherent susceptibility of different coral species (e.g. Loya et al. 2001). However, ongoing climate change and increasing disturbance will not necessarily favour those species that are most resistant to temperature-induced coral bleaching (Baker et al. 2008), as it is important to consider the ultimate fate of bleached corals (i.e. rates of mortality in the aftermath of coral bleaching episodes) and the capacity for population recovery (e.g., Linares et al. 2011) in predicting changes in assemblage structure. Moreover, it must be noted that the susceptibility of a coral to thermal stress does not necessarily equate to susceptibility of other stresses (but see Chapter 5); therefore, changes in community composition will be dependent upon specific stressors at the location, including the specific history of stress and presence or absence of additional stressors.

7.1 Variation in bleaching susceptibility

Considerable anecdotal information suggests that there is marked inter- and intra-specific variation in bleaching susceptibility (Hughes et al. 2003). For example, only a portion of colonies within any given population will actually bleach and even fewer actually die (e.g., Baird and Marshall 2002); however, there are instances where 100% of colonies of a given species or type have exhibited bleaching. For example, in 1998 at Sesoko Island, Japan, 100% of colonies of several different corals species (e.g., *Acropora digitifera*, *Pocillopora damicornis*, *Seriatopora hystrix*, and *Porites cylindrical*) bleached and died (Loya et al. 2001). However, when averaged across multiple locations and studies, the mean percentage of colonies that bleach (even for the most susceptible coral taxa) is only 60% (Figure 6.2). This shows that there is marked variation in bleaching susceptibility among individual colonies, providing some scope for populations to persist and adapt to changing environmental conditions. There are however, few studies that have explicitly quantified variation in bleaching susceptibility within or among different coral populations, partly due to the lack of a rigorous, quantitative metric for comparing the exact timing and severity of bleaching among individual coral colonies. It is also important to consider the ultimate fate of bleached corals (Chapter 6), because selective forcing on population and community structure will only occur through differential mortality; if corals bleach, but then recover and subsequently reproduce, there will be no selective removal of susceptible genotypes that will lead to increases in resistance to bleaching (Chapter 5).

Until now, variation in bleaching susceptibility has mostly been compared among coral taxa (e.g., Loya et al. 2001), identifying specific taxa (mostly species or genera) that are more or less affected by anomalous environmental conditions, mostly elevated temperatures. Based on the proportion of colonies that bleach and/ or die, it is clear that there are marked inter-specific differences in bleaching susceptibility (Figure 6.2, but see also Chapter 5,

Brown and Suharsono 1990, McClanahan 2000, Baird and Marshall 2002, Loya et al. 2001, Riegl 2002). However, the capacity of populations and species to persist into the future and withstand changing environmental conditions is more appropriately measured based on the overall variation in bleaching susceptibility among individual colonies from a given population; those species that have greatest phenotypic diversity in their responses to adverse environmental conditions will have the greatest capacity for adaptation, rather than the species with the largest proportion of individuals that can simply withstand a given level of stress (e.g., Pandolfi et al. 2011).

The occurrence and severity of bleaching among wild populations is often quantified based on the proportion of colonies that actually exhibit conspicuous "paling" or actually die in the aftermath of major bleaching episodes (e.g., Marshall and Baird 2000). These measures provide population-level estimates of bleaching susceptibility, but do not account for the full range of responses within each population and only distinguish between those colonies that do or don't bleach. Quantifying changes in the density of zooxanthellae within individual coral colonies provides the most unambiguous and definitive measure of bleaching incidence and severity (Chapter 2 and 3, see also Fitt et al. 2001). However, this requires that either there is a very detailed baseline of zooxanthellae densities for individual coral populations (requiring extensive monitoring in time and space) or individual corals are sampled both before and after bleaching events. Importantly, absolute estimates of zooxanthellae densities are strongly dependent upon the selection of alternative methods in common use (Chapter 2, Chapter 3). For this reason, absolute measures of zooxanthellae densities are often not comparable among different studies, and that there is strong imperative for standardisation of key methods (Chapter 3). If consistent methods are applied through time, then proportional changes in the zooxanthellae densities provide a clear measure of bleaching severity, which can be used to compare among colonies (Chapter 4), among populations and through time

(Chapter 5). Further work is required to experimentally test exact thresholds, but the extent of zooxanthellae loss may also provide an indication as to whether colonies will die or recover (Figure 3.1). There are other less intrusive measures of bleaching incidence, which correlate closely with declines in zooxanthellae densities (e.g., mean phosynthetic yield, measured using PAM flurometry), but these methods still require long-term sampling of individual coral colonies in order to establish the incidence and severity of bleaching (Chapter 4).

Clearly, there is substantial (though rarely quantified) variation in bleaching susceptibility within and among coral taxa (Chapter 4, Chapter 6), enabling corals to live in a range of environments with often very extreme temperature regimes (Coles and Brown 2003, Bauman et al. 2013a, 2013b). The question is whether this variation is attributable to extrinsic or intrinsic factors. It is suggested for example, that intra-specific variation in responses of corals (even closely positioned colonies from within the same habitat) may be attributable to fine-scale differences in environmental conditions (e.g., Jokiel and Brown 2004), or prior exposure to extreme temperatures. By quantifying differential bleaching susceptibility under carefully controlled conditions (e.g., Chapter 4) it is possible to largely eliminate the confounding influences of fine-scale differences in environmental conditions, thereby demonstrating that there is a component of bleaching susceptibility that is determined by intrinsic factors, such as the zooxanthellae clade types and condition of individual colonies (Edmunds 1994, Marshall and Baird 2000, Loya et al. 2001, Stimson et al. 2002). There are plenty of examples of physiological responses in corals, such as increasing levels of fluorescent proteins, mycosporine-like amino acids, or antioxidant enzymes, that moderate the effects of temperature extremes (Baird et al. 2009). There is also an increasing realisation that bleaching susceptibility may be determined by characteristics of the entire holobiont, representing the coral itself, associated zooxanthellae (*Symbiodinium*) as well as a whole suite of complex microbiota (Weis 2010). If these intrinsic factors that influence bleaching

susceptibility have a genotypic basis, and are determined to be heritable, then this provides considerable scope for adaptation (Csaszar et al. 2010), as future generations may be more competent at delaying the stress response (e.g. increased production of mycosporine-like amino acids, changes in the symbiont community).

Intrinsic variation in bleaching susceptibility is largely attributed to differences in the predominant type of zooxanthellae hosted by corals (Glynn et al. 2001, Baker et al. 2008). In the eastern Pacific, for example, increasing thermal tolerance of *Pocillopora* was linked to increased prevalence of colonies that host a thermally tolerant clade D symbiont (Glynn et al. 2001). Similarly, *Pocillopora* in French Polynesia host a diversity of symbionts, including clade D (Magalon et al. 2007), which may explain their low level of bleaching susceptibility compared with many other geographic locations (Chapter 5). For other coral genera, which may be incapable of switching symbionts (Goulet 2006), prior exposure to environmental extremes can stimulate photo-protective mechanisms (e.g., increased concentrations of certain pigments) that reduce bleaching susceptibility (Dunne and Brown 2001, Brown et al. 2002, Baird et al. 2009). There is not however, any clear evidence that individual colonies undergo persistent changes in their physiology (e.g., symbiont shuffling, or sustained increases in concentrations of fluorescent proteins) that contribute to increasing bleaching resistance as temperatures continue to rise (Hoegh-Guldberg et al. 2002). Rather than individual colonies achieving greater bleaching resistance, it may be that increased exposure to adverse environmental conditions will selectively remove highly sensitive phenotypes, thereby naturally increasing the proportion of individuals that possess these important adaptations for global climate change (e.g., Sampayo et al. 2008).

7.2 Maximising adaptation to changing environmental conditions

Coral reefs are considered to be among the most vulnerable ecosystems to sustained and ongoing effects of global climate change (Walther et al. 2002) owing to the extreme thermal

sensitivities of scleractinian corals, which bleach and often die when temperatures increase by only 1-2^{o}C above the local summer maxima (Jokiel and Coles 1990). Mass-bleaching episodes do not however, necessarily spell the end for scleractinian corals. Rather, these large-scale and highly selective disturbances represent an important selective pressure that is likely to lead to rapid changes in thermal tolerance among these organisms (Buddemeier and Smith 1999, Pandolfi et al. 2011). There are however, likely to be limits to thermal adaptation and acclimatization, and these may incur tradeoffs in the overall fitness of coral populations (Pandolfi et al. 2011), such that sustained increases in greenhouse gas emissions will increasingly challenge the persistence of reef organisms (and especially corals) and the degradation and loss of corals will be very patchy in time and space. It is also clear that effects of climate change on coral reefs are operating against a backdrop of many other more direct anthropogenic disturbances, which either undermine the capacity of coral to acclimate and adapt (e.g., Chapter 5) or increase vulnerability to observed and projected environmental changes.

Effects of climate-induced coral bleaching are compounding pre-existing pressures from natural and direct anthropogenic stresses (e.g., overfishing, pollution, excess nutrients and disease epidemics) to accelerate and exacerbate widespread degradation of coral-reef ecosystems (Nyström et al. 2000, Kleypas et al. 2001, Nyström & Folke 2001, Hughes et al. 2003, Pandolfi et al. 2003). However, this provides opportunities to mitigate the effects of ongoing climate change by directly addressing more direct anthropogenic threats. For example, Wooldridge (2009) estimated that significant improvements in water quality on the inshore Great Barrier Reef would enable local corals to withstand a further 2.0–2.5^{o}C increase in ocean temperatures. Until now, the effects of climate change have been relatively minor compared with the extended history and often-extensive reef degradation caused by more direct anthropogenic impacts (Pandolfi et al. 2003). However, ongoing increases in

ocean temperatures, together with changes in seawater chemistry, will increase over time to become the most important threat to coral reef ecosystems (Hoegh-Guldberg et al. 2007). Minimising global greenhouse-gas emissions is therefore, crticial to minimise longer-term and more extreme impacts of climatic change on coral reefs, all the while reducing direct effects of direct anthropogenic disturbances to restore ecosystem function and resilience to these ecosystems.

7.3 Conclusions

Documented increases in the frequency and extent of mass coral bleaching are often cited as evidence that climate-related changes in environmental conditions have now exceeded the tolerance of most (if not all) scleractinian corals, and that there is extremely limited capacity for corals to adapt (e.g., Hoegh-Guldberg et al. 2002, 2007). If so, projected increases in ocean temperatures, and other climatic disturbances (especially ocean acidification), will lead to increasing loss of coral species and accelerated degradation of coral reef environments (Hoegh-Guldberg and Bruno 2010). However, this study adds to increasing evidence (reviewed by Pandolfi et al. 2011) that there is substantial phenotypic and genotypic plasticity in the responses of corals to increasing temperature. In Moorea, for example, there is clear evidence of declines in bleaching susceptibility through successive bleaching events, especially among the most susceptible corals (e.g., *Acropora* and *Montipora*). This was likely due to selective removal of the most susceptible genotypes during initial bleaching events, such that the thermal tolerance of the remaining community has been enhanced. However, it is also possible, that coral that bleached, but survived, now have a much higher tolerance to temperature anomalies (e.g., Guest et al. 2012). Ongoing research is necessary to quantify the overall extent of adaptive capacity among different coral populations and species, while clearly documenting the responses of corals (i.e., proportional declines in zooxanthellae densities; Chapter 3) to test for evidence of adaptation. Long-term experimental studies are

also required, as undertaken for fishes (Donelson et al. 2011) to test for trans-generational changes in the bleaching susceptibility of corals, which is the most direct test of adaptive capacity. Meanwhile, urgent action is needed to reduce global greenhouse emissions, as well as minimizing all direct anthropogenic threats to coral reef ecosystems, in order to maximize the capacity of corals to adapt to changing environmental conditions.

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