Effects of water quality on the health and condition of inshore corals

Thesis submitted by

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Statement on the Contribution of Others

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"Just keep swimming…" – Dory, Finding Nemo

GENERAL ABSTRACT

Coral species are threatened by environmental change, both from global pressures, particularly ocean warming and acidification, and local pressures, such as poor water quality and pollution. Species with broad distributions experience a range of different environments. For example, distribution ranges that span the continental shelf expose coral populations to oligotrophic offshore conditions and turbid, nutrient-rich inshore reef waters. Such study systems provide a unique opportunity to understand how species modify their biochemical and molecular phenotype in response to varying environmental conditions. Organisms can optimise their performance and fitness (i.e. growth, survival and reproduction) under local environmental regimes through physiological acclimatisation within their lifetimes and/or through genetic adaptation at the population or species level across generations. Studies of species with broad habitat distributions can provide insights into the fundamental mechanisms that underpin acclimatisation and adaptation, which collectively enable corals to respond to changing environments in the future.

Biochemical attributes of corals, and estimates of their growth and survival, can be used to describe the health of the coral holobiont under different environmental conditions. In ***Chapter 2,*** I compared survival, growth, and five biochemical health attributes of *Acropora tenuis* among habitats characterised by different water quality regimes in the central Great Barrier Reef, Australia. Health attributes of the coral host and its' *Symbiodinium* were monitored over three seasons along a strong and a weak inshore water quality gradient, each with three locations at increasing distances from the coast and major rivers. Along the strong water quality gradient, corals had the highest symbiont densities and tissue energetic concentrations closest to the coast and river source, where particulate concentrations were the highest. In contrast, corals found at the site with clearer water had slower growth and increased skeletal density. Differences in coral and *Symbiodinium* attributes were less pronounced along

the weaker gradient. According to most of the physiological and biochemical attributes measured, high concentrations of dissolved and particulate nutrients were not detrimental to this robust coral species over the timeframe of the study. Decreased skeletal densities associated with higher growth rates at more turbid sites are likely to cause higher susceptibility to physical damage from storms, which occur seasonally and are predicted to increase in frequency in the future. These results highlight the importance of assessing multiple coral attributes when monitoring coral health.

Fatty acids (FA), the building blocks of lipids, have been proposed as biomarkers of coral health and stress, as they play a vital role in the metabolism and stress resistance of a broad range of organisms. In corals, FA can also potentially reveal sources of nutrition and host-symbiont resource sharing, which can elucidate basal mechanisms of biochemical and physiological functioning of corals. In ***Chapter 3,*** I explore seasonal and spatial variation in tissue concentrations and composition of FA in *Acropora tenuis* along the two aforementioned water quality gradients. FA health indicator ratios varied similarly within both regions along the respective water quality gradients. Corals exposed to clear, nutrient-poor conditions at sites along the afore studied water quality gradients (defined as good or very good water quality by the Reef Rescue Marine Monitoring Program at the Australian Institute of Marine Science) had the highest ratios, while corals from sites of moderate WQ had the lowest ratios, suggesting heterotrophic food sources within turbid, nutrient-enriched conditions along these same water quality gradients can supplement reduced autotrophy. Percentages of essential FA (EPA and ARA) were highest in corals from clearer water and were negatively correlated with *Symbiodinium* density. Strong seasonal divergence occurred in polyunsaturated fatty acid (PUFA) concentrations, with greater percentage of n-3 PUFA found in the dry seasons (June 2013 and October 2013) compared to greater percentage of n-6 PUFA found during the wet seasons (February 2013 and February 2014). This study demonstrated essential FA and their

derived health ratios respond to changes in seasonal and environmental conditions supporting FA as biomarkers of coral holobiont health.

Predation is a major source of coral mortality, and corallivorous fishes can significantly influence growth and survival of coral populations. In ***Chapter 4,*** I document how corals transplanted from a highly turbid and nutrient-enriched environment of moderate water quality to a low turbidity, non-nutrient-enriched environment of good water quality suffered high mortality and skeletal loss from predation. Specifically, colonies of *Acropora tenuis* transplanted from the site closest to the coast and river source to the site with clearer water along the stronger water quality gradient sustained significantly greater loss of coral skeleton, when compared to control colonies and their reciprocally transplanted counterparts. These results suggest marked intraspecific differences in the physiological condition and palatability of coral colonies underlying selective predation on corals originating from a high turbidity, nutrient-enriched environment. Further studies are needed to understand the underlying biochemical or physiological attributes that incite selective predation within coral populations and their ecological consequences.

To investigate drivers of plasticity in the biochemical and physiological attributes of *A. tenuis*, a suite of FA and biochemical attributes were monitored over the course of the reciprocal transplant experiment described in *Chapter 4*. In ***Chapter 5,*** I evaluated the degree to which coral populations from different environmental regimes acclimated to a novel environment four months after transplantation. To partition the effects of source population genetics, long-term acclimatisation and the environment, I quantified variation in global gene expression (GE) and FA composition of surviving experimental fragments. There was a strong influence of source population on GE profiles enriched with higher relative expression of genes associated with translation, ribosome biogenesis and ribosome cellular components in corals sourced from moderate water quality compared to lower relative expression in corals sourced

from an environment defined by good water quality. Environment was a major driver of change in FA composition; all major FA classes, with the exception of short-chain PUFA, decreased in concentration when corals were exposed to moderate water quality and increased in concentration when corals were exposed to a good water quality environment, regardless of source population. There was also evidence of plasticity in the responses of coral genes relating to elevated health and immunity due to environmental change. This chapter demonstrates the plasticity of corals in response to environmental change, but also a limit to that plasticity dictated by their source population either as a consequence of underlying genetic differences or long-term acclimatisation. Therefore, there may be potential hope for future corals, if we can reduce anthropogenic water quality stressors on coral health and condition.

In summary, comparisons of a range of molecular and biochemical health attributes among experimental colonies of *Acropora tenuis* originating from a range of water quality regimes reveal new insights into environmental drivers and the underlying genetic basis of coral health. This research demonstrates that common coral species on inshore reefs, such as *A. tenuis,* can grow rapidly under water quality conditions characterised by high concentrations of particulate and dissolved nutrients. However, negative correlations among attributes commonly associated with good health (e.g. growth rate and skeletal density) highlight the need to carefully define health attributes. Inshore populations of the coral *A. tenuis* can acclimatise and/or adapt to local conditions through variation in growth rate, symbiont type and density, skeletal density and organic tissue components, including FA composition. Yet, coral populations still maintain a genetic signature of their origins, which enables differentiation and identification of inshore populations. By integrating physiological attributes, biochemical composition and genomics, this research improves our understanding of the capacity of corals to acclimatise and/or adapt to a range of environmental conditions, most notably terrestrial runoff and climate change into the future.

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CHAPTER 1: General Introduction

1.1 Background

Coral species are threatened world-wide, both from global pressures, particularly ocean warming and acidification (Hoegh-Guldberg et al. 2007), and local pressures, such as poor water quality and pollution (Fabricius 2005). Negative impacts of poor water quality include altered species composition or loss of diversity (Fabricius et al. 2012), reduced recruitment success (Thompson et al. 2014b), restricted depth distributions (Cooper et al. 2007) and greater bleaching susceptibility (Wooldridge and Done 2009; Cunning and Baker 2013; Wiedenmann et al. 2013). In particular, recent declines in coral cover along Australia's Great Barrier Reef (GBR; De'ath et al. 2012) raise the need for further exploration of causes and consequences of these pressures.

Coastal or inshore coral populations, which comprise approximately one-third of the GBR (Hopley et al. 2007), are predicted to be the most affected by local environmental and anthropogenic stressors (Wooldridge 2009; Brodie et al. 2010). Generally, inshore corals are exposed to acute disturbances, including elevated extreme summer temperatures that lead to more severe bleaching events (Berkelmans 2002), as well as wet season river plumes with associated decreased salinities, increased suspended solid loads and enhanced nutrient enrichment (Anthony, 2006; De'ath and Fabricius, 2010; Fabricius et al., 2013). In other reef regions, inshore environments are subjected to chronic pressures associated with coastal development and deteriorated water quality (Singapore, Browne et al. (2015); Madagascar, Maina et al. (2013); Palau, Golbuu et al. (2011); Panama, Seemann et al. (2014); Florida Keys, Kenkel et al. (2015). In combination, these stressors have a high potential to affect the health and survival of corals on inshore reefs.

Species with broad distributions that encompass a range of different environments, for example coral species that are distributed from oligotrophic offshore waters to turbid

inshore reef waters, provide opportunities to understand how acclimatisation and adaptation to varying environmental conditions occurs within species. Physiological and biochemical studies of these species can also provide insights into potential mechanisms an organism might possess to enable it to respond to changing environments in the future.

Organisms can optimise their survival, performance and fitness under local environmental regimes through physiological acclimatisation (i.e. plasticity of the phenotype, which may reflect epigenetic mechanisms) and/or genetic adaptation. Physiological acclimatisation, also commonly known as phenotypic plasticity, is the capacity of an organism to tune its phenotype, biochemical attributes and physiological performance to a variety of environmental conditions within its lifetime (Coles and Brown 2003; Weis 2010; Brown and Cossins 2011; Sanford and Kelly 2011). Acclimatisation can provide a rapid response to environmental change. Beyond acclimatisation, there is genetic adaptation. Adaptation is the result of natural selection, whereby populations (not individuals) evolve over generations and optimise traits via novel mutations or selection on standing genetic variation, such that fitness is enhanced under local environmental conditions (Sanford and Kelly 2011). Locally adapted individuals, or genotypes, are predicted to have greater fitness than foreign individuals, resulting in significant genotypic-environmental interactions (Brown and Cossins 2011). Epigenetic modifications, which are modifications of gene expression rather than alterations of the genetic code itself, can be inherited from one generation to the next, and can lead to long-term phenotypic changes in individuals induced by intrinsic and environmental changes within previous generations (Feil and Fraga 2012; Granados-Cifuentes et al. 2013). Furthermore, genetic differentiation can arise through developmental canalisation and other epigenetic longer-term acclimatisation mechanisms (Pigliucci et al. 2006). Either physiological acclimatisation (including epigenetic modifications), genetic adaptation, or some combination of these processes are required for corals to persist in the

face of increasing local and global impacts (Donner et al. 2005).

Acclimatisation and adaptation are not required in response to variation in environmental conditions alone. It is when these environmental conditions change beyond local tolerance thresholds that organisms and populations, particularly corals, need to respond to such changes (Edmunds and Gates 2008; Brown and Cossins 2011). Rates of environmental change caused by anthropogenic sources have risen at unprecedented rates compared to recent evolutionary history (Brohan et al. 2006; Meissner et al. 2012) and continue to rise. As such, it has been predicted that corals may be unable to adjust their physiologically responses within the time frame predicted (Donner et al. 2005; Hoegh-Guldberg et al. 2007). If the threshold of physiological tolerance of inshore coral populations is exceeded, these corals must acclimatise and/or adapt to these novel conditions for survival.

Survival, fitness and stress tolerance of corals are affected by environmental conditions, including declining water quality and climate change. The health and stress tolerance of corals can be measured and potentially predicted from biochemical and physiological attributes of the coral holobiont (Little et al. 2004; Rosenberg et al. 2007; Mieog et al. 2009; Rocker et al. 2012). Optimal health is defined here as the ideal performance and maintenance of physiological, biochemical and genetic functioning within the coral holobiont and is dependent upon local environmental conditions. Healthy corals are suggested to have optimal symbiont densities between ~10 and 30 x 10^5 cells cm^{-2} (Wooldridge et al. 2016), high organic reserves (suggested thresholds for *Acropora* species range between 2.9 - 5.6 mg tissue cm^{-2}; Thornhill et al. 2011) and structurally sound skeletons (although skeletal density is found to be highly plastic in response to the environment; Smith et al. 2007). Therefore, it is important to evaluate the degree of phenotypic and genotypic variation in fitness-related traits within coral populations to reveal their adaptation potential to particular environmental stressors. Partial colony mortality is one

useful indicator of coral health; however, healthy corals can be killed by external stressors regardless of their health status, including predation or physical disturbances (Rotjan and Lewis 2005; Fabricius et al. 2008). Relating biochemical, physiological and genetic attributes of corals and their endosymbionts to overall survival and stress tolerance of the holobiont will help us understand the health status of corals and their potential capacity to acclimate and/or adapt to different environmental conditions. Understanding the degree to which water quality and climate change impact the health status of corals will improve future predictions of the capacity of corals to resist, respond, recover and survive when confronted with environmental change.

Coral growth, survival and reproduction are key processes underlying changes in coral cover and diversity (Connell et al. 1997) and are governed by physiological and biochemical attributes of coral colony health and condition. Commonly used indicators of coral health and condition include types and densities of associated dinoflagellate symbionts (Berkelmans and van Oppen 2006; Baird et al. 2007), rates of photosynthesis and respiration (Anthony and Hoegh-Guldberg 2003), bleaching tolerance (Berkelmans 2002; Cunning and Baker 2013), disease resistance (Harvell et al. 2007), skeletal integrity (Hughes 1987; Lough and Barnes 1992; Pratchett et al. 2015), and nutritional status (Anthony and Fabricius 2000; Hoogenboom et al. 2011). Comparisons of these health indicators among corals and populations within a species can reveal functional trade-offs and energy investments made by the coral holobiont to cope with environmental variation (Mydlarz et al. 2010), and provide a comprehensive assessment of the health of individual species of reef corals.

The potential for corals to survive depends largely on local environmental conditions, particularly water quality parameters, to which the holobiont is exposed (Fabricius 2005). For example, exposure to high sedimentation levels can create anoxic environments that lead to smothering of corals (Anthony et al. 2002; Philipp and Fabricius 2003; Weber et al. 2012), in

addition to greater susceptibility to disease (Haapkylä et al. 2011; Pollock et al. 2014) and bleaching (Wooldridge and Done 2009; Cunning and Baker 2013). Increased nutrients and organic material, in combination with decreased irradiance, can have deleterious effects on coral calcification, photo-physiology and energy stores (Anthony et al. 2007; Carilli et al. 2009; Cunning and Baker 2013; Fabricius et al. 2013a). Specifically, disruptions in photosynthetic functioning and coral-symbiont relations (Erftemeijer et al. 2012; Flores et al. 2012) induced by decreased light levels from high turbidity, can cause further deterioration of coral holobiont health by increasing bleaching susceptibility (Wooldridge and Done 2009; Cunning and Baker 2013) and depleting lipid reserves (Porter et al. 1989; Fitt et al. 1993; Yamashiro et al. 2005; Rodrigues and Grottoli 2007). In contrast, moderate levels of nutrients and shading can provide benefits for some corals, depending on the levels of these parameters and the species in question (Fabricius, 2005; Fabricius et al., 2013; Wooldridge, 2014). Some corals exposed to deteriorated water quality have improved nutritional status (Anthony et al. 2007), higher photosynthetic efficiency (Browne et al. 2015) and increased lipid reserves (Szmant-Froelich and Pilson 1980). Thus, poor water quality does not necessarily cause deterioration in coral health. To gain insights into the capacity of corals to acclimatise and/or adapt to different water quality conditions, it is important to understand how biochemical and physiological attributes of corals vary in association with changes in these environmental regimes.

1.2 Study species and design

This study was designed to investigate spatio-temporal variation and change in the overall health and condition of inshore corals, which are increasingly exposed to mounting environmental and water quality pressures associated with coastal activities and river runoff. The common branching coral *Acropora tenuis* (found in the Indo-Pacific, Red Sea and Indian

Ocean; Veron and Stafford-Smith 2000) was chosen as the focal study species because its distribution spans a broad range of water quality environments throughout the entire Great Barrier Reef, Australia; it occurs on both offshore clear-water reefs, where corals are exposed to comparatively high light levels but low levels of nutrients from sediments and organic matter (Fabricius et al. 2005), and inshore reefs, where corals are typically exposed to high levels of particulates and organic matter, combined with decreased light levels (Fabricius et al. 2005). Acroporid corals are known to be highly susceptible to bleaching (Marshall and Baird 2000; Berkelmans 2002) and relatively slow to recover from environmental stressors (Loya et al. 2001; van Woesik et al. 2011) compared to massive species. For *A. tenuis* to thrive and survive within this wide spectrum of environmental conditions, this coral species has presumably evolved plasticity in a range of biochemical parameters and physiological processes.

Working in conjunction with the Marine Monitoring Program (MMP) run by the Australian Institute of Marine Science (AIMS), coral populations in two regions in the central sector of the GBR were studied over a 2-year period. As part of the MMP, a suite of environmental and ecological variables are monitored regularly along four inshore water quality gradients emanating from selected rivers and increasing in distance from the North Queensland coast (Thompson et al. 2014a). Given the steep gradients in environmental conditions experienced on these inshore reefs (Thompson et al. 2014a) and the plethora of environmental and ecological data complied by the MMP, studies of inshore corals along these gradients provide a convenient *in situ* experimental system for understanding the effects of climate and anthropogenic stressors and their interactions on corals.

Two inshore water quality gradients were selected for study (see Fig. 2.1). Coral populations within both the Burdekin and Whitsunday regions are exposed to a wide range of water quality conditions (Thompson et al. 2014a) and will continually be defined throughout

this thesis by the water quality ratings set forth in Thompson et al. (2014a) and presented to the public through the 'Great Barrier Reef Report Card' (Queensland Government 2015). In doing so, comparisons to long-term environmental and ecological data associated with AIMS and MMP outputs can be established and used to inform of long-term water quality effects on coral physiology and susceptibility.

The more northerly Burdekin region contains a large catchment centred around a single river, where land use is dominated by pastures for cattle grazing (Brodie et al. 2003). The large flow of the Burdekin River contributes the highest input of total suspended sediments to the GBR lagoon (approximately 3.93 million tonnes $year^{-1}$) of all catchments adjacent to the GBR (Kuhnert et al. 2012); flood plumes can extend > 200 km to the north, transporting fine sediments and associated nutrients as muddy marine snow (Bainbridge et al. 2012). Within the Burdekin region, water quality at each site was defined as very good (B3), good (B2) and moderate (B1) according to Thompson et al. (2014a). Aggregate scores of particulate nitrogen concentrations, particulate phosphorus concentrations, chlorophyll concentrations, and a water clarity indicator (combining suspended solids, turbidity and Secchi depth) are used to define this water quality index. According to the 2014 'Great Barrier Reef Report Card' (Queensland Government 2015), the Burdekin region was defined by higher particulate nitrogen, particulate phosphorus and sediment levels in the marine environment from 2013 - 2014. The more southerly Whitsunday region has several smaller rivers (Proserpine River, O'Connell River and Pioneer River) flowing into the GBR lagoon and the catchment is dominated by crop cultivation (Drewry et al. 2009). Although the rivers in this region are relatively small, their nutrient loads are significantly elevated due to widespread fertiliser use within the catchment (Mitchell et al. 2005). Within the Whitsunday region, water quality at each site was defined as good (W3), moderate (W2) and poor (W1; Thompson et al. 2014a), where increased inorganic nutrient and pesticide levels were the

drivers of declining water quality from 2013 - 2014 (Queensland Government 2015).

1.3 Overall aims and significance

Given environmental changes predicted to affect coral reefs in the coming decades and the vulnerability of inshore corals exposed to both local and global stressors, the primary goal of this thesis is to improve current knowledge and understanding of the drivers of coral health and condition across a range of local water quality regimes. Using naturally occurring, inshore water quality gradients in two regions of the central sector of Australia's Great Barrier Reef as model systems, my specific aims were to: (1) investigate spatial and temporal variation in the growth and survival of inshore corals and identify correlated physiological health attributes; and (2) examine the potential for acclimatisation in coral health parameters and gene expression in response to changing water quality. This thesis enhances the current state of knowledge of coral health and condition, as well as explores the capacity of inshore corals to acclimatise to deteriorating water quality in coastal environments.

1.4 Objectives and thesis outline

My specific objectives for each chapter are as follows:

In ***Chapter 2,*** I examine spatio-temporal variation in indicators of coral heath and condition over two years along two inshore water quality gradients in the scleractinian coral *Acropora tenuis,* by (1) characterising environmental variation along the two environmental gradients, (2) quantifying physiological and biochemical attributes associated with coral health and condition, and (3) identifying influential environmental parameters affecting overall coral health and condition. This study provides important background on *in situ*

patterns of coral health for inshore coral populations and their potential to acclimatise to local water quality environments.

In ***Chapter* 3**, I investigate spatial and temporal variation in the energetic content and nutritional status of inshore populations of *A. tenuis*, as well as assess the viability of fatty acids (FA) as bioindicators for monitoring the health status of inshore coral populations. Results increase current knowledge of coral nutritional status in relation to local environmental parameters and enhance our understanding of the ability of corals to resist and recover from future environmental stressors.

In ***Chapter 4,*** I assess the growth, mortality and survival of inshore corals in response to changing water quality in a reciprocal transplant study involving turbid-water (moderate water quality) and clear-water (good water quality) populations of *A. tenuis*. Results provide new insights into intraspecific differences in the condition of coral colonies in response to changed water quality conditions. Intriguingly, such differences can lead to differing levels of selective predation pressures.

Chapter 5 builds on *Chapter 4* through further investigations of biochemical and genetic attributes that may lead to acclimatisation and/or adaptation in response to changing water quality. In this chapter, I assess changes FA composition and quantify gene expression (GE) in reciprocally transplanted corals of *A. tenuis*. Results enhance and clarify our knowledge of the molecular mechanisms that underpin changes in coral colony health and condition.

Finally, in ***Chapter 6,*** I synthesise key findings from this thesis and highlight the

significance of local environmental parameters to the overall health and condition of corals in an uncertain future. In addition to discussing implications of the research conducted in this thesis, remaining knowledge gaps for future research topics are identified.

CHAPTER 2: Variation in the health and biochemical condition of the coral *Acropora tenuis* along two water quality gradients on the Great Barrier Reef, Australia

Under review in *Marine Pollution Bulletin*

2.1 Synopsis

Biochemical and physiological attributes of corals can be used as indicators to monitor spatio-temporal changes in their health and condition. This study explores how plasticity in various health attributes has enabled the coral *Acropora tenuis* to respond to differing water quality conditions on inshore reefs of the central Great Barrier Reef. Coral health attributes were monitored along a strong and a weak water quality gradient, each with three reefs at increasing distances from the coast and from major river sources. Significant differences in coral health attributes were detected along the strong water quality gradient; corals grew fastest, had the least dense skeletons, highest symbiont densities, and highest lipid concentrations closest to the coast and river mouth, where water quality was considered moderate. Variations in coral health attributes were less pronounced along the second, much weaker, water quality gradient. Although high nutrient and particulate loads were not detrimental to the overall health of this robust coral species under ambient conditions, potentially deleterious consequences of concomitant increases in symbiont densities and decreases in skeletal densities include higher susceptibility to photo-stressors and physical damage. Therefore, differences in coral health attributes across populations, either as a consequence of acclimatisation (plasticity) and/or adaptation, may result in trade-offs between tolerance to high nutrient loads and low light levels versus tolerance to stressors (causing bleaching) or investment in structural strength. Spatio-temporal variation in coral health indicators reveals how water quality drives coral condition and highlights the importance of assessing multiple coral health attributes in coral reef monitoring.

2.2 Introduction

Coral species are threatened world-wide by environmental change, both from global pressures, particularly ocean warming and acidification (Hoegh-Guldberg et al. 2007), and local pressures, such as poor water quality and pollution (Fabricius 2005; Burke et al. 2011). One mechanism for responding rapidly to environmental change is physiological acclimatisation - the capacity of an organism to tune its phenotype and physiological performance to a varying environment within its lifetime (Coles and Brown 2003; Weis 2010; Brown and Cossins 2011). Studies of species with broad distributions that encompass a range of different environments, for example coral species that are distributed from oligotrophic offshore waters to turbid inshore reef waters, provide an important opportunity to understand how species have acclimatised or adapted to varying environmental conditions. Such studies can also provide insights into potential mechanisms an organism might possess through local acclimatisation or adaptation to enable it to respond to changing environments in the future.

Inshore and nearshore coral reefs comprise approximately one-third of Australia's Great Barrier Reef (GBR; Hopley et al., 2007). Inshore coral populations are predicted to be the most affected by the individual and cumulative impacts of global and local pressures (Wooldridge 2009; Brodie et al. 2010). Generally, inshore corals are located in shallow water and are exposed to higher temperature extremes, potentially leading to more severe bleaching in a warming climate compared to offshore corals (Berkelmans 2002). Inshore reefs are also more likely to be exposed to land runoff and associated decreased salinities, and increased loads of suspended solids and nutrients leading to regional organic enrichment. Increased organic material and decreased irradiance have the potential to suppress coral calcification and productivity (Anthony et al., 2007; Cunning and Baker, 2013; Fabricius et al., 2013). The steep gradients in environmental conditions experienced on GBR inshore reefs (Thompson et

al. 2014a) provide a convenient *in situ* experimental system for understanding the effects of global and local pressures and their interactions on inshore coral populations.

Many water quality parameters can modify the health and condition of corals, especially with regard to energy acquisition and expenditure (Fabricius 2005). Negative impacts of poor water quality include altered species composition or loss of diversity (Fabricius et al. 2012), reduced recruitment success (Thompson et al. 2014b), restricted depth distributions (Cooper et al. 2007) and greater bleaching susceptibility (Wooldridge and Done 2009; Cunning and Baker 2013; Wiedenmann et al. 2013). High sedimentation can create anoxic environments and smother corals (Anthony et al. 2002; Philipp and Fabricius 2003; Weber et al. 2012; Jones et al. 2016). High nutrient concentrations found in terrestrial runoff have had deleterious effects on coral survival, photo-physiology and skeletal growth (Carilli et al., 2009; Fabricius et al., 2013). Decreased light levels from high turbidity can disrupt photosynthetic functioning and coral-symbiont relations (Erftemeijer et al. 2012; Flores et al. 2012; Jones et al. 2016). However, some species may benefit from water quality conditions deemed poor as responses depend on species' physiological niches and local acclimatisation (Fabricius, 2005; Fabricius et al., 2013; Wooldridge, 2014). For example, some coral species show improved nutritional status (Anthony et al. 2007) and higher photosynthetic efficiency (Browne et al. 2015) on reefs with high sediment and nutrient loads. As a consequence of these seemingly contradictory results, and the correlations and interactions that have been found among many environmental variables (Crain et al. 2008), multiple coral responses need to be considered to understand how corals modulate energy acquisition and expenditure in relation to their surrounding environmental conditions.

Coral cover is commonly used as an indicator of the health of coral assemblages (Done 1982; De'ath et al. 2012), however, change in cover is not a predictive measure and can occur in response to many different processes, including exposure to storms and

predation. Therefore, change in cover is not diagnostic of coral health, especially at the species and population level. Coral growth, survival and reproduction are drivers of changes in coral cover and diversity (Connell et al. 1997), and these measures are directly driven and influenced by coral colony health and condition (Cooper et al. 2009). These processes depend largely on biochemical and physiological attributes of the individual coral holobiont (Rohwer et al. 2002; Rosenberg et al. 2007). Thus, more specific indicators of coral health and condition include types and density of associated dinoflagellate symbionts (Berkelmans and van Oppen 2006; Baird et al. 2007; Cunning and Baker 2014), rates of photosynthesis and respiration (Anthony and Hoegh-Guldberg 2003), bleaching tolerance (Berkelmans 2002; Cunning and Baker 2013), disease resistance (Harvell et al. 2007), skeletal integrity (Hughes 1987; Lough and Barnes 1992; Madin et al. 2012; Pratchett et al. 2015), and nutritional status (Anthony and Fabricius 2000; Hoogenboom et al. 2011). Combined, these health indicators enable more detailed evaluation of functional trade-offs and energy investments made by the coral holobiont (Mydlarz et al. 2010), and provide a more appropriate assessment of the health of individual species of reef corals.

This study investigates how biochemical attributes, growth and survival of the branching coral *Acropora tenuis* varies among sites along two water quality gradients, associated with distance to the coast and river discharges, across two years. *A. tenuis* is a widely distributed species in the western Pacific Ocean that occurs on the upper reef slope (Veron and Stafford-Smith 2000). It is common on both offshore clear-water reefs where corals are exposed to comparatively high light levels but low levels of nutrients from sediments and organic matter, and in shallow water on inshore reefs where colonies are typically exposed to high levels of particulate and organic matter (Anthony et al. 2004). Importantly, environmental conditions at inshore reefs (i.e. water quality, turbidity, organic matter, light) are more variable (De'ath 2007; De'ath and Fabricius 2010; Schaffelke et al.

2012; Thompson et al. 2014a) compared to offshore reefs. To thrive and survive within this wide environmental spectrum, individuals and populations of this species have acclimatised and/or adapted to suit local conditions through a range of biochemical attributes and physiological processes.

The aims of this study were to understand local drivers of the health and condition of *A. tenuis* on inshore reefs by: 1) characterising environmental variation along a strong and a weak environmental gradient at varying distances from the coast and river influences; 2) quantifying different coral health and biochemical attributes amongst water quality environments and seasons; and 3) correlating environmental parameters with *A. tenuis* health and condition. By documenting a range of health indicators, this study aims to add valuable information regarding relationships among coral attributes, to better understand which water quality parameters are of most consequence to coral colony health.

2.3 Methods

Study sites

The study was conducted at three sites within each of two regions in the central sector of Australia's Great Barrier Reef (GBR), designated as sites B1-B3 in the Burdekin region and sites W1-W3 in the Whitsundays region. The numerical sequence corresponds to increasing distances of sites from a river source and distance from the coast (Fig. 2.1). All six sites are part of the established long-term Marine Monitoring Program (MMP) undertaken by the Australian Institute of Marine Science (AIMS) over the past 10 years. This program monitors a suite of environmental and ecological variables along inshore water quality gradients away from priority rivers along the North Queensland coast (Thompson et al. 2014a). In this study, the more northerly Burdekin region contains a large catchment centred around a single river, where land use is dominated by pastures for cattle grazing (Brodie et al.

2003). The large flow of the Burdekin River contributes the highest input of total suspended sediments to the GBR lagoon (approximately 3.9 million tonnes $year^{-1}$) of any catchment (Kuhnert et al. 2012). Its flood plumes can extend > 200 km to the north of the river mouth, transporting fine sediments and associated nutrients as muddy marine snow (Bainbridge et al. 2012). The Burdekin sites studied here are Geoffrey Bay on Magnetic Island (B1: S19°09.264' E146°52.083'), Pandora Reef (B2: S18°48.980' E146°26.059'), and Pelorus Island (B3: S18°32.435' E146°29.326'), located approximately 60 km, 115 km, and 125 km from the Burdekin River mouth, respectively. Water quality at each site was defined as very good (B3), good (B2) and moderate (B1; Thompson et al. 2014a), with the latter location characterised by higher levels of turbidity. In addition, Geoffrey Bay (B1) is located in a shallow bay adjacent to a large suburb. The more southerly Whitsunday region is influenced by several smaller rivers (Proserpine River, O'Connell River and Pioneer River) and their catchments are dominated by grazing and sugarcane cultivation (Brodie et al. 2003; Drewry et al. 2009). Although the rivers are relatively small, their nutrient loads are significantly elevated due to widespread fertiliser use within the catchment (Mitchell et al. 2005). The Whitsunday sites studied here are Pine Island (W1: S20°22.685' E148°53.311'), Daydream Island (W2: S20°15.343' E148°48.749'), and Double Cone Island (W3: S20°06.281' E148°43.298'), which are approximately 20 km, 30 km, and 50 km away from river sources, respectively. Water quality at each site within this region was defined as good (W3), moderate (W2) and poor (W1; Thompson et al. 2014a), where nutrient enrichment drives the decline in water quality. Furthermore, Daydream Island (W2) is home to a resort, which may also affect surrounding environmental conditions. These inshore sites are exposed to a wide range of water quality conditions that vary among sites according to their proximity to the coast and source of river run-off (Thompson et al. 2014a).

At each of the six sites, fragments of approximately 15 × 15 cm^2, a size typically

below reproductive size to minimise the likelihood that changes in coral health attributes were due to spawning and reproduction, were collected from twelve to twenty colonies of *Acropora tenuis* naturally growing at depths of 1 – 3 m below datum. Fragments were individually tagged, and randomly allocated and attached to a stainless steel rack at each site following Howells et al. (2013). Racks were located at 2 m depth and less than 0.5 m from the substrate, thus experimental corals were within a similar depth range to native coral colonies within the area and approximately 5 – 10 m from the corresponding MMP site, where environmental data and permanent survey transects are monitored regularly (Thompson et al. 2014a). Although racks elevated corals slightly off the bottom, which could have had implications for exposure to sediments, experimental corals were affected equally across the water quality gradients and all analyses were comparative. Placement of corals on racks facilitated repeated sampling of genotypes over time in the highly turbid (< 1 m visibility) conditions occasionally experienced at the study sites. It was assumed that the health and physiology of the corals on the rack was similar to native, wild colonies although this was not tested. Racks were established in October 2012 for B1, B2, B3, and W1, but for logistical reasons not until February 2013 at W2 and June 2013 for W3.

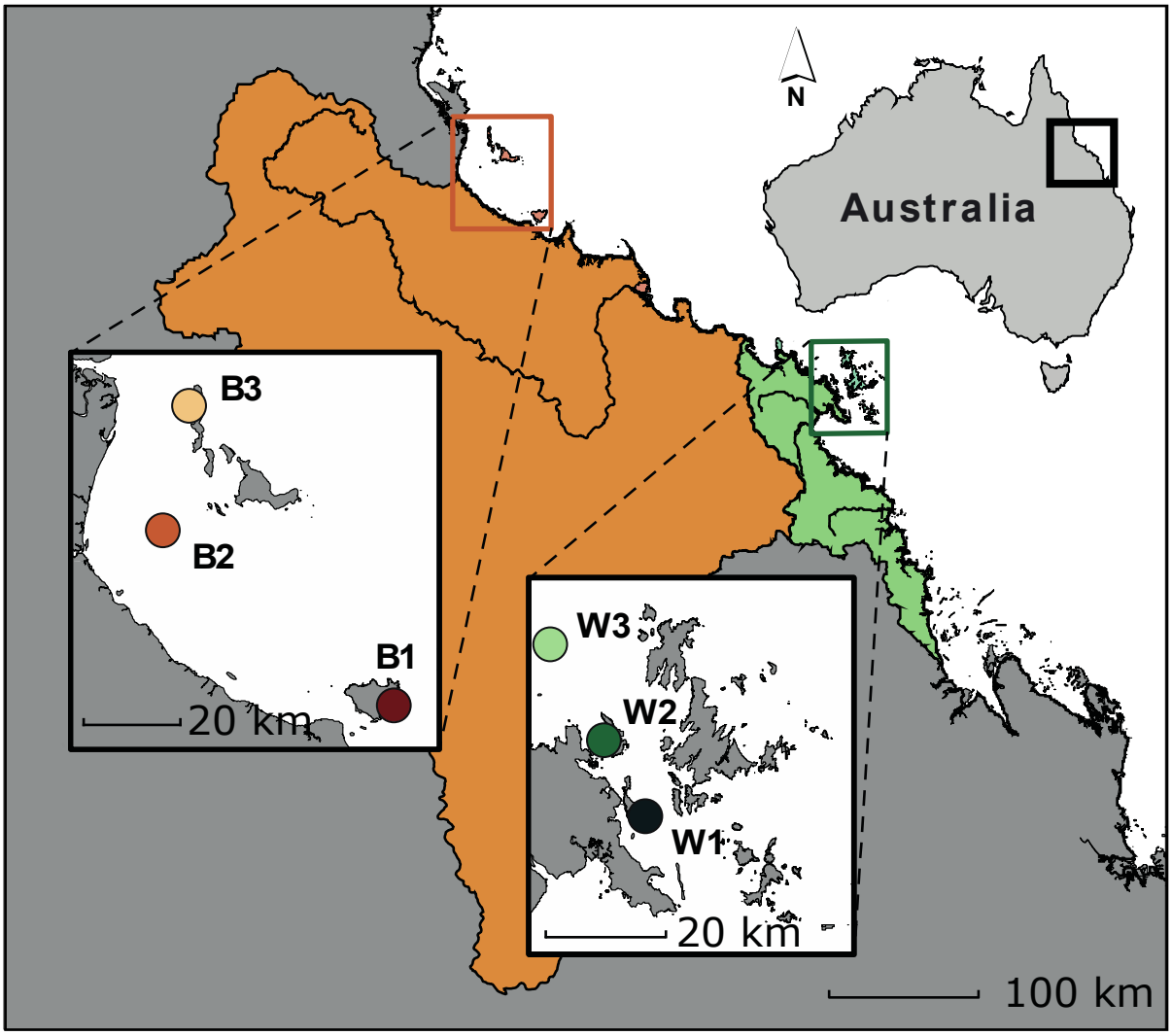

Fig. 2.1 Map of Australia and the central Great Barrier Reef showing the location of six inshore sites within the Burdekin region (orange; B1-B3), and the Whitsunday region (green; W1-W3). Each region contains three sites along gradients of increasing distances from the coast and source of river runoff selected for assessment of inshore coral health and water quality conditions. The main rivers, the Burdekin River in the Burdekin region and the Proserpine, O'Connell and Pioneer Rivers in the Whitsunday region, are marked as black lines.

Coral sampling

To examine variation in coral health and biochemical attributes (Table 2.1), all colonies were placed on racks and sampled three times per year (n = 12 to 20 colonies per site per time point) over two years in the late dry season in spring (October), the wet season in summer (February), and the early dry season in winter (June): 7-10 October 2012, 19-22 February 2013, 15-18 June 2013, 4-9 October 2013, 10-22 February 2014, and 25-30 June 2014. Growth, survival and visible condition (i.e. partial mortality, disease and bleaching) were monitored at each sampling time from photographs with a Canon S90 digital camera. Photographs were taken from directly above each coral colony at a height of approximately 0.5 m and at 90° to a plane defined by the top of each colony; each photograph included a reference scale bar and coral colour health chart (Siebeck et al. 2006). Photography was not possible at W1 in June 2014 because of high turbidity (< 0.25 m visibility). Three samples (single branches ~ 2 to 6 cm long) were collected from the middle of each colony at each sampling time. Samples were immediately snap-frozen and stored in liquid nitrogen (-80 °C) until further analysis (see Fig. 2.2).

Table 2.1 List of coral health and biochemical traits, environmental parameters, and explanatory factors within this study.

Coral health and biochemical traits	
Growth (cm day^{-1})	
Mortality (%)	
Symbiont density (cells cm^{-2})	
Skeletal density (g mL^{-1})	
Ash-free dry weight (% organics)	
Total lipids (mg lipid g dry $weight^{-1}$)	
Symbiont type (clade)	
Fatty acids (mg g $lipid^{-1}$)	
Environmental parameters	
Temperature (°C)	
Salinity (ppt)	
Dissolved organic carbon (μM)	
Particulates	
Chlorophyll a (μg L^{-1})	*Particulate organic carbon* (μM)
Turbidity (NTU)	*Particulate nitrogen* (μM)
Total suspended solids (mg L^{-1})	*Particulate phosphorus* (μM)
Dissolved organic nutrients	
Dissolved organic nitrogen (μM)	*Dissolved organic phosphorus* (μM)
Dissolved inorganic nutrients	
Dissolved organic nitrogen (μM)	*Dissolved organic phosphorus* (μM)
Silicate (μM)	
Explanatory factors	
Coral health and biochemical traits (GLMMs)	
Date	
Distance along gradient	
Colony (random)	
Environmental PCA – principal components (GLMs)	
Season	
Region	
Distance along gradient	
Environmental parameters – means (GLMs)	
Season	
Distance along gradient	

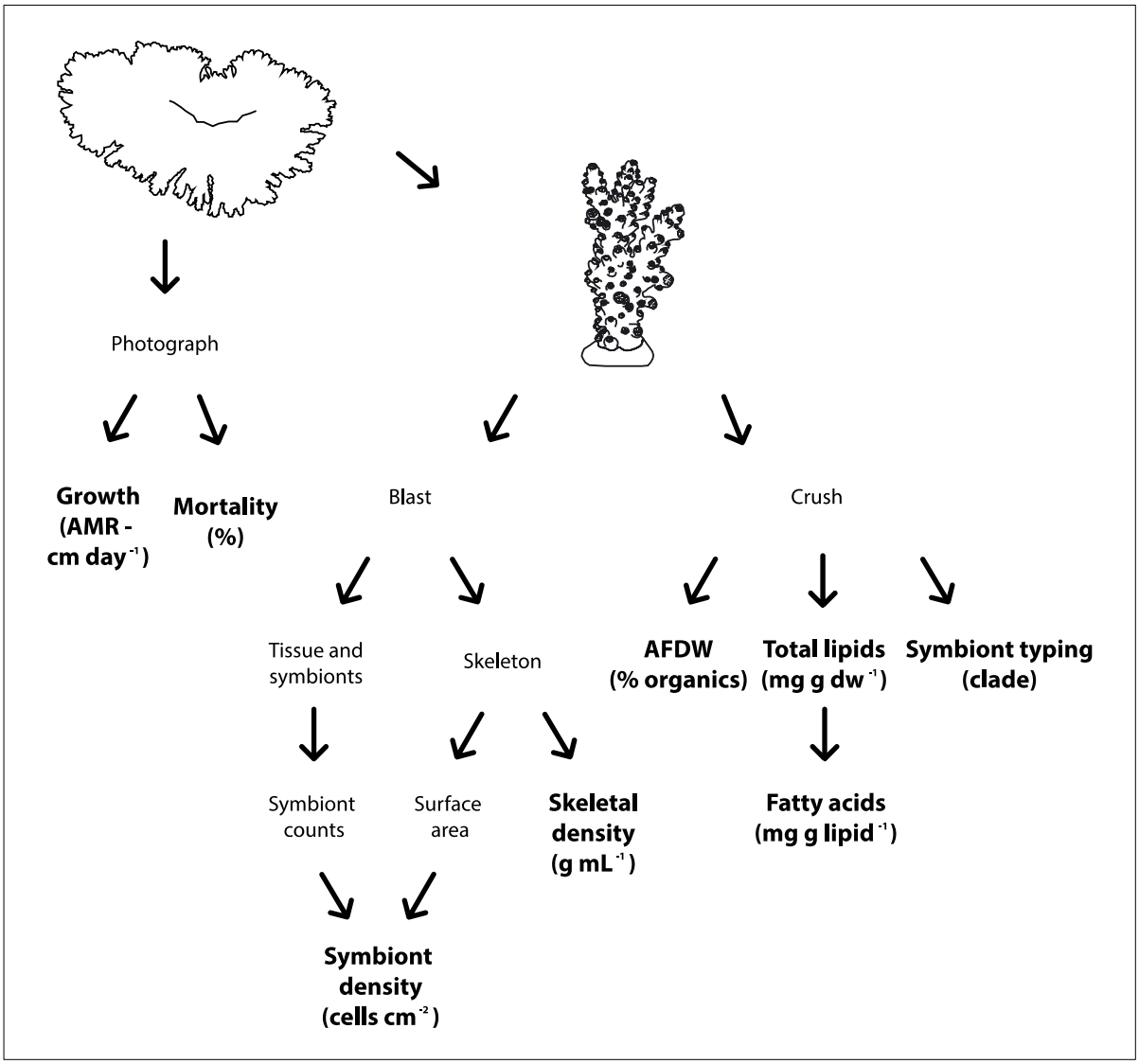

Fig. 2.2 Schematic diagram of coral health attribute data collection and sample processing within this study.

Analyses of coral health and condition

Coral growth and mortality

Growth rates of coral colonies were estimated from the arithmetic mean radius (AMR) following Pratchett et al. (2015). The two-dimensional planar area of each coral colony was determined from the photographs by tracing their outline using the software package 'ImageJ' (version 1.46r, National Institutes of Health, USA) and scaling to the reference bar. Increases in projected AMR of corals (referred to as growth rates in subsequent chapters) were expressed as the change in AMR between consecutive measurements. Partial or full colony mortality was quantified by tracing the outline of the total colony area (combined live and dead), tracing the outline of the dead area (if present) and then subtracting the dead area from the total area for each colony. Once colony mortality reached 100 %, the colony was removed from further analyses. Changes in colony size between monitoring periods were standardised to the number of days between sampling points. Negative relative growth indicates a reduction in overall colony size following partial mortality.

Symbiont and skeletal densities

To quantify *Symbiodinium* density, coral tissue was separated from the skeleton using an airpick and approximately 15.0 mL of filtered seawater and homogenised for 20 seconds. For microscopic analysis of symbiont density, a 0.25 mL subsample was preserved in 1.5 % formalin solution and four replicate counts were conducted using a Neubauer haemocytometer (Fitt et al. 2000). Total symbionts per area were calculated using the average of the symbiont counts, total volume of the coral slurry and surface area of the coral branch. Coral surface area was calculated by the wax dipping method following Stimson and Kinzie (1991). Coral skeletons were weighed and their volumes determined by the water displacement method (Oliver et al. 1983). Skeletal density was obtained by dividing the

weight by volume of each sample.

Total lipid content and ash-free dry weight

Total lipid content was determined following Conlan et al. (2014). Branches were crushed using a pneumatic press and ground to a fine powder with a mortar and pestle on liquid nitrogen. Samples were freeze-dried for 48 hours until completely dry. Frozen dry samples were subsampled for total lipid and (~ 90 % of the coral sample) and ash-free dry weight analyses (~ 10 %).

Ash-free dry weight was determined following Fitt et al. (2000). Freeze-dried aliquots were weighed to the nearest 0.1 mg, placed in quartz cups, and then exposed to 250 °C for one hour, followed by 12 hours at 475 °C. Samples were placed in a desiccator and subsequently weighed to the nearest 0.1 mg. Ash-free dry weight was calculated as the change in weight divided by the total dry weight of each sample after heating and expressed as percent organic content.

Total lipid was extracted from freeze-dried coral samples with three repeat 3.0 mL of dichloromethane:methanol (2:1 DCM) extractions. Cells were disrupted with sonication for 5 minutes, and then allowed to cool for 5 minutes. Samples were filtered into a clean test tube using a solvent-cleaned, cotton-filled glass Pasteur pipette. An additional rinse of 1.0 mL of DCM solvent was washed through the filter. The 10 mL samples were washed with 5.0 mL of 3:1 0.44 % KCl:Methanol, agitated for 10 seconds and left to separate for 12 hours. Samples were centrifuged at 1000 rpm for 5 minutes at room temperature, after which the top layer was removed and discarded. Samples were recovered using a glass 1.0 mL syringe and placed into a clean, pre-weighed test tube. Samples were dried with nitrogen evaporation for approximately 45 minutes. Total lipid extracts were then weighed to the nearest 0.1 mg, extrapolated to the total crushed sample and standardised to dry weight (mg lipid g dw^{-1}).

Symbiont type

The dominant *Symbiodinium* type in each colony was determined in samples collected in June 2013. DNA was extracted from 2 mm^3 frozen subsamples with a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (excluding the phenol step), followed by standard ethanol precipitation and re-suspension in 30.0 µL of 10mM Tris-HCL (pH 8.0).

PCR was used to amplify the large subunit (23S)-rDNA following van Oppen et al. (2001) with forward lsu primer (5'-CCCGCTAATTTAAGCATATAAGTA-3') and reverse lsu primer (5'-GTTAGACTCCTTGGTCGTGTTTCA-3'). Seventy ng of diluted DNA (1:30) was added to 10.0 µL PCR reactions containing 10 µM of each primer, 2 mM dNTPs (Scientifix), 1 X reaction buffer with 20 mM $MgCl_2$ (Scientifix), 0.385 U *Taq* polymerase (5.5 U/µL; Scientifix), and 6.0 µL milliQ H_2O. The PCR profile consisted of 5 minutes at 95 °C, followed by 35 cycles of 20 seconds at 95 °C, 30 seconds at 60 °C, and 90 seconds at 72 °C, followed by a final 10 minutes at 72 °C.

Agarose gel electrophoresis confirmed PCR amplification of a 670 base pair product. Restriction digestion was carried out in 10.0 µL reactions consisting of 5.0 µL of PCR product, 1.5 U *Taq 1* (ThermoScientific) and 10 X buffer Taq1 (ThermoScientific). The restriction digestions were incubated for 2 hours at 65 °C, then 3.0 µL was loaded on a 2 % high resolution agarose gel with a 1.0 X TAE buffer and electrophoretically separated for 60 minutes at 100 V. *Symbiodinium* type in each sample was determined by comparison to known reference samples of *Symbiodinium* clades C1, C2 and D.

Quantification of water quality parameters

Environmental data from February 2008 to June 2014 was provided by the MMP (see Schaffelke et al. (2012) and Thompson et al. (2014a) for details of sampling and analyses).

Sea temperature, seawater chlorophyll fluorescence and turbidity were continuously monitored every 10 minutes *in situ* at 2 m depth below datum (reef crest) at all six sites using Sensus Ultra Temperature loggers and WETLabs Eco FLNTUSB Combination Fluorometer and Turbidity Sensors. Discrete water samples were also collected using Niskin bottles at 2 m below datum every four months (February, June, and October). Water samples were analysed for dissolved inorganic nutrients, dissolved organic nutrients, particulates, and salinity at the AIMS Analytical Services Laboratory. (See Appendix A Suppl. Fig. 2.1 and Suppl. Fig. 2.2 for individual water quality parameters.)

Statistical analyses

All analyses were performed using the statistical package R (Version 2.15.3, R Core Team 2015). Spatial and temporal patterns in environmental parameters at the six study sites were characterised with principal component analyses (PCA) using the R package 'vegan' (Oksanen et al. 2015). When all 14 water quality parameters were analysed together in one PCA, three groupings of similar environmental variables were detected based on the direction and magnitude of eigenvectors (Appendix A Suppl. Fig. 2.3). To develop ecologically relevant composite water quality metrics and reduce the number of parameters in subsequent analyses, separate PCAs were performed on the three groups of similar chemical or physical environmental parameters acting in with same direction within the PCA (Legendre and Gallagher 2001). Scores along the first axis of each of the three PCAs were used to derive the following three composite variables (following methods of Thompson et al. (2013)): 1) particulates (PART - chlorophyll, turbidity, total suspended solids, particulate organic carbon, particulate nitrogen, and particulate phosphorus), 2) dissolved organic nutrients (DO - dissolved organic nitrogen and dissolved organic phosphorus), and 3) dissolved inorganic nutrients (DI - silicate, dissolved inorganic nitrogen, and dissolved inorganic phosphorus).

The remaining environmental variables, temperature (TEMP), salinity (SAL), and dissolved organic carbon (DOC) were considered individually in subsequent analyses. Seasonal averages were calculated for February, June and October across the six years of data (February 2008 until June 2014) for each of the single (i.e. TEMP, SAL, DOC) and composite (i.e. PART, DI, DO) environmental parameters. PCAs were used to characterise how environmental conditions varied among sites and seasons.

Generalised linear models (GLMs) were performed on principal components considered important, i.e. when standard deviations were greater than 1.0, for quantitative analyses of these data. Standard deviations greater than 1.0 establish a principal component that explains more of the total variance than would be expected by chance (Al-Kandari and Jolliffe 2005). The three explanatory factors in the model – season, region and distance along environmental gradient (nested within region) – were all treated as fixed factors. GLMs were also used to test for differences in means of the individual water quality parameters (three single and three composite means) among seasons and distances along environmental gradient (nested within each region). All model fits were assessed through examination of residual plots.

To test for differences in means of the five coral health attributes split by region in response to the two explanatory factors (date and distance along environmental gradient), generalised linear mixed models (GLMMs) were used within the packages 'lme4' (Bates et al. 2015) and 'afex' (Singmann et al. 2016) with a Gaussian error distribution. Colony (or genotype) was included as a random factor in the model to account for non-independence of sampling. For symbiont density, a log-link function was included in order to meet model assumptions. Within the Whitsunday region, GLMMs were only performed on data from dates when all three sites were sampled.

Spearman's rank correlation analysis was used to test for associations between each pair of the five coral health and biochemical attributes. Only the variable symbiont density did not meet assumptions of normality and homoscedasticity of variance and was therefore log10-transformed. Differences in the relative frequency of symbiont types among sites within regions were tested with a χ^2-test.

Generalised additive mixed effects models (GAMMs) were used to partition effects of water quality parameters (TEMP, SAL, PART, DI, DO and DOC) on coral health and biochemical attributes. Covariates included region and distance of sites along environmental gradient (within region). Colony (or genotype) was included as a random factor to account for repeated sampling and maintain assumptions of independence. Coral health and biochemical attributes were matched with environmental water quality variables from 3 months prior to the sampling date to account for the likelihood that prior environmental conditions influenced coral responses. As residuals of data were not linear, GAMMs were fitted using the 'mgcv' package following Fabricius et al. (2013) and model selection was based on minimisation of the Akaike information criterion (AICc). The model including all environmental parameters and top two models based on AICc values were presented for comparison, including changes in AICc with respect to top-ranked model (ΔAICc).

2.4 Results

Environmental condition of inshore sites

In combination, the three single and three composite water quality variables described inshore environments in the Burdekin and Whitsunday regions, with two important principal component axes explaining 84.2 % of the variation in the data (Fig. 2.2; Table 2.1). All sites grouped together in June due to consistently high values of dissolved organic nutrients (DO), low temperatures (TEMP) and low values of the composite water quality parameter for

particulates (PART). Sites also grouped together in October due to consistently high salinity (SAL) and low values of both dissolved inorganic nutrients (DI) and dissolved organic carbon (DOC). The six sites were best differentiated in February (the wet season). Specifically, high values of DOC and DI, combined with low SAL, separated sites within the Burdekin region and ordered them according to decreasing distance from the river and coast. In contrast, high values of TEMP and PART and low values of DO described the Whitsunday sites, although the separation of sites was not as strong as the Burdekin sites. GLM on the principal component scores of the first axis revealed that water quality parameters varied significantly with increasing distance from along environmental gradient and among seasons (Table 2.1).

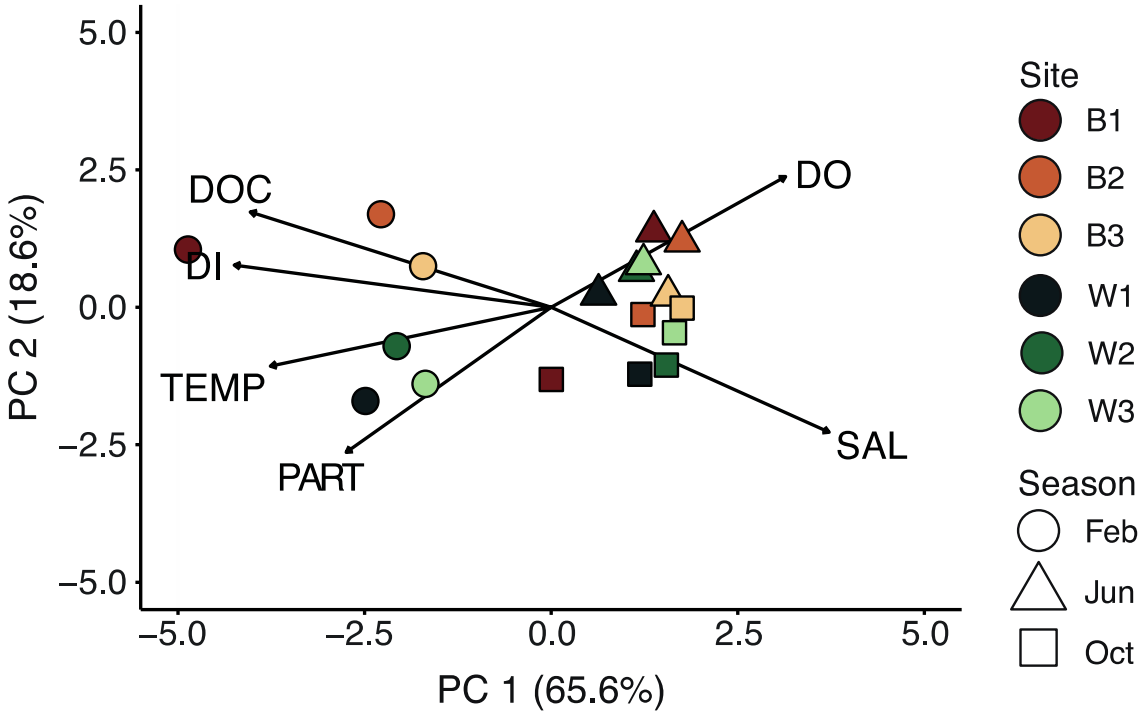

Fig. 2.3 Principal component analysis biplot of water quality variables measured at six inshore sites from the Burdekin and the Whitsunday regions. Data were averaged for each season (February, June and October) and across six years (February 2008 until June 2014). Correlated and clustered variables were analysed in individual PCAs to define three composite water quality parameters based on scores along the first PCA axis of the respective analysis. Composite environmental parameters are particulates (PART), dissolved organic nutrients (DO) and dissolved inorganic nutrients (DI). Environmental parameters considered individually are temperature (TEMP), salinity (SAL) and dissolved organic carbon (DOC).

Table 2.2 Standard deviation and proportion of variance explained by each axis in a principal component analysis (PCA) of water quality parameters defining three sites within each of the Burdekin and Whitsunday regions. Effects of seasons, regions and distance within regions on important principal components (standard deviation > 1.0) of generalised linear model outputs (GLM) using a Gaussian error distribution. *** denotes significance at α = 0.001. ** denotes significance at α = 0.01. * denotes significance at α = 0.05.

Importance of components						
	PC1	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*
Standard deviation	1.98*	1.06*	0.761	0.518	0.263	0.187
Proportion of variance	0.656	0.186	0.097	0.045	0.012	0.006
Cumulative proportion	0.635	0.842	0.938	0.983	0.994	1.000
Principal component 1						
	df	Deviance	F value	P		
Season	2	56.99	106.9	<0.001	***	
Region	1	0.302	1.132	0.312		
Season* Region	2	1.639	3.074	0.091		
Region(Distance)	2	5.313	9.964	0.004	**	
Principal component 2						
	df	Deviance	F value	P		
Season	2	6.303	12.36	0.004	**	
Region	1	5.206	20.41	0.002	**	
Season* Region	2	4.136	8.110	0.012	*	
Region(Distance)	2	1.237	1.213	0.377		

Temperatures were warmest in February and coolest in June in both regions, and slightly cooler in the southern Whitsunday region compared with the Burdekin region (Fig. 2.3a, g). Salinity did not differ among sites within regions but was significantly lower in February than in June and October in both regions (more so in the Burdekin than the Whitsunday; Fig. 2.3b, h; Table 2.2). Values of PART consistently decreased with increasing distance from the coast and river, (Fig. 2.3c, i; Table 2.2). At B1, PART values were significantly higher at the site closest to the coast and river source (B1) in February and October than in June (Fig. 2.3c; Table 2.2). At all other sites, PART values were more similar through time, with a trend towards lower PART values in the dry season (Fig. 2.3i). DI differed by distance within the Burdekin region (Fig. 2.3d; Table 2.2), with highest values in February and at the site closest to the coast and river source (B1). This was not the case in the Whitsunday region (Fig. 2.3j), although there was a decline from February to October. DO values did not differ significantly with increasing distance from river source within regions (Table 2.2); however, lowest levels were found in February in the Whitsunday region (Fig. 2.3e, k). Patterns in DOC varied seasonally within both regions (Table 2.2), with a peak in February (Fig. 2.3f, l).

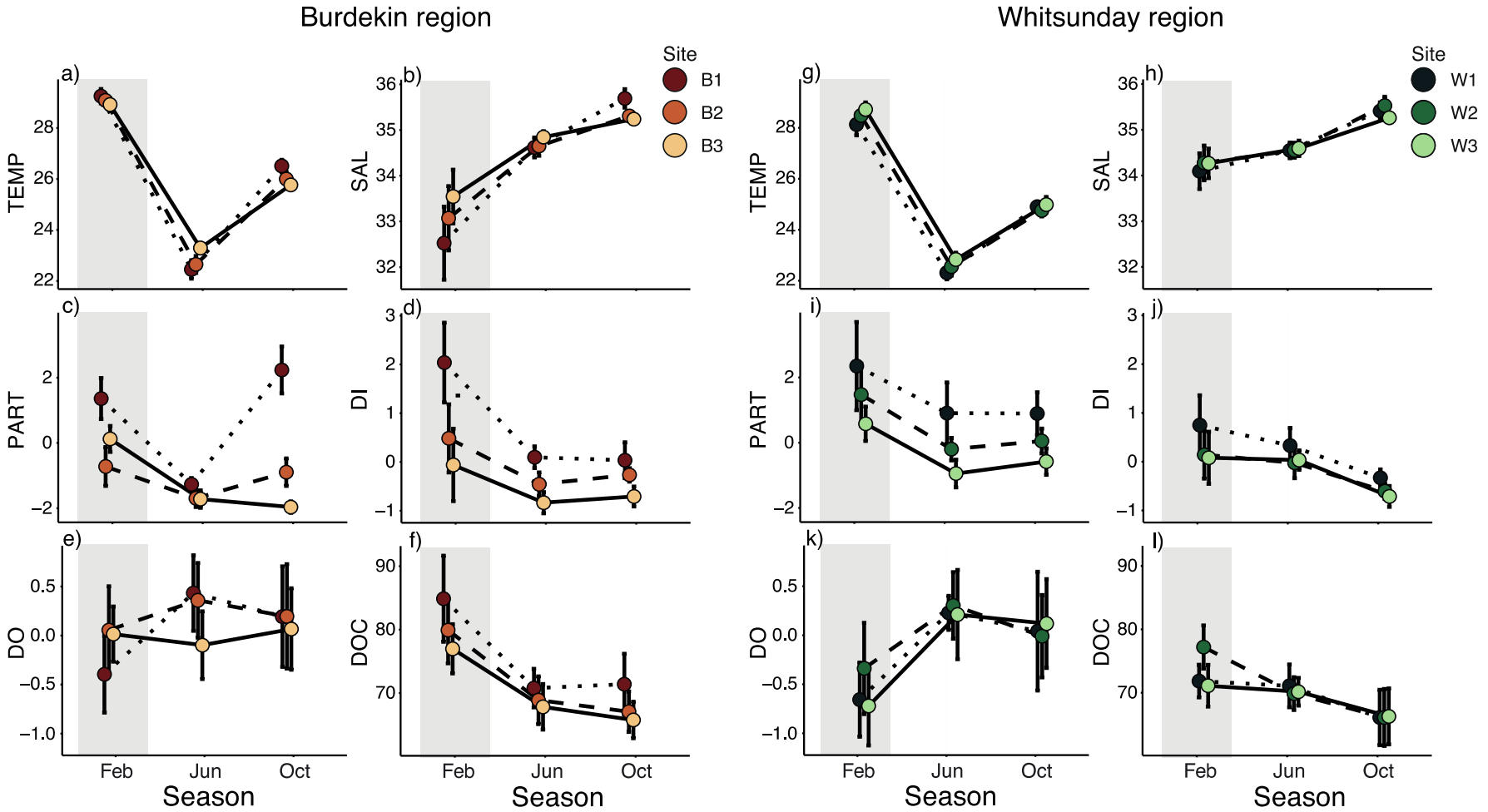

Fig. 2.4 Variations in environmental water quality parameters at six inshore sites (Burdekin region - left; Whitsunday region - right). Data points are means ± SE of samples collected in February, June, and October from 2008 until 2014. Environmental water quality parameters include: (a, g) Temperature (°C; TEMP); (b, h) Salinity (ppt; SAL); (c, i) Particulates (first PCA axis units; PART); (d, j) Dissolved Inorganic nutrients (first PCA axis units; DI); (e, k) Dissolved Organic nutrients (first PCA axis units; DO); (f, l) Dissolved Organic Carbon (µM; DOC). Wet season is indicated by light grey panels.

Table 2.3 Variation in the environmental parameters at the Burdekin and Whitsunday sites in response to seasons, regions and distance from rivers within the regions. Parameter estimates based on generalised linear models (GLM, with Gaussian error distribution) of single and composite variables. *** denotes significance at α = 0.001. ** denotes significance at α = 0.01. * denotes significance at α = 0.05.

Temperature					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	29.16	0.276	105.5	<0.001	***
Date (Jun)	-6.288	0.226	-27.86	<0.001	***
Date (Oct)	-2.993	0.226	-13.26	<0.001	***
Region (Whitsunday)	-1.116	0.391	-2.854	0.005	***
Date (Jun)	0.407	0.319	1.275	0.205	
Date (Oct)	-0.569	0.319	-1.782	0.077	
Distance (Burdekin)	-0.038	0.113	-0.340	0.735	
Distance (Whitsunday)	0.199	0.113	1.762	0.081	
Salinity					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	32.78	0.343	95.70	<0.001	***
Date (Jun)	1.656	0.280	5.921	<0.001	***
Date (Oct)	2.364	0.280	8.453	<0.001	***
Region (Whitsunday)	1.406	0.484	2.901	0.004	***
Date (Jun)	-1.302	0.396	-3.291	0.001	***
Date (Oct)	-1.175	0.396	-2.971	0.003	***
Distance (Burdekin)	0.132	0.140	0.945	0.347	
Distance (Whitsunday)	0.011	0.140	0.080	0.936	
Particulates					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	-2.219	0.629	-3.530	<0.001	***
Date (Jun)	1.815	0.513	3.536	<0.001	***
Date (Oct)	0.462	0.513	0.901	0.370	
Region (Whitsunday)	-0.948	0.889	-1.066	0.289	
Date (Jun)	-0.269	0.726	-0.370	0.712	
Date (Oct)	0.883	0.726	1.216	0.226	
Distance (Burdekin)	0.982	0.257	3.827	<0.001	***
Distance (Whitsunday)	0.849	0.257	3.308	0.001	***
Dissolved Inorganic Nutrients					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	2.079	0.421	4.941	<0.001	***
Date (Jun)	-1.219	0.344	-3.549	<0.001	***
Date (Oct)	-1.133	0.344	-3.300	0.001	***
Region (Whitsunday)	-1.305	0.595	-2.194	0.030	*
Date (Jun)	1.009	0.486	2.076	0.040	*
Date (Oct)	0.260	0.486	0.534	0.594	
Distance (Burdekin)	-0.629	0.172	-3.663	<0.001	***
Distance (Whitsunday)	-0.224	0.172	-1.307	0.194	
Dissolved Organic Nutrients					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	-0.026	0.407	-0.063	0.950	
Date (Jun)	0.340	0.332	1.022	0.309	
Date (Oct)	0.260	0.332	0.781	0.436	
Region (Whitsunday)	-0.544	0.575	-0.946	0.346	

Date (Jun)	0.480	0.470	1.022	0.309	
Date (Oct)	0.363	0.470	0.772	0.442	
Distance (Burdekin)	-0.041	0.166	-0.249	0.803	
Distance (Whitsunday)	0.000	0.166	-0.004	0.996	
Dissolved Organic Carbon					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	86.08	3.793	22.697	<0.001	***
Date (Jun)	-11.43	3.097	-3.690	<0.001	***
Date (Oct)	-12.52	3.097	-4.044	<0.001	***
Region (Whitsunday)	-12.20	5.364	-2.274	0.025	*
Date (Jun)	8.417	4.379	1.922	0.057	
Date (Oct)	5.295	4.379	1.209	0.229	
Distance (Burdekin)	-2.749	1.548	-1.775	0.078	
Distance (Whitsunday)	-0.247	1.548	-0.159	0.874	

Variation in coral condition indicators along inshore environmental gradients

Dominant symbiont type differed significantly among sites within the Burdekin region ($\chi^2 = 37.3$, $p < 0.001$), but not within the Whitsunday region ($\chi^2 = 4.22$, $p = 0.377$; Fig. 2.4). In both regions, symbiont populations tended to be C1-dominant at the closest inshore site and C2-dominant at the site furthest from the coast and the river source, with a more pronounced trend for Burdekin sites. Only one individual at the mid-gradient Burdekin site (B2) had mixed type D and C1 symbionts.

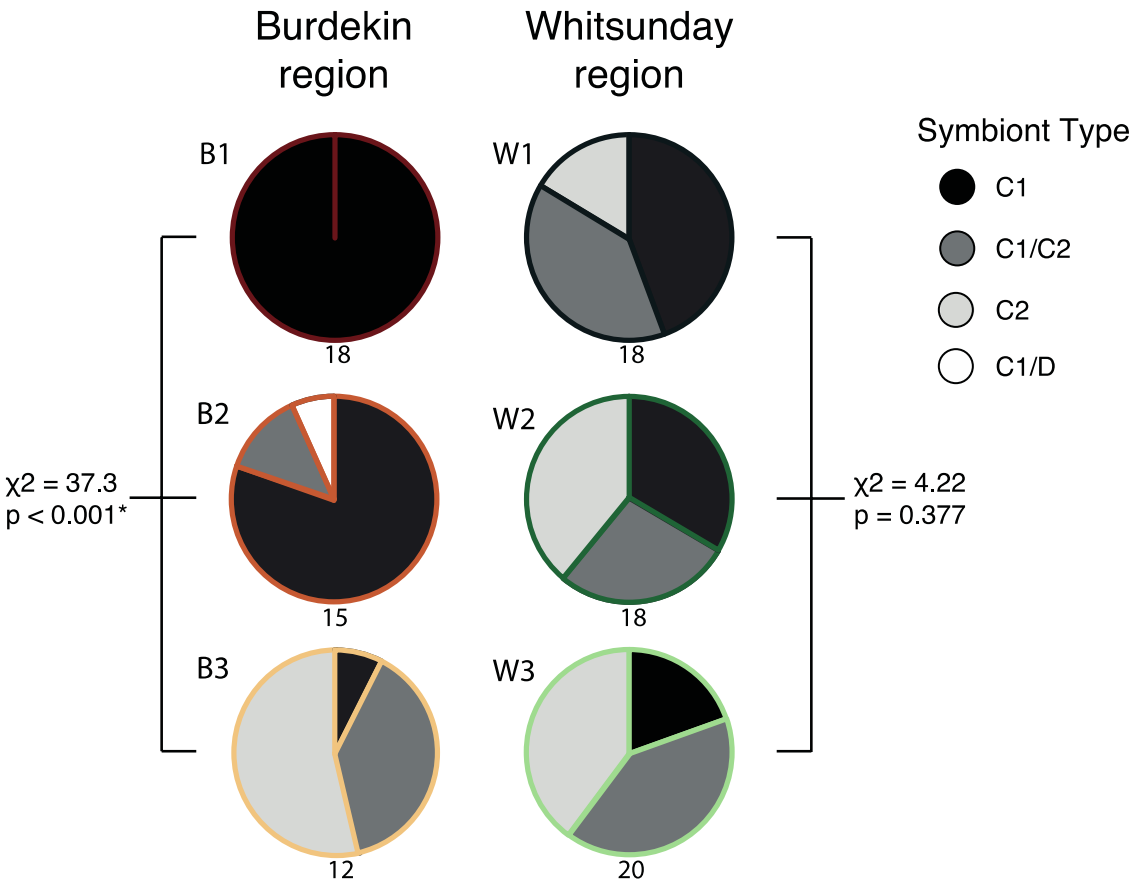

Fig. 2.5 Symbiont associations of *Acropora tenuis* colonies found within the Burdekin and Whitsunday regions. Pie charts represent the proportion of adult colonies dominated by different *Symbiodinium* types. Numbers below charts represent the sample size (n) at each site.

Survivorship of corals monitored was 100 % in the Burdekin region, and no partial mortality was recorded. In contrast, in the Whitsunday region, although no partial mortality was recorded, whole coral colony mortality was recorded for six colonies at W2 between February 2013 and June 2013, and one colony at W1 between October 2013 and February 2014.

In the Burdekin region, corals at the B1 and B2 sites (0.0173 ± 0.0010 cm day^{-1} and 0.0176 ± 0.0011 cm day^{-1}, respectively) had approximately 2 times higher growth rates than at the more offshore B3 site (0.0081 ± 0.0009 cm day^{-1}) from February 2013 to June 2014 (Fig. 2.5a; Table 2.3). In the Whitsunday region, growth rates were consistently lowest at the middle site (W2) across all dates (Fig. 2.5f; Table 2.3). W3 generally had the highest growth rates, however, W1 had similar growth rates to W3 in February 2014.

Within the Burdekin region, corals at the nearshore B1 site had 3 times higher mean symbiont density (Fig. 2.5b) and 25 % lower mean skeletal density compared to those at the more distant B3 site (Fig. 2.5c; Table 2.3). In contrast, in the Whitsunday region, patterns in symbiont density with distance from the river source differed depending on the date (Table 2.3), with highest (13.3 ± 1.33 x 10^5 cells cm^{-2}) and lowest (4.0 ± 0.80 x 10^5 cells cm^{-2}) symbiont densities associated with W2 corals in June 2013 and February 2014, respectively (Fig. 2.5g). Skeletal density within the Whitsunday region was not affected by distance from river source (Fig. 2.5h).

Patterns in the two indicators of energy stores, ash-free dry weight and lipid content, differed significantly across the Burdekin region sites, but did not differ across the Whitsunday region sites (Fig. 2.5; Table 2.3). Corals at the nearshore B1 site had the highest ash-free dry weight across the study, with a maximum in June 2013 (8.4 ± 0.39 %; Fig. 2.5d); B1 corals also consistently had the highest lipid content (13.6 ± 1.80 mg lipid g dw^{-1}; Fig. 2.5e). The more distant B3 corals had the lowest values of all sites, and values were

comparatively stable (ash-free dry weight: 5.6 ± 0.31 %; lipid content: 7.13 ± 1.13 mg lipid g dw^{-1}). In the Whitsunday region, neither ash-free dry weight nor lipid content differed significantly with distance from river source, but lipid content decreased through time (Fig. 2.5i, j; Table 2.3).

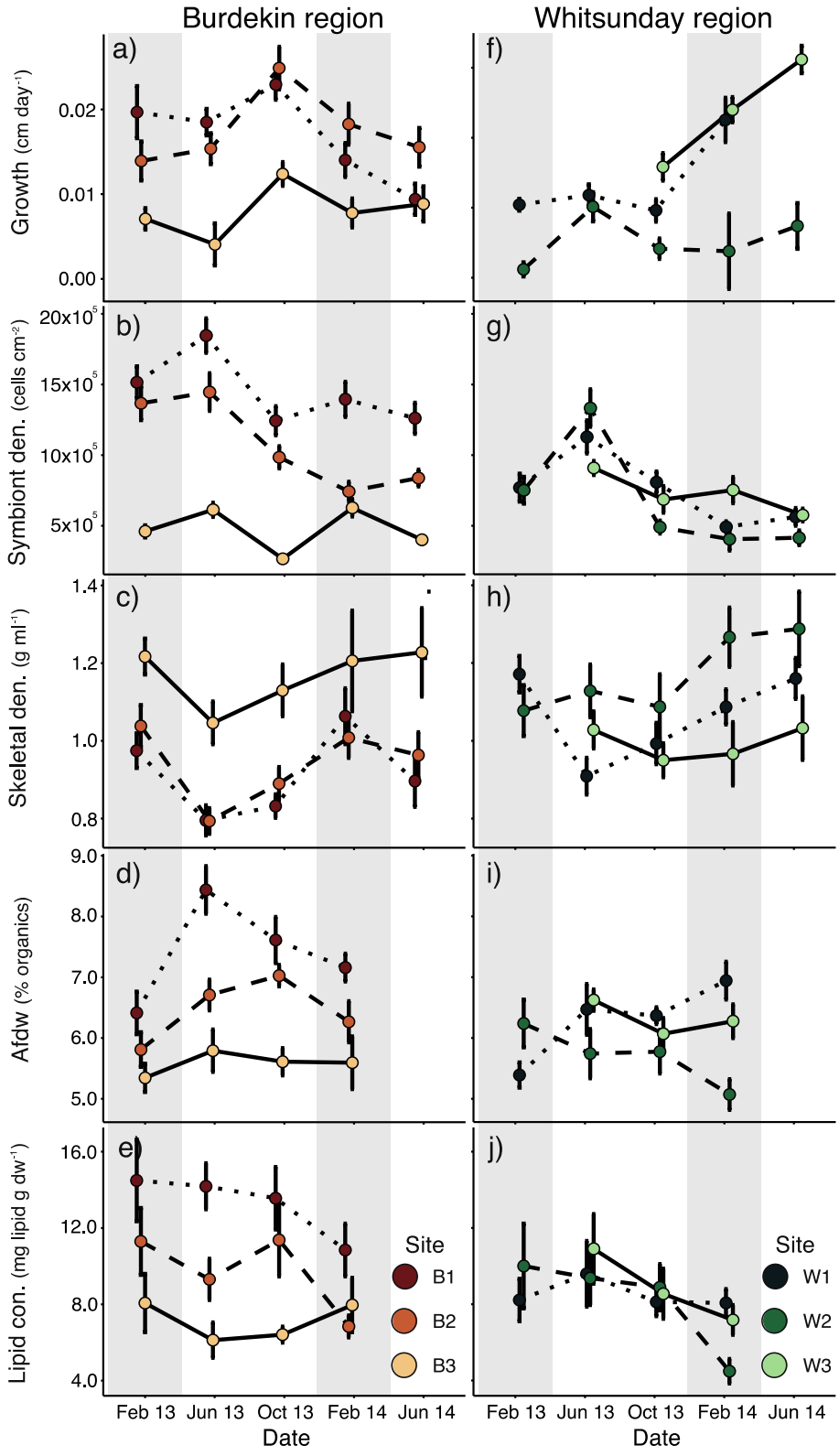

Fig. 2.6 Coral condition and health attributes among sites and sampling dates separated by region (Burdekin - orange; Whitsunday - green). Condition and health attributes were: (a, f) proportional growth (cm day^{-1}); (b, g) symbiont density (cells cm^{-2}) and (c, h) skeletal density (g mL^{-1}) from February 2013 to June 2014 and (d, i) ash-free dry weight (percent organics) and (e, j) total lipid content (mg lipid g dw^{-1}) from February 2013 to February 2014. Wet seasons are indicated by light grey panels. Circles indicate site means ± SE (n = ~ 15).

Table 2.4 Coral health and biochemical attributes in response to dates, regions and distance from river in the Burdekin and Whitsunday regions using generalised linear mixed models (GLMMs) with Gaussian error distributions. For symbiont density, a log-link function was used. *** denotes significance at α = 0.001. ** denotes significance at α = 0.01. * denotes significance at α = 0.05.

	Burdekin Region				Whitsunday Region			
Growth								
	df	χ^2 value	P		*df*	χ^2 value	P	
Date	4	18.913	<0.001	***	2	12.731	0.002	**
Distance	1	11.678	<0.001	***	1	11.130	<0.001	***
Date*Distance	4	15.700	0.003	**	2	12.775	0.002	**
Symbiont density								
	df	χ^2 value	P		*df*	χ^2 value	P	
Date	4	1.8305	0.767		3	26.489	<0.001	***
Distance	1	1271.8	<0.001	***	1	0.3548	0.551	
Date*Distance	4	1.4975	0.827		3	11.347	<0.010	**
Skeletal density								
	df	χ^2 value	P		*df*	χ^2 value	P	
Date	4	10.503	0.032	*	3	9.0878	0.028	*
Distance	1	12.169	<0.001	***	1	1.4037	0.236	
Date*Distance	4	4.7509	0.314		3	6.1131	0.106	
Ash-free dry weight								
	df	χ^2 value	P		*df*	χ^2 value	P	
Date	3	20.344	<0.001	***	2	1.3012	0.522	
Distance	1	23.899	<0.001	***	1	0.3005	0.584	
Date*Distance	3	11.499	0.009	**	2	1.8842	0.390	
Lipid Content								
	df	χ^2 value	P		*df*	χ^2 value	P	
Date	3	3.0891	0.378		2	0.0049	0.998	
Distance	1	17.307	<0.001	***	1	0.1702	0.680	
Date*Distance	3	1.6728	0.643		2	0.6659	0.717	

Both positive and negative correlations were detected between pairs of coral attributes for measurements combined across the six sites and all observation times (Fig. 2.6). Growth rate positively correlated with symbiont density, ash-free dry weight and lipid content, but negatively correlated with skeletal density. A negative correlation was also found between skeletal density and the other attributes (symbiont density, ash-free dry weight and lipid content). Symbiont density was positively correlated with ash-free dry weight and lipid content. Ash-free dry weight and total lipid content were also positively correlated (Fig. 2.6).

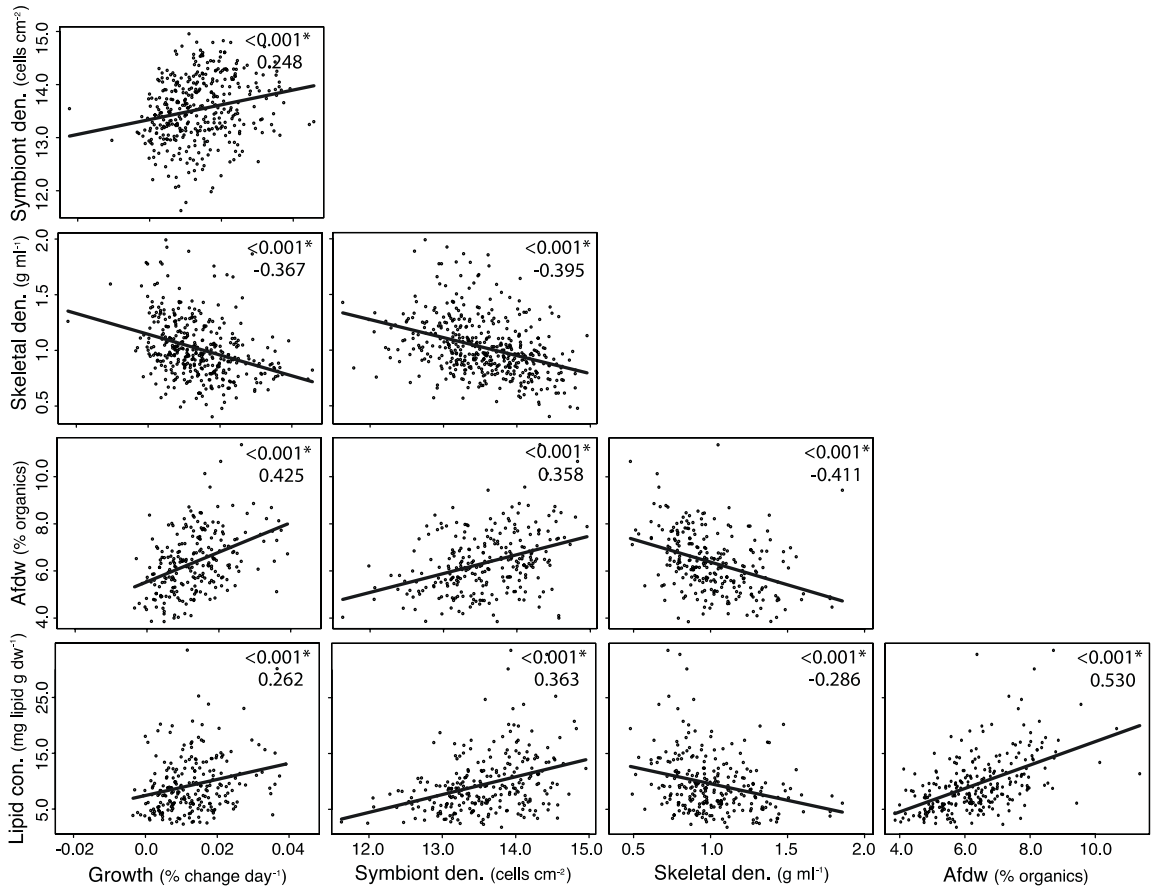

Fig. 2.7 Correlations between the different coral condition and health attributes from the six study sites and all observation times. Symbiont densities are log-10 transformed. Black lines indicate the slopes of the relationships. Significances and rho are shown in the top right corners of each plot. * denotes significance at $\alpha = 0.05$.

Environmental drivers of coral health and biochemical attributes

No single environmental parameter was associated with all of the measured coral health and biochemical attributes (Table 2.4; Fig. 2.7). Symbiont density and ash-free dry weight were best explained by the environmental parameters (R^2 = 0.458 and 0.312, respectively). Coral growth was weakly related to the environmental variables measured (R^2 = 0.196), initially increasing with PART, but declining at very high levels of PART and SAL, and with increasing DI. Symbiont density increased with increases in TEMP and DOC but declined with PART. Skeletal density increased with increasing SAL. Ash-free dry weight was highest at the lowest and highest levels of TEMP and decreased with increasing DOC, but plateaued at and above medium levels of DOC. Increases in total lipid content were associated with an increasing trend in DI.

Table 2.5 Optimal and all-inclusive generalised additive mixed models (GAMMs) for predicting coral health and biochemical condition attributes within the Burdekin and Whitsunday regions. Models presented are those with the lowest values of the Akaike information criterion (AICc) from GAMMs that evaluated the influence of environmental variables. Environmental variables are listed in Fig. 2.2. Covariates within GAMMs include: region and distance of sites along environmental gradient nested within region. Top-ranked model is in bold. * denotes significance in top model at $\alpha = 0.05$ (see Fig. 2.7).

Growth					
	df	logLik	AICc	ΔAICc	wAICc
SAL* + PART* + DI*	12	8.5910	7.6	0.00	0.336
SAL + PART + DI + DO	14	10.044	9.0	1.38	0.168
TEMP + SAL + PART + DI + DO + DOC	18	10.346	17.1	9.50	0.003
Symbiont density					
	df	logLik	AICc	ΔAICc	wAICc
TEMP* + PART* + DOC*	12	-255.51	537.7	0.00	0.462
TEMP + PART + DOC + DI	14	-254.07	537.1	1.39	0.230
TEMP + SAL + PART + DI + DO + DOC	18	-253.70	545.0	9.28	0.004
Skeletal density					
	df	logLik	AICc	ΔAICc	wAICc
SAL* + DOC	10	13.928	-7.4	0.00	0.162
SAL	8	11.554	-6.8	0.57	0.122
TEMP + SAL + PART + DI + DO + DOC	18	16.570	4.5	11.81	0.000
Ash-free dry weight					
	df	logLik	AICc	ΔAICc	wAICc
TEMP* + DO + DOC*	12	839.58	-1653.9	0.00	0.194
TEMP + DOC	10	837.30	-1653.7	0.21	0.175
TEMP + SAL + PART + DI + DO + DOC	18	841.50	-1644.2	9.70	0.002
Lipid content					
	df	logLik	AICc	ΔAICc	wAICc
DI	8	1017.9	-2019.1	0.00	0.251
null	6	1015.3	-2018.3	0.83	0.166
TEMP + SAL + PART + DI + DO + DOC	18	1019.5	-2000.1	19.04	0.000

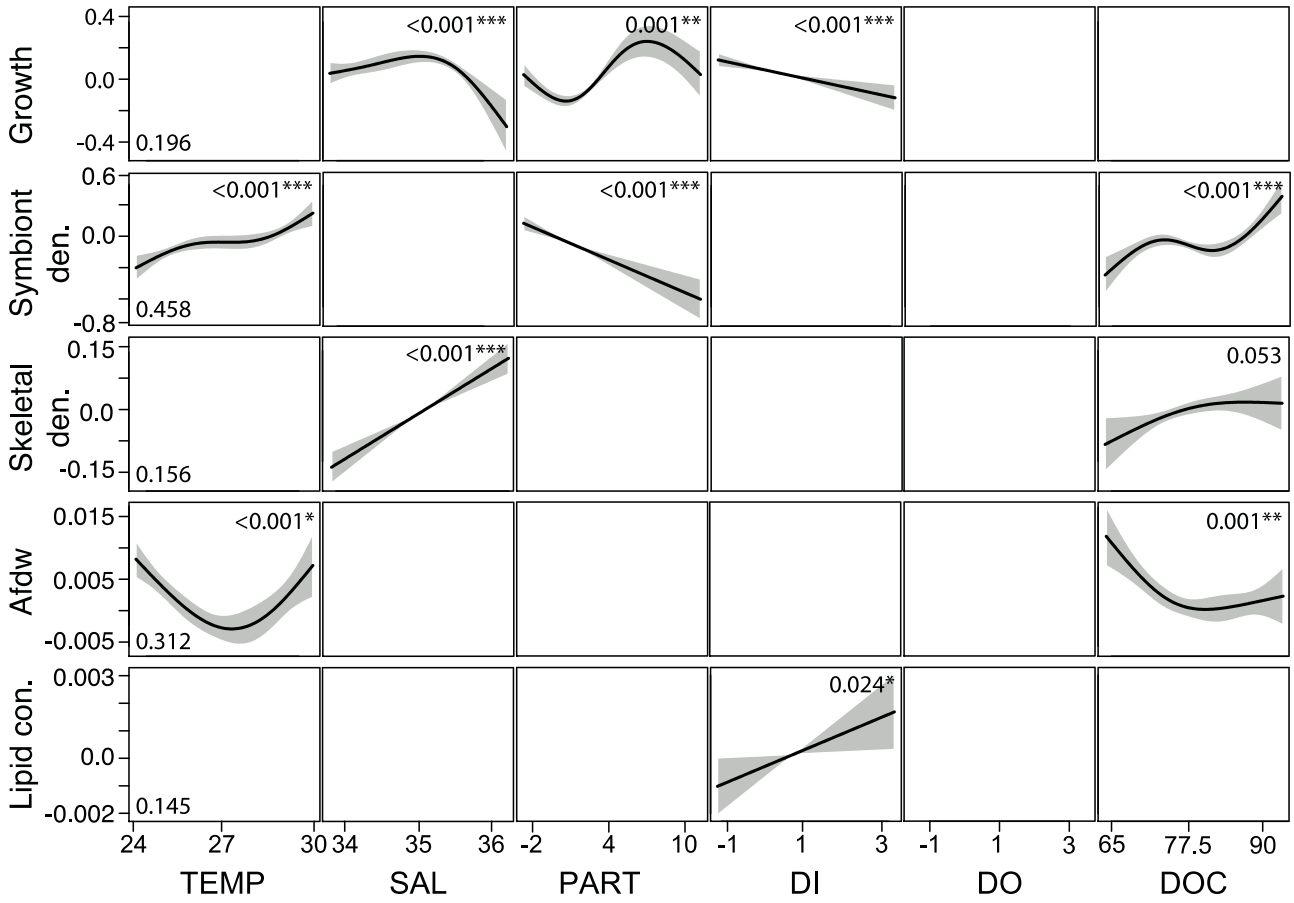

Fig. 2.8 Changes in mean coral health and condition atrributes as a function of environmental variables in the Burdekin and Whitsunday regions. Plots are partial effect plots from the optimal GAMM for each attribute (Table 2.4). Fitted black lines represent the mean coral health attribute estimates and grey regions represent 95 % confidence intervals. The environmental variables are listed in Fig. 2.2. Covariates within GAMMs include: region and site within region. Also shown is the proportion of variation accounted for by the models (R^2, bottom left of first plot of each model) and the P-value for the effect of these water quality variables (top right).

2.5 Discussion

This study documents temporal and spatial variation in coral health and biochemical attributes of the ubiquitous coral *Acropora tenuis* in shallow water along two inshore water quality gradients within the GBR. High levels of variation in coral health and biochemical attributes were detected along the relatively stronger water quality gradient in the Burdekin region. Corals had the highest symbiont density and highest lipid content at the site closest to the coast and river source, where water quality was defined as moderate (Thompson et al. 2014a). At the site farthest along this environmental gradient, corals grew slower, had more dense skeletons, lower symbiont density and lower lipid content. These correlated health and biochemical attributes indicate that, at least in shallow water, this species of coral has the capacity to invest disparately in physiological and biochemical attributes necessary for survival in contrasting environmental conditions, either as a consequence of acclimatisation or local adaptation.

Interactions of coral health and biochemical attributes

Skeletal extension (lengthening of skeletal elements) and skeletal density (a measure of infilling of the skeleton) both result from newly synthesised calcium carbonate through light-enhanced and dark calcification (Vago et al. 1997; Abramovitch-Gottlib et al. 2005; Jokiel 2011). Dark calcification results in randomly-oriented granular crystals that are fragile extensions of the skeletal framework, while light-enhanced calcification fills in this framework (i.e. Gladfelter, 1983, 1982). The less dense skeletons of *A. tenuis* from the nearshore and mid-Burdekin sites (B1 and B2, respectively) could suggest that the balance between dark calcification (extension) and light-enhanced calcification (infilling) is skewed towards dark calcification at these sites. Structural integrity of coral skeletons could become compromised if extension rates are greater than infilling rates over extended periods of time,

leading to weakening of the structural framework (Madin et al. 2012).

Skeletal density has been proposed to be a 'supertrait' defined by Madin et al. (2016) as an easy to measure trait, which is correlated with both biological and ecological processes (i.e. growth rate, physical damage susceptibility, predation) of the coral. The significant negative relationship found between growth (change in AMR; cm day^{-1}) and skeletal density (g mL^{-1}) for corals within the inshore Burdekin and Whitsunday regions had a slope of -0.012. This is similar to the slope of -0.015 found for a relationship between growth rate (mm $year^{-1}$) and estimated CMA (colony mass per area; g cm^{-2}) for a range of colony growth forms, including branching and corymbose corals like *A. tenuis* (Madin et al. 2016) suggesting the consistency of this relationship across and within coral species. Variation in skeletal density within a single species was high (*A. tenuis*) across environmental conditions (R^2 = 0.108). This may restrict the use of a single, general relationship between skeletal density and growth across species and growth forms set forth by Madin et al. (2016), as well as the ability to confidently interpret growth rates from skeletal density. Skeletal density of coral colonies has the potential to be a useful indicator of coral health and condition that can be used in monitoring but requires measurement within populations as it can vary greatly within species, however environmental conditions should be assessed and taken into account when interpreting growth rate of individual coral species and populations within those species.

Decreased linear extension and skeletal density were associated with low salinity levels at the beginning of the dry season. A potential role for salinity in governing these aspects of coral growth is supported by findings of other studies, which reported high extension rates paired with low skeletal density of multiple *Porites* species and *Orbicella faveolata* at more inshore sites (Lough and Barnes 1992; Manzello et al. 2015, respectively), as well as high overall reef growth when exposed to high turbidity and terrigenous sediment

loads (Perry et al. 2012). Suspended particulate matter and light influence growth and photo-physiology in foliose corals (Browne et al. 2015). However, skeletal growth rate was found to decrease when *Pocillopora damicornis* was exposed to high sediment loads and reduced water quality (Fabricius et al. 2012), or be relatively insensitive to high sediment loads in *Porites cylindrica* (Anthony and Fabricius 2000). Highly influential environmental parameters not examined within this study that may affect skeletal extension and density are water motion and wave energy. These environmental parameters directly affect coral health traits (van Woesik et al. 1999; Sofonia 2006; Hoogenboom et al. 2011; Browne 2012), and interact with other environmental conditions, such as sedimentation that increases turbidity and suspended particulates (van Woesik et al. 1999; Orpin and Ridd 2012; Fabricius et al. 2013b). The negative correlations found between skeletal density and skeletal extension (measured as increase in projected planar surface area of corals in this study) and between skeletal density and symbiont density, highlight potential trade-offs between investment in maintenance versus growth by corals that enable them to survive across water quality gradients in coastal environments. These results suggest that changes in coral cover driven by rapid skeletal extension rates can potentially increase the vulnerability of populations to physical damage. Inshore sites are found to be more susceptible to less intense storms and cyclones, with > 80 % of branching and foliose corals being broken and inshore coral cover decreasing from 40 % to 8 % compared to offshore coral cover decreasing from 42 % to 20 % (Fabricius et al. 2008). Although more rapidly extending corals with less dense skeletons may quickly return a reef to high coral cover, these corals maybe more susceptible to physical disturbances, such as storms and cyclones. Therefore, if increased skeletal extension rates are correlated with decreased skeletal density, fast growth does not necessarily indicate good coral colony health; instead, rapid growth could indicate a high risk of damage following physical disturbances (i.e. storm damage).

Highest concentrations of dissolved inorganic nutrients had a significant negative effect on extension rates of *A. tenuis* on these inshore reefs. This provides further support that dissolved inorganic nutrients, such as dissolved inorganic nitrogen and dissolved inorganic phosphate, decrease calcification (and hence extension; Koop et al., 2001; Stambler et al., 1991). Phosphates are considered a crystalline poison (Simkiss 1964) and may lead to reduced extension and/or less dense, more fragile skeletons at high concentrations. These finding that the lowest skeletal densities and highest extension rates were found at nearshore B1, where levels of inorganic nutrients were highest (dissolved inorganic phosphate 0.122 ± 0.015 μM; dissolved inorganic nitrogen 0.593 ± 0.115 μM; Appendix A Suppl. Fig. 2.1) is consistent with increased skeletal fragility when exposed to increased phosphate concentrations (Simkiss 1964). Water quality guidelines suggest trigger values for total phosphorus should not exceed 15.0 μg L^{-1} in inshore marine environments and 10.0 μg L^{-1} in offshore marine environments (ANZECC 2000). Furthermore, total nitrogen should not exceed 100.0 μg L^{-1} with NO_x and NH_4^+ not exceeding 8.0 and 10.0 μg L^{-1}, respectively (ANZECC 2000). Results from this study suggest that inshore corals were exposed to nutrient levels below recommended water quality guidelines during the study period and changes in coral cover driven by rapid skeletal extension rates can increase the vulnerability of populations.

Along these inshore reefs, high levels of suspended particulates (i.e. 0.030 – 5.073 mg L^{-1}; Appendix A Suppl. Fig. 2.1; Suppl. Fig. 2.2) did not induce detrimental effects such as mortality or bleaching in *A. tenuis,* however no colonies of this species occur in water depths greater than 5 m on the most turbid reefs (Thompson et al. 2014a). None of these inshore sites within the Burdekin and Whitsunday regions exceeded the GBRMPA trigger value for suspended solids of 15.0 and 2.0 mg L^{-1} for coastal and mid-shelf reefs, respectively (GBRMPA 2010). Turbidity levels did not exceed 7 NTU along the inshore Burdekin and

Whitsunday regions (Appendix A Suppl. Fig. 2.1 and 2.2). These reefs were well below the recommended guidelines levels of less than 20 NTU for marine environments (ANZECC 2000). It is likely that light limitation in these turbid waters rather than high particle and nutrient concentrations are the main drivers limiting their depth distribution suggesting that benthic irradiance rather than turbidity might be a better predictor of ecosystem health (ANZECC 2000). Increased concentrations of particulates have the potential to either stress or benefit scleractinian corals (Anthony and Fabricius 2000; Fabricius et al. 2014), depending on the optimisation of the relationship between the coral host and its photosynthetic symbionts. Highest symbiont densities found at the most turbid site (B1) in the Burdekin region further suggest that suspended particulates and decreased light have caused a shift to higher symbiont densities, as found for *Pocillopora* corals and *Acropora* corals in studies of spatial and seasonal environmental variation (Fabricius et al., 2012; Hinrichs et al., 2013, respectively). Increases in symbiont density have also been found in conjunction with lower metabolic rates and energy levels (Hinrichs et al., 2013). Highest lipid content and ash-free dry weight found at the most turbid site (B1) in the Burdekin region indicate that these corals do not have reduced energy stores. Energy stores and lipids are influenced by the fecundity of colonies and the time before spawning (Stimson 1987; Leuzinger et al. 2003; Grottoli et al. 2006) but reproductive traits were not assessed here. The potential influence of reproductive activity was minimised by standardising colony size to approximately 15 × 15 cm^2, a size typically below reproductive size. Further analyses are required to explore the relative proportions of structural lipids, which are known to be relatively stable (Saunders et al. 2005), and storage lipids, which increase in improved environmental conditions (Harland et al. 1992).

Increased symbiont densities were associated with increased temperature and dissolved organic carbon. Seasonal fluctuations in symbiont density and biomass occur in

many coral species (Fitt et al., 2000; Hinrichs et al., 2013; Thornhill et al., 2011). Environmental influences can have opposing effects on symbiont density, with highest densities occurring in the winter when temperatures are coldest (Fitt et al., 2000 and Ulstrup et al., 2008) and highest densities occurring in the summer with increased temperatures (Hinrichs et al., 2013). Symbiont density was positively correlated with ash-free dry weight and total lipid content, suggesting that symbiont communities may substantially contribute to the organic and lipid reserves within coral tissues; however, symbiont communities have been found to account for < 5 % of total tissue biomass (Thornhill et al. 2011). One-third higher organic content and 2-fold higher lipid concentrations were found in corals with the highest symbiont densities. Presumably, increases in symbiont densities were necessary for optimisation of energy production and photosynthetic rates in lower light conditions. Positive correlations between symbiont density and organic and lipid content in this study suggest additional organic and lipid reserves are the result of larger symbiont populations and the necessary coral tissue to contain these populations. Large symbiont populations and high organic and lipid reserves may indicate enhanced coral holobiont functioning through higher photosynthetic rates (Browne et al. 2015). Optimal symbiont densities have been suggested to be between ~10 and 30 x 10^5 cells cm^{-2} (Wooldridge et al. 2016). All corals of *A. tenuis* within this study did not exceed the suggested values and symbiont densities peaked in B1 in June 2013 at 18.5 x 10^5 cells cm^{-2}, indicating these inshore corals may not be at increased risk of bleaching due to high symbiont densities alone. Occasionally, unusually high light levels do occur on inshore coral reefs during calm weather events (Anthony and Connolly 2004). As sedimentation drops out of suspension, corals in turbid waters can be exposed to light levels beyond the norm, which may induce bleaching.

If an extreme thermal or light event inducing a stress response did occur, high organic and lipid reserves may aid in the recovery from these stressors (Thornhill et al. 2011). High

organic and lipid content found in the coral holobiont may come not only from the symbionts but also from the surrounding environment through heterotrophic feeding (Anthony and Fabricius, 2000; Hinrichs et al., 2013; Seemann et al., 2012; Thornhill et al., 2011). Particulate and dissolved organic nutrients can act as a supplemental food source in corals (Stephens 1962; Ferrier 1991; Rosenfeld et al. 1999; Anthony and Fabricius 2000). Corresponding increases in symbiont density and dissolved organic carbon suggests dissolved organic nutrients may also be directly available to the symbionts, as proposed by Wiedenmann et al. (2012). Light and feeding are important factors for assimilation of dissolved organic nutrients into corals (Al-Moghrabi et al. 1993), which further interlink environmental water quality conditions when assessing overall coral health and condition. Therefore, autotrophic indices (e.g. symbiont density, chlorophyll a concentration) alone are poor indicators of coral health and condition, but in conjunction with lipid content and biomass, a more detailed indication of coral health and condition can be achieved.

Coral-*Symbiodinium* associations (density and type) can affect the overall health and condition of corals (Little et al. 2004; Berkelmans and van Oppen 2006; Abrego 2008). These associations were significantly different across the sites in the Burdekin region and had a similar, but insignificant trend in the Whitsunday region. *A. tenuis* harboured clade C *Symbiodinium* at these sites, as has been found throughout the central and southern GBR in other studies (van Oppen et al. 2005; Cooper et al. 2007; Tonk et al. 2013). Overall, clade C1 *Symbiodinium* are typically more common at inshore sites (Ulstrup and van Oppen 2003). Furthermore, variation in dominant *Symbiodinium* clades, potential background *Symbiodinium* clades (i.e. low density clades C and D) and lipid concentrations along these inshore gradients suggest *Symbiodinium* clades may differentially influence lipid concentrations, as different clades (i.e. clade C and clade D) can have different lipid and fatty acid concentrations (Francis, *pers. comm.*), as well as respond differently in lipid and fatty

acid composition under thermal stress (Kneeland et al. 2013) Therefore, differences in observed changes of coral holobiont health may be driven by environmental parameters acting on the coral host, and their *Symbiodinium* populations.

Environmental drivers of coral health and condition

Parallel variation in multiple water quality parameters makes it difficult to identify the single most influential environmental parameter affecting coral health and condition (Cooper et al. 2007; Fabricius et al. 2014). To compensate for high levels of correlation in water quality parameters, many studies have created WQ indices (Fabricius et al. 2005; Cooper et al. 2007; Browne et al. 2015). The principal component analyses used in this study aimed to reduce the complexity of highly correlated parameters, while still maintaining differentiation between the main water quality parameters defined by Fabricius (2005). Even with parameter reduction, influential environmental parameters were specific to the coral health attribute being examined. As such, individual coral health and biochemical attributes were each influenced by different water quality parameters (e.g. salinity, particulates, and dissolved inorganic nutrients affected coral growth rates; dissolved organic nutrients only negatively influenced ash-free dry weight). Similarly, no single environmental parameter was found to drive coral condition along an anthropogenic gradient in coastal waters of Singapore (Browne et al. 2015) nor seasonally at Ningaloo Reef, Australia (Hinrichs et al., 2013).

Environmental conditions were relatively benign during the two-year observation period and no extreme events, such as anomalously warm summer temperatures, decreased salinity or extensive runoff, occurred. As a consequence of the absence of such extreme events, water quality impacts alone could be evaluated. Observed variation in water quality did not directly cause detrimental effects to coral colonies of *A. tenuis* in shallow water, as there was no coral mortality along the strong Burdekin gradient and limited mortality along

the weaker Whitsunday gradient. This may explain the relatively stable biochemical and physiological responses found for the coral *A. tenuis*. It remains unclear how inshore corals will respond during extreme events, as these inshore corals appear to have either acclimatised and/or adapted to the benign conditions.

Conclusions

Results revealed that *A. tenuis* can be hardy in shallow water with reduced water quality conditions and indicated that high concentrations of particulate and dissolved nutrients do not necessarily reduce coral health. Furthermore, inshore populations of the coral *A. tenuis* conform to their local conditions by adjusting symbiont density and skeletal investments. When undertaking coral health assessments, careful consideration should be given to selecting suitable health attributes, as components of coral health and condition can be negatively correlated. For example, the fastest growing corals had the least dense skeletons. It is important to remember the potential consequences of localised biochemical acclimatisation or adaptation in coral health attributes. High symbiont densities increase coral vulnerability to thermal and light stress that cause photo-inhibition, lower skeletal densities increase coral susceptibility to physical damage (i.e. cyclones, destructive fishing), and higher organic content has the potential to promote recovery of corals after extreme stress events. The findings of this study enhance understanding of water quality parameters that are of most consequence to coral holobiont health and could be useful for monitoring the status and trend of GBR corals and reporting on reef health. Furthermore, this study provides support for prioritisation of actions that improve water quality parameters and identifies which components of water quality have greatest impact on coral health. Future research should investigate whether corals from different water quality environments have different susceptibilities to bleaching from heat and light stress.

CHAPTER 3: Temporal and spatial variation in fatty acid composition in corals along water quality gradients on the Great Barrier Reef

3.1 Synopsis

Fatty acids (FA), the building blocks of lipids, play a vital role in coral metabolism and stress resistance and have been proposed as biomarkers of environmental stress and coral health. An appropriate balance of essential polyunsaturated fatty acids (PUFA) is necessary to promote proper biochemical and physiological functioning. These include omega-3 (n-3) PUFA (anti-inflammatory functioning), and omega-6 (n-6) PUFA (pro-inflammation and immune responses). This study explores seasonal and spatial variation in the abundance of 17 FA in the coral *Acropora tenuis*, and evaluates if FA ratios could be used to reveal sources of nutrition and evidence of host-symbiont sharing. Variations in lipid investment and storage in corals along two water quality gradients on the central GBR were documented to assess the viability of FA as biomarkers for monitoring the health of coral populations. Ratios of key FA varied along the two water quality gradients in a similar manner, suggesting that FA ratios may be useful indicators of coral health. Corals in comparatively good water quality conditions (sites farthest from the coast and the source of river runoff with low turbidity and nutrient-enrichment) had the highest putative health ratios (i.e. n-3:n-6). Strong differences in PUFA composition were found between wet and dry seasons, with high percentage n-3 PUFA defining the dry seasons (June 2013 and October 2013) and high percentage n-6 PUFA defining the wet seasons (February 2013 and 2014). Saturated FA (SFA) and monounsaturated FA (MUFA) concentrations varied with season and were positively correlated with *Symbiodinium* density, but SFA and MUFA were also dominant in corals exposed to the highest particulate and nutrient loads, thus reducing our ability to distinguish between potential autotrophic versus heterotrophic origins of FA. Overall, results

demonstrate that essential FA and their derived health ratios vary with season and environmental conditions supporting FA as biomarkers of coral holobiont health.

3.2 Introduction

Identifying healthy environments for corals and thresholds beyond which increases in a parameter become stressful require an understanding of coral responses under a range of environmental conditions. For example, moderate levels of nutrients and shading can provide benefits for some corals through higher photosynthetic efficiency (Browne et al. 2015) and improved nutritional status (Anthony et al. 2007), but poor water quality can smother corals with particulates (Anthony et al. 2002; Philipp and Fabricius 2003; Weber et al. 2012), decrease photosynthesis, calcification and energy stores (Anthony et al. 2007; Carilli et al. 2009; Cunning and Baker 2013; Fabricius et al. 2013a), and disrupt coral-*Symbiodinium* symbioses (Erftemeijer et al. 2012; Flores et al. 2012). Indicators currently measured, like growth and survival, while providing coarse measures of overall reef health, provide limited information of coral health at the colony or species level. Biochemical and physiological markers of coral health can respond more rapidly, provide early warning of deterioration in overall condition, and provide enhanced understanding of the fitness of coral populations.

Understanding sources of nutrition and energy is fundamental to elucidating the fitness of an organism. Corals have high trophic plasticity, acquiring nutrition both from endosymbiotic dinoflagellates through autotrophy (Muscatine and Porter 1977) and from plankton, particulate matter and dissolved nutrients through heterotrophy (Anthony and Fabricius 2000; Ferrier-Pagès et al. 2003; Houlbrèque and Ferrier-Pagès 2009; Hinrichs et al. 2013b). However, turbidity (characterised by light and particulate levels) and nutrients can represent either a nutritional resource or a source of stress, dependent on concentrations and the species of coral (Anthony and Fabricius 2000). Furthermore, heterotrophic feeding efforts can be influenced by morphology of coral species (i.e. large versus small polyps and mounding versus branching), surface area to volume ratio, type of tentacles and nematocysts and even *Symbiodinium* abundance (Houlbrèque and Ferrier-Pagès 2009). Fatty acids (FA)

may aid in distinguishing between autotrophic and heterotrophic sources of nutrition of corals (Al-Moghrabi et al. 1995; Treignier et al. 2008) suggesting that studies of FA composition and key FA ratios can provide useful insights into coral health and condition.

Scleractinian corals have relatively high lipid content, ranging between 10 and 46 % of organic dry weight (Harland et al. 1992). Lipids provide a nearly 2-fold greater source of energy (39.5 kJ kg^{-1}) compared to both proteins (23.6 kJ kg^{-1}) and carbohydrates (17.2 kJ kg^{-1}; Bureau et al. 2002), thus knowledge of deviations from baselines levels of lipids is an important indicator of the nutritional status of a coral. In general, half of coral lipids are committed to long-term energy storage (i.e. wax esters), while the other half are structural (i.e. phospholipids) and involved in maintaining cell structure and integrity (Joseph 1979; Edmunds and Davies 1986; Latyshev et al. 1991; Harland et al. 1993; Saunders et al. 2005; Imbs et al. 2010). FA are the building blocks of lipids, and the types of individual FA and FA classes and their relative abundances can be unique among taxa (Volkman 1999). FA are used in the majority of physiological processes in animals (e.g. respiration, growth, reproduction; studies on corals include: Stimson, 1987; Ward, 1995; Yamashiro et al., 1999). And play a vital role in coral metabolism and stress resistance (Hulbert 2003; Imbs et al. 2015). Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) are both efficiently metabolised, thus they are typically used for rapid energy production. In comparison, polyunsaturated fatty acids (PUFA) are more structurally complex and are used to produce signalling molecules and compounds involved in cellular activity and health hormones (Tocher 2003). Elevated levels of PUFA are necessary for a wide range of physiological actions, including immune responses, inflammatory responses, neural functioning and reproduction (Funk 2001; Tocher 2003). Thus, monitoring changes in the FA composition of coral tissues may be a valuable tool for assessing the health of corals.

An appropriate balance of essential long-chain polyunsaturated fatty acids (LC

PUFA) is necessary to promote proper biochemical and physiological functioning, maintain structural integrity of cellular membranes, and retain energy reserves (Russo 2009; Richier et al. 2010). Essential FA can be divided into omega-3 (n-3) and omega-6 (n-6) FA with the precursors 18:3n-3 and 18:2n-6 of the two biosynthetic pathways, respectively. These precursors compete for rate-limiting desaturase Δ6 in the synthesis of LC PUFA suggesting that n-3 PUFA and n-6 PUFA can be differentially and/or preferentially synthesised (Bergé and Barnathan 2005). n-3 LC PUFA are essential precursors to compounds involved in anti-inflammatory regulation, and include eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA; Dalsgaard et al., 2003; Guil-Guerrero, 2007; Sargent et al., 1990). EPA functions in anti-inflammatory processes and DHA functions in fluidity of cell membranes (Stubbs 1992; Russo 2009; Swanson et al. 2012); however, these n-3 PUFA are unable to be substituted for one another in metabolic processes, although low levels of retro-conversion are possible (Nettleton 1995). Conversely, bioactive arachidonic acid (20:4n-6, ARA), an n-6 PUFA, is required for the synthesis of pro-inflammation health hormones, responsible for water transport across membranes and an important component of immune responses (Sargent et al. 1990; Nettleton 1995; Tocher 2003; Russo 2009).

Syntheses of FA occur in both the coral host and its endosymbiont *Symbiodinium* (Oku et al. 2003; Dunn et al. 2012), and represent a principal biochemical link between these two symbiotic partners (Kellogg and Patton 1983; Patton and Burris 1983). Despite misconceptions based on generalisations, lower animals (including corals) are able to synthesise PUFA (Monroig et al. 2013); however, the direct biosynthetic mechanisms are far from clear in invertebrates. Although vertebrates do not possess Δ12 and Δ15 desaturases (Dewick 1997), which metabolise FA by removing hydrogen atoms and create carbon-carbon double bonds, the presence and function of these desaturases is currently under investigation in invertebrates (Dunn et al. 2012; Monroig et al. 2013). Generally in photosynthesising

invertebrates, 16:0 (palmitic acid; PA), the most common FA found in animals and other organisms, is converted into 18:3n-3 and 18:2n-6 (n-3 and n-6 FA, respectively), which are precursors to LC PUFA (Fig. 3.1). Specifically, 16:0 is elongated into 18:0 (stearic acid, SA) and metabolised by Δ9 desaturase into 18:1n-9 (oleic acid, OA). 18:1n-9 is further metabolised by Δ12 desaturase into 18:2n-6 (linoleic acid; LA) followed by Δ15 desaturase into 18:3n-3 (α-linolenic acid; ALA; Kellogg and Patton 1983; Dalsgaard et al. 2003). It has been suggested that 18:3n-3 and 18:2n-6 are produced in *Symbiodinium* cells and then translocated to tissues of the coral host (Kellogg and Patton 1983; Patton and Burris 1983; Harland et al. 1993; Dalsgaard et al. 2003; Papina et al. 2003; Bachok et al. 2006). However, heterotrophic feeding is an additional source of n-3, n-6 and n-9 FA (18:1n-9, 18:2n-6 and 18:3n-3; Al-Moghrabi et al., 1993; Hinrichs et al., 2013; Meyers, 1979; Seemann et al., 2013) and hence might be elevated under enhanced particulate and nutrient conditions. Determining the source of these FA could provide further information on the health status of the coral holobiont, as 18:2n-6 and 18:3n-6 are essential for proper physiological functioning, metabolism and stress resilience.

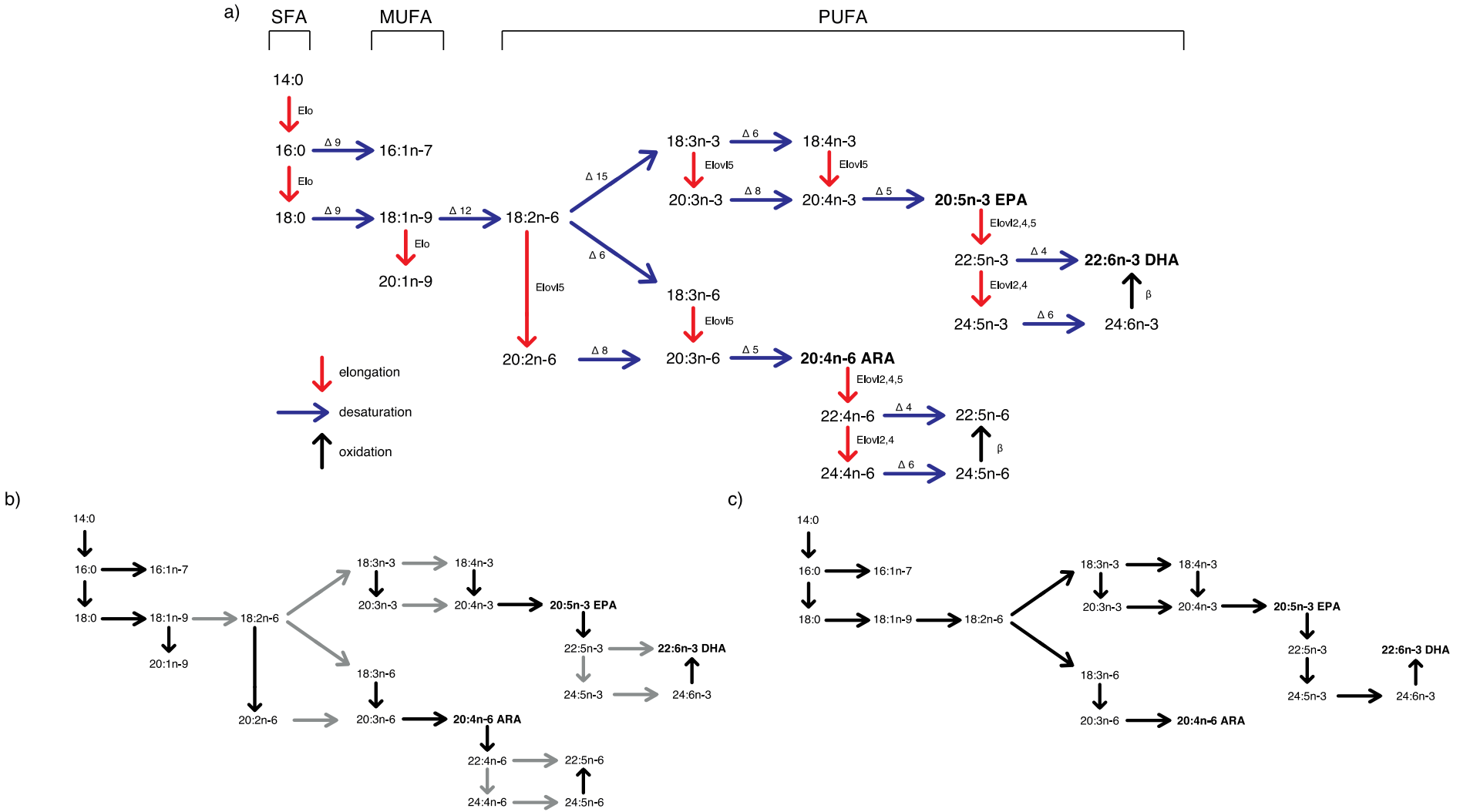

Fig. 3.1 Schematic diagram of FA biosynthetic pathways potentially occurring in (a) the coral holobiont, (b) the coral host tissues, with grey arrows as proposed reactions, and (c) the algal endosymbiont. **Bold** denotes essential FA. (Modified from Dalsgaard et al. 2003 and Monroig et al. 2013). Blue arrows represent desaturation by specified desaturases (ΔX), red arrows represent elongation by specified elongases (EloX), and black arrows represent peroxisomal β-oxidation (β), which cleaves the FA chain.

Concentrations of n-3 and n-6 PUFA, as well as relative proportions and ratios of these PUFA, can provide insights into overall health and functioning through nutritional and oxidative values, as well as trophic interactions and indicators of potential disease (Bergé and Barnathan 2005; Simopoulos 2008; Ayas et al. 2013; Nomura et al. 2013). The following FA ratios have been suggested as putative FA health indicators: n-3:n-6, LC n-3:LC n-6, EPA:ARA and EPA:DHA. Increased n-3 PUFA are important for cellular fluidity, anti-inflammation and immunity (Tocher 2003; Russo 2009). Many marine studies that use FA as health indicators often assume stable or constant spatio-temporal patterns (Dethier et al. 2013), therefore it is vital to determine if FA profiles of corals vary across time and space. Assessment of seasonal (Oku et al. 2003) and spatial variation (Seemann et al. 2013) in FA composition of corals has been limited, restricting our capacity to evaluate the potential of FA ratios as health indicators.

The quantity of lipid reserves combined with FA composition of marine organisms reflect the biochemical and ecological conditions of their environment (Bergé and Barnathan 2005). Environmental factors that can influence lipid reserves and FA composition include light (Harland et al. 1992; Al-Moghrabi et al. 1995; Oku et al. 2003), depth (Harland et al. 1992), temperature (Ward 1995; Ben-David-Zaslow and Benayahu 1999; Oku et al. 2003) and feeding strategy (Meyers et al. 1978; Meyers 1979; Szmant-Froelich and Pilson 1980). Lipid reserves decrease in bleached corals (Porter et al. 1989; Fitt et al. 1993; Yamashiro et al. 2005; Rodrigues and Grottoli 2007), yet increase when exposed to additional particulate nutritional sources (Meyers et al. 1978; Meyers 1979; Szmant-Froelich and Pilson 1980). However, some studies have found environmental factors, such as depth, temperature and pCO_2, do not necessarily affect lipid reserves (Harland et al. 1991, 1992; Grottoli et al. 2004; Strahl et al. 2016). As many of these environmental factors are correlated and interact (Crain

et al. 2008), determining responses to seasonal and environmental variation will provide further indication of how coral health and condition vary with environmental change.

To evaluate the viability of FA composition as a health indicator of corals, spatial and temporal changes in FA composition of the coral *Acropora tenuis* were examined along *in situ* water quality gradients in two inshore regions of the Great Barrier Reef. The aim was to determine if variation in FA composition was correlated with variation in biochemical attributes of coral health and condition (assessed and defined in ***Chapter 2***). This study highlights the seasonal nature of FA composition in corals, the potential for FA ratios as coral health indicators and the relationships between FA composition and coral health attributes, including growth and survival.

3.3 Methods

Study site and sampling design

This study was conducted in conjunction with the spatial and temporal study of biochemical and physiological health attributes described in Chapter 2. Briefly, corals were sampled at three sites, located at increasing distances from the coast and a river source, within each of two regions in the central Great Barrier Reef, Australia (Fig. 2.1). All sites were part of the established Marine Monitoring Program (MMP) run by the Australian Institute of Marine Science (AIMS). The strong water quality gradient within the Burdekin region included Geoffrey Bay, Magnetic Island (B1), where water quality was moderate, Pandora Reef (B2), midway along the gradient, and Pelorus Island (B3), where water quality was good (***Chapter 2;*** Thompson et al. 2014a). The weaker gradient in the Whitsunday region ranged from good to poor water quality, and included Pine Island (W1), closest to the river source, Day Dream Island (W2), and Double Cone Island (W3), farthest from the river source (***Chapter 2;*** Thompson et al. 2014a).

Between twelve and twenty experimental coral colonies of *Acropora tenuis* were collected and placed on stainless steel racks at 2 m depth, approximately five to ten meters from the start of the MMP permanent transects (Thompson et al. 2014a). Fragments were approximately 15 × 15 cm^2 in size, a size typically below reproductive size to minimise the likelihood that changes in lipid and FA content were due to reproduction. Experimental colonies were sampled three times per year to document variation in FA composition in the wet season (February), the early dry season (June), and the late dry season (October): 19-22 February 2013, 15-18 June 2013, 4-9 October 2013 and 10-22 February 2014. Branches, ~ 2 to 6 cm long, were collected from the middle of eight to twelve colonies at each site at each sampling time point. Samples were immediately snap-frozen in liquid nitrogen (-80 °C) for further analyses.

Fatty acid composition

Total lipid content and FA composition of coral colonies were determined following Conlan et al. (2014). Briefly, branches were crushed using a pneumatic press and ground to a fine powder on liquid nitrogen. Samples were transferred to a freeze-dryer for 48 hours until completely dry. Frozen dry samples were subsampled and weighed for total lipid and FA (~ 90 % of the coral sample) and ash-free dry weight analyses (~ 10 %). Total lipid content and ash-free dry weight data were analysed in ***Chapter 2***.

Total lipid extracts from the coral holobiont samples were reconstituted in 1.0 mL of dichloromethane:methanol (2:1) for saponification. Two mL of 5 % potassium hydroxide (KOH) in 80/20 methanol (MeOH)/MilliQ water was added, and samples were heated to 60 °C for 3 hours. Samples were allowed to cool and 1.0 mL MilliQ water was added. Samples were purified three times by adding 1.8 mL 4:1 hexane:$CHCl_3$ (DCM), vortexing for 10

seconds, centrifuging for 3 minutes at 1000 rpm, and collecting the aqueous MeOH/H_2O extract.

Remaining aqueous MeOH/H_2O extracts from the samples were methylated at 100 °C for 1 hour with 2.0 mL acetyl chloride:methanol (MeOH) after addition of 100 uL internal standard C23:0 (0.75 mg mL^{-1}). Two mL of potassium carbonate (1 M) and 1.7 mL hexane were added and samples were vortexed for 10 seconds, then centrifuged at 1000 rpm for 3 minutes at room temperature. FA concentrations were analysed with gas chromatography (Agilent Technologies 7890A, USA) following Conlan et al. (2014). Areas of resulting individual FA peaks were corrected by theoretical relative response factors (Ackman 2002) and identified and quantified against known external standards (mixed and individual standards from Sigma-Aldrich, Inc., St. Louis, USA and NuChek Prep Inc., Elysian, USA) using GC ChemStation software (Agilent Technologies, USA).

FA content was standardised to weight of total lipid content and expressed as mg FA g $lipid^{-1}$ for quantitative comparisons. Individual FA content, sums of FA classes, percent composition of FA, and ratios of FA health indicators (i.e. n-3:n-6, LC n-3:LC n-6, EPA:ARA and EPA:DHA) were calculated to further explore how investment in FA varies with water quality and season.

Statistical analysis

Analyses were performed using the statistical software R 2.15.3 (R Core Team 2015). Spatio-temporal trends in FA composition were characterised with multivariate principal component analyses (PCA) using the package 'vegan' (Oksanen et al. 2015) to determine energetic investments of corals associated with the two inshore regions (Burdekin and Whitsunday). PCAs were performed on percentage composition of individual FA from both regions to explore variation in FA composition of colonies within populations across time

and space. Generalised linear models (GLMs) were performed on principal components considered important (standard deviations greater than 1.0), with the three explanatory factors in the model – date, region and site (nested within region) – all treated as fixed factors.

To test for differences in mean concentrations of total FA, FA classes, individual FA (mg FA g lipid^{-1}), and FA health indicators among the three potential explanatory factors (date, region and site), generalised linear mixed models (GLMMs) were run using the packages 'lme4' (Bates et al. 2015) and 'afex' (Singmann et al. 2016) with a Gamma error distribution and log-link function in order to meet model assumptions. FA health indicator ratios were run with a Gaussian error distribution. Colony identification was included as a random factor to account for repeated sampling and maintain assumptions of independence. Post-hoc analyses were used to reveal homogenous dates and sites.

Spearman's rank correlation analyses were performed to test for associations between FA concentrations and coral growth, and between FA and overall condition, as measured by a range of coral health attributes, including symbiont density, skeletal density and ash-free dry weight from ***Chapter 2***. To control for type 1 error in statistical tests of FA composition and coral health attributes, the Benjamini-Yukutieli (BY) multiple correction was used (FDR corrected $\alpha = 0.009$; Benjamini and Yekutieli, 2001).

3.4 Results

Fatty acid composition of the coral holobiont Acropora tenuis

Seventeen FA were used in analyses of coral holobionts from the Burdekin and Whitsunday regions (split by region for ease of visualisation; Fig. 3.2). The relative contributions of these FA to overall FA composition of experimental *Acropora tenuis* colonies varied among sites and between seasons. Burdekin corals were separated along PC1, which explained 47.6% of the variation in FA composition (Fig. 3.2a; Table 3.1); corals at

site B1 (closest to coast and river source) had the highest PC1 scores and corals at site B3 (furthest from coast and river source) had the lowest PC1 scores. The FA composition of B1 corals was characterised by higher percentages of FA that are generally precursors to LC PUFA (14:0, 16:0, 16:1n-7, 18:1n-9, 18:2n-6, 18:3n-6 and 18:4n-3 with the exception of 20:3n-6; Fig. 3.3a). In contrast, B3 corals were characterised by FA that included LC PUFA and their derivatives (20:5n-3, 22:5n-3, 20:4n-6 and 22:4n-6; Fig. 3.3b). Less differentiation was detected among sites within the Whitsunday region along PC1 (Fig. 3.2b).

Seasonal variation in FA composition separated coral samples along PC2, which explained 14.2% of the overall variation. Generally, corals sampled in dry seasons (July 2013 and October 2013) had positive scores, whereas corals sampled in wet seasons (February 2013 and February 2014) had negative scores. FA associated with the dry season were 21:0, 16:1n-7, 18:1n-9, 20:1n-9, 20:5n-3, 22:5n-3 and 22:6n-3 (Fig. 3.3c). Fatty acids associated with the wet season were 14:0, 16:0, 17:0, 18:0, 18:4n-3, 18:2n-6, 20:3n-6, 20:4n-6 and 22:4n-6 (Fig. 3.3d). With the exception of 18:4n-3, a strong seasonal split of PUFA was detected, with n-3 FA associating with the dry season (20:5n-3, 22:5n-3 and 22:6n-3) and n-6 FA associating with the wet season (18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6 and 22:4n-6).

Overall, concentrations of individual FA and FA classes (mg FA g lipid^{-1}), as well as percent FA composition (Appendix B Suppl. Table 3.2), of *A. tenuis* within the two inshore regions of the GBR were generally affected by date. FA concentrations were significantly higher in dry seasons (June and October 2013) compared to wet seasons (February 2013 and February 2014; Appendix B Suppl. Fig. 3.1; Suppl. Fig. 3.2; Suppl. Table 3.1).

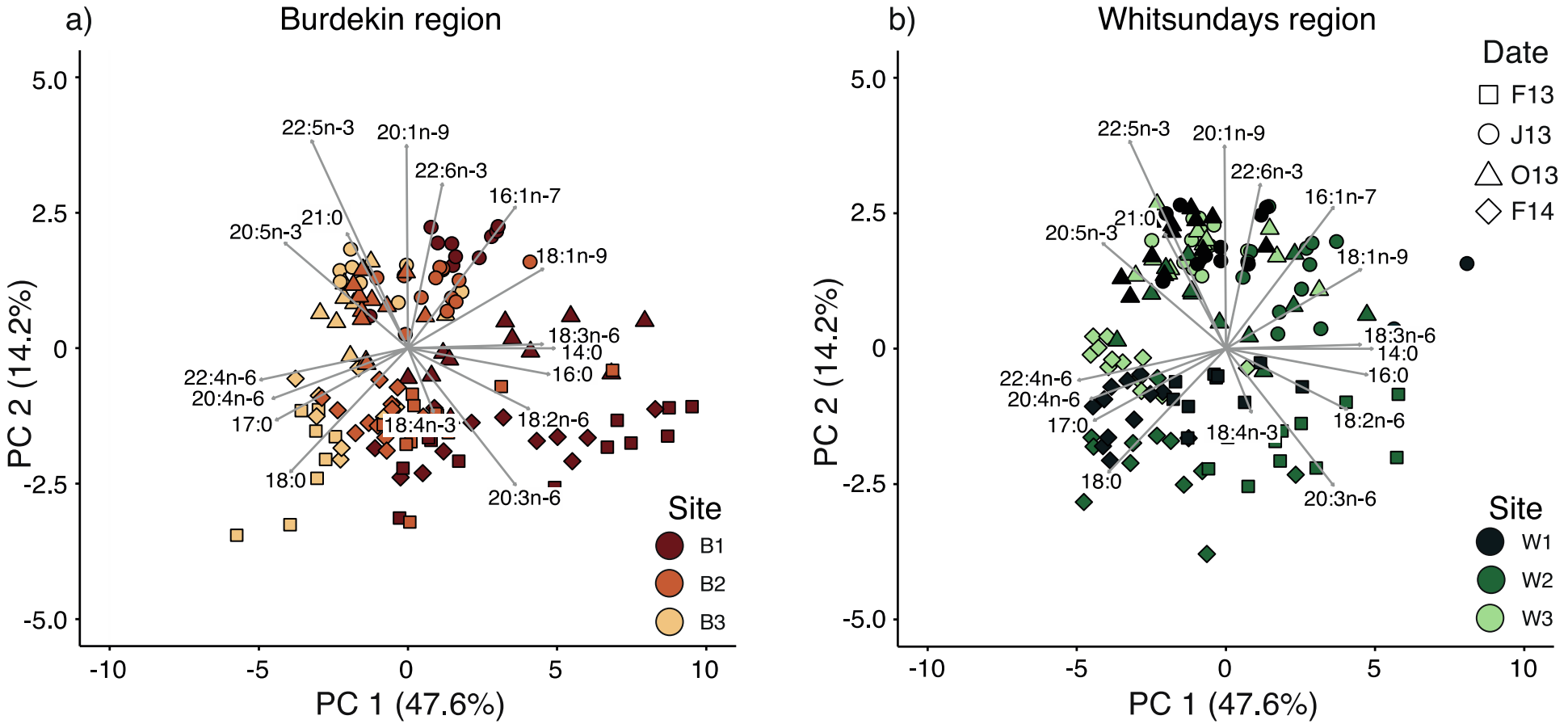

Fig. 3.2 Biplot of principal component analysis of the FA compositions of *Acropora tenuis* experimental colonies separated by region: (a) Burdekin region and (b) Whitsunday region. The percent composition of FA within lipid extractions were from coral holobionts sampled in February 2013 (□), June 2013 (O), October 2013 (Δ) and February 2014 (◇).

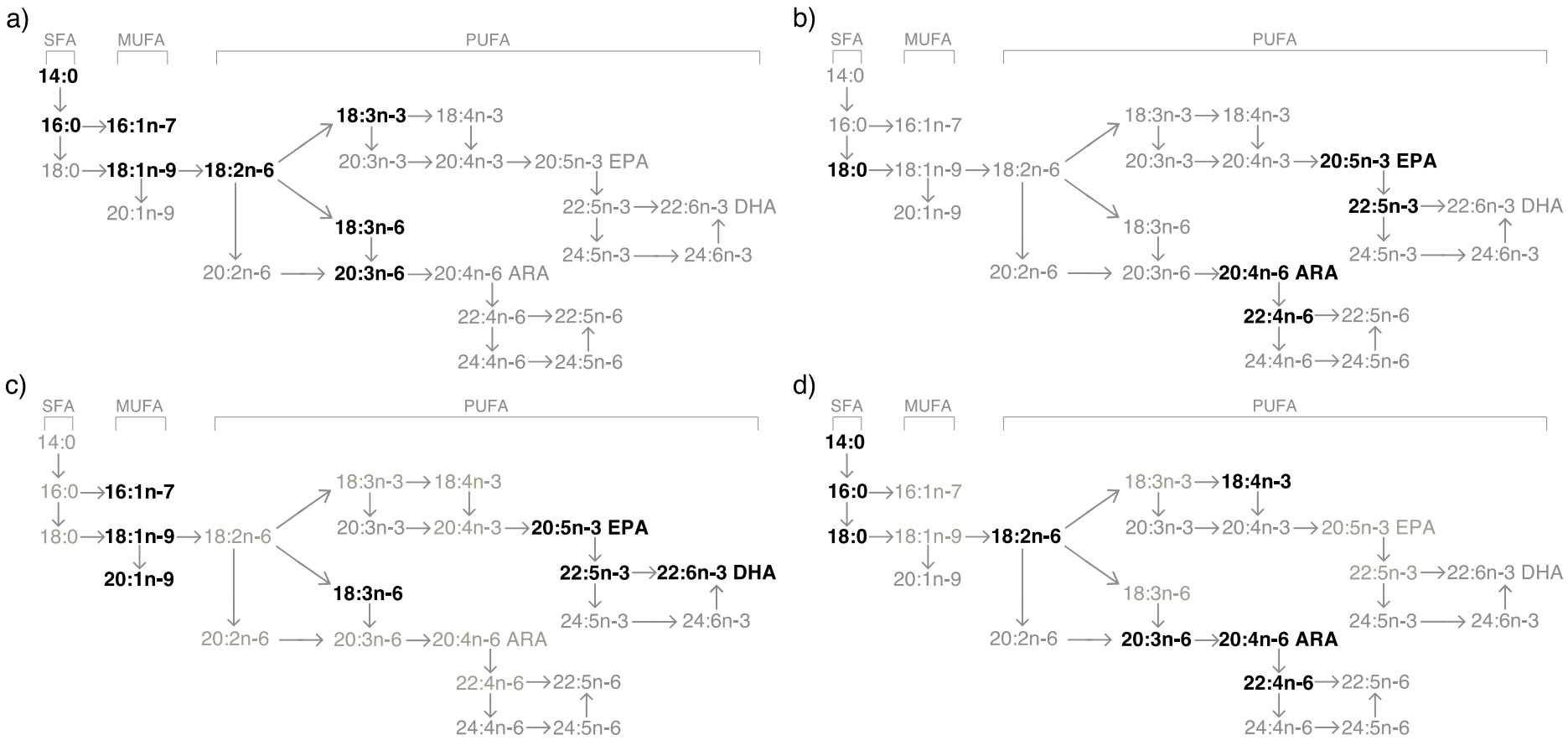

Fig. 3.3 FA biosynthesis schematic schematic of FA associated with PC1 and PC2 scores in the PCA of FA compositions for experimental colonies of *Acropora tenuis* bolded: (a) positive PC1 scores, (b) negative PC1 scores, (c) positive PC2 scores, and (d) negative PC2 scores.

Table 3.1 Standard deviation and proportion of variance explained in a principal component analysis (PCA) of the FA percentage composition of *Acropora tenuis* colonies at three sites within each of the Burdekin and Whitsunday regions. Generalised linear models (GLMs) using a Gaussian error distribution of important principal components (> 1.0 standard deviation) were used to determine significant differences among dates, regions and sites (nested within region). *** denotes significance at α = 0.001. ** denotes significance at α = 0.01. * denotes significance at α = 0.05.

Importance of components					
	PC1	*PC2*	*PC3*	*PC4*	
Standard deviation	2.85 *	1.55*	1.30*	1.16*	
Proportion of variance	0.476	0.142	0.100	0.079	
Cumulative proportion	0.476	0.618	0.718	0.797	
Principal component 1					
	df	Deviance	F value	P	
Date	3	258.3	21.11	<0.001	***
Region	1	91.47	22.43	<0.001	***
Date*Region	3	97.10	7.935	<0.001	***
Region(Site)	4	634.4	38.89	<0.001	***
Principal component 2					
	df	Deviance	F value	P	
Date	3	476.6	432.2	<0.001	***
Region	1	16.97	46.18	<0.001	***
Date*Region	3	7.570	6.868	<0.001	***
Region(Site)	4	29.37	19.98	<0.001	***
Principal component 3					
	df	Deviance	F value	P	
Date	3	131.9	53.87	<0.001	***
Region	1	16.10	19.72	<0.001	***
Date*Region	3	12.19	4.979	0.002	**
Region(Site)	4	75.40	23.10	<0.001	***
Principal component 4					
	df	Deviance	F value	P	
Date	3	12.47	6.390	<0.001	***
Region	1	49.35	75.84	0.001	***
Date*Region	3	7.122	3.648	0.013	*
Region(Site)	4	118.7	45.60	<0.001	***

Putative fatty acid health indicator ratios

Overall, the ratio of n-3:n-6 FA in tissues of *A. tenuis* was 0.864 ± 0.011 (mean ± SE). The overall ratio for LC PUFA was higher than this (LC n-3:LC n-6 ratio = 1.046 ± 0.016), whereas the overall EPA:ARA ratio was slightly lower (0.805 ± 0.015). The overall EPA:DHA ratio (which examines the relative proportions of two n-3 LC PUFA) was 2.073 ± 0.458. All four FA coral health indicator ratios were significantly affected by sampling date, region and site (within region; Fig. 3.3; Table 3.2).

Ratios for three of the FA health indicators (n-3:n-6, LC n-3:LC n-6 and EPA:ARA) differed significantly among dates and sites (Fig. 3.3a-i; Table 3.2). Ratios were significantly higher in the early dry season (June 2013) compared to the wet seasons (February 2013 and February 2014; Fig. 3.3a, d, g) and significantly higher in the Whitsunday region compared to the Burdekin region (Fig. 3.3b, e, h). Sites with relatively good water quality in both the Burdekin and Whitsunday regions (B3 and W3) had higher ratios, mid-gradient sites (B2 and W2) generally had lower ratios and moderate and poor water quality sites (B1 and W1) generally had higher or similar ratios compared to corals at mid-gradient sites (Fig. 3.3c, f, i).

Ratios for the EPA:DHA coral health indicator also differed among sampling dates; significantly higher ratios were detected in February 2014 compared to all other sampling dates (Fig. 3.3j; Table 3.2). Corals in the Whitsunday region had higher EPA:DHA ratios compared to those in the Burdekin region (Fig. 3.3k) with the exception of B3 being similar to W3. In both regions, corals at sites with better water quality had the highest ratios (Fig. 3.3l). In the Whitsunday region, the EPA:DHA ratio was approximately 1.5-fold higher at W1 compared to W2 and W3. In the Burdekin region, the EPA:DHA ratio for corals at B3 was approximately 2-fold higher than for corals at B1 and B2.

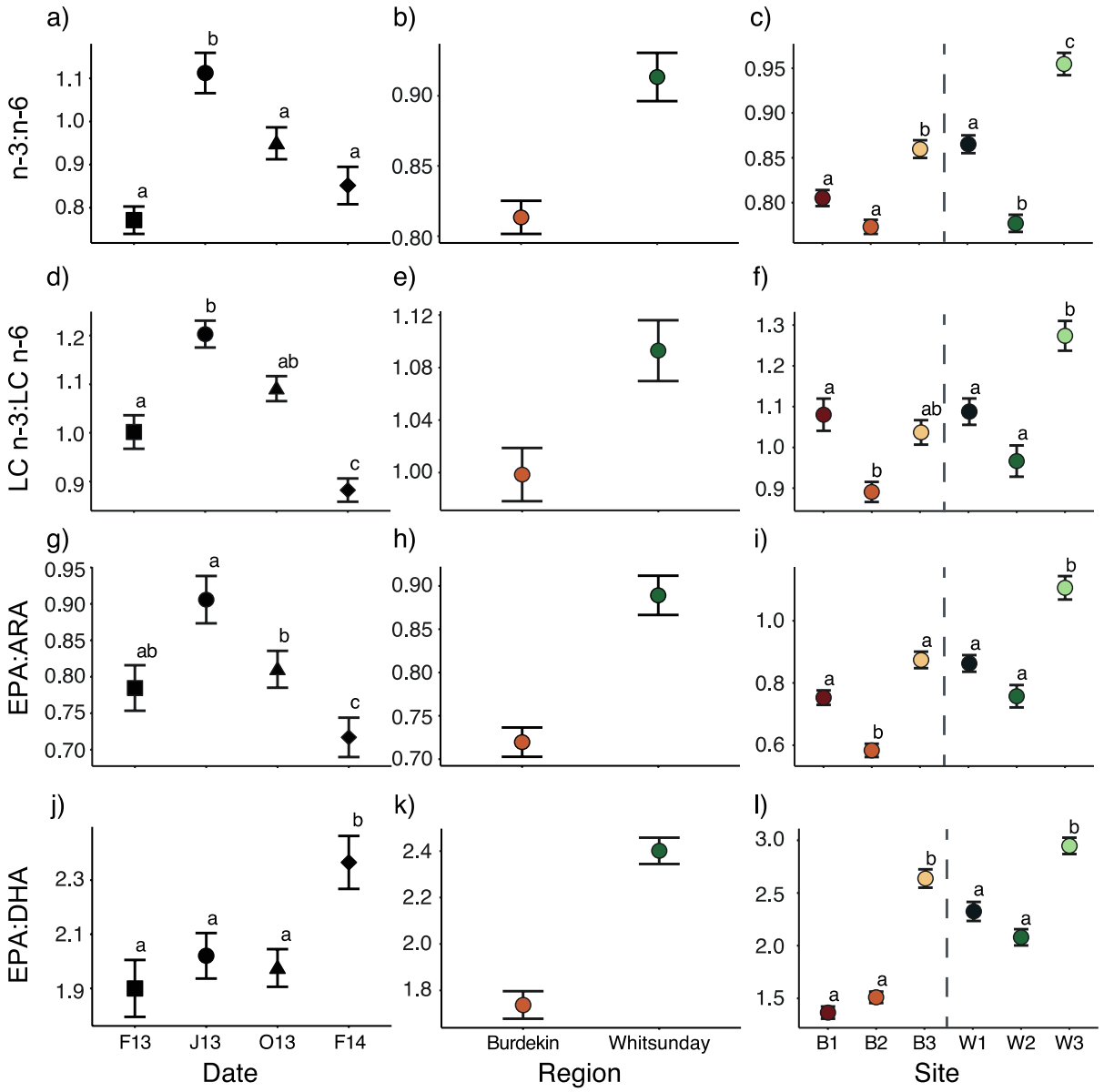

Fig. 3.4 Comparisons of FA ratios investigated as coral health indicators for the coral holobiont *Acropora tenuis*: (a, b, c) n-3:n-6; (d, e, f) LC n-3:LC n-6; (g, h, i) EPA:ARA; and (j, k, l) EPA:DHA. Mean FA ratios (± SE) are compared among sampling dates (February 2013 (□), June 2013 (O), October 2013 (Δ) and February 2014 (◇)); between regions (Burdekin (orange) and Whitsunday (green)); and among sites within regions (B1, B2, B3, W1, W2 and W3). Letters indicate homogenous groups identified by pairwise post-hoc analyses. Individual FA and FA classes are shown in Appendix A Suppl. Fig. 3.1 and Suppl. Fig. 3.2.

Table 3.2 Effects of date, region and site (within region) on FA ratios, investigated as coral health indicators for *Acropora tenuis* in the Burdekin and Whitsunday regions. Generalised linear mixed model (GLMM) outputs with a Gaussian error distribution. *** denotes significance at α = 0.001. ** denotes significance at α = 0.01. * denotes significance at α = 0.05.

Omega-3 to Omega-6 Ratio					
	df	Deviance	F value	P	
Date	3	1.030	24.99	<0.001	***
Region	1	0.567	41.26	<0.001	***
Date*Region	3	0.014	0.335	0.800	
Region(Site)	4	2.954	53.78	<0.001	***
Long Chain Omega-3 to Long Chain Omega-6 Ratio					
	df	Deviance	F value	P	
Date	3	3.745	33.70	<0.001	***
Region	1	0.539	14.54	<0.001	***
Date*Region	3	0.156	1.399	0.244	
Region(Site)	4	2.838	19.16	<0.001	***
EPA to ARA Ratio					
	df	Deviance	F value	P	
Date	3	1.245	13.80	<0.001	***
Region	1	1.833	60.96	<0.001	***
Date*Region	3	0.179	1.979	0.118	
Region(Site)	4	4.516	37.55	<0.001	***
EPA to DHA Ratio					
	df	Deviance	F value	P	
Date	3	8.332	12.96	<0.001	***
Region	1	27.36	127.7	<0.001	***
Date*Region	3	1.351	2.103	0.100	
Region(Site)	4	49.99	58.34	<0.001	***

Fatty acid correlations with coral health attributes

Both positive and negative correlations were detected between FA concentrations (individual, classes and health indicator ratios) and coral health attributes (i.e. growth, symbiont density, skeletal density and ash-free dry weight) of *A. tenuis* colonies located along water quality gradients in the Burdekin and Whitsunday regions (Table 3.3). Total FA concentration did not correlate with any of the health attributes, however symbiont density was positively correlated (FDR corrected-$\alpha = 0.009$) with eight individual FA (14:0, 16:0, 16:1n-7, 18:1n-9, 18:2n-6, 18:3n-6, 18:4n-3 and 22:6n-3), two FA classes (SFA and MUFA) and one health indicator ratio (LC n-3:LC n-6). Symbiont density was also negatively correlated with three individual FA (17:0, 20:5n-3 (EPA) and 20:4n-6 (ARA)) and one health indicator ratio (EPA:DHA). Growth of coral colonies was not correlated with any components of FA composition. Skeletal density was positively correlated with one health indicator ratio (EPA:DHA). Ash-free dry weight was positively correlated with one individual FA, 18:4n-3 (Table 3.3).

Table 3.3 Summary of significant correlations between FA composition of *Acropora tenuis* in the Burdekin and Whitsunday regions and coral health attributes: (a) symbiont density, (b) growth, (c) skeletal density, and (d) ash-free dry weight. Samples were collected between February 2013 and February 2014. ***Italics*** indicate a negative correlation. *** denotes significance at α = 0.001. ** denotes significance at α = 0.01. * denotes significance at α = 0.05. Bold denotes significance at FDR corrected-α = 0.009. Non-significant relationships not shown.

a) Symbiont density			
	rho	P	
Fatty Acid Classes			
SFA	**0.163**	**0.009**	**
MUFA	**0.235**	**< 0.001**	***
LC n-6	*-0.142*	0.023	*
Individual Fatty Acids			
14:0	**0.348**	**< 0.001**	***
16:0	**0.181**	**0.004**	**
17:0	***-0.202***	**0.001**	**
16:1n-7	**0.179**	**0.004**	**
18:1n-9	**0.296**	**< 0.001**	***
20:1n-9	0.125	0.045	*
18:2n-6	**0.304**	**< 0.001**	***
18:4n-3	**0.397**	**< 0.001**	***
20:5n-3	***-0.178***	**0.004**	**
22:6n-3	**0.196**	**0.002**	**
18:3n-6	**0.377**	**< 0.001**	***
20:3n-6	0.135	0.031	*
20:4n-6	***-0.162***	**0.009**	**
22:4n-6	*-0.160*	0.010	*
Health Indicator Ratios			
LC n-3:LC n-6	**0.162**	**0.009**	**
EPA:DHA	***-0.528***	**< 0.001**	***

b) Growth			
	rho	P	
Individual Fatty Acids			
18:4n-3	0.163	0.013	*

c) Skeletal density			
	rho	P	
Individual Fatty Acids			
20:1n-9	*-0.146*	0.019	*
18:4n-3	*-0.140*	0.025	*
22:6n-3	*-0.136*	0.029	*
Health Indicator Ratios			
EPA:DHA	**0.187**	**0.003**	**

d) Ash-free dry weight			
	rho	P	
Individual Fatty Acids			
20:1n-9	0.134	0.031	*
18:4n-3	**0.206**	**< 0.001**	***
Health Indicator Ratios			
EPA:DHA	*-0.146*	0.019	*

3.5 Discussion

This study documents temporal and spatial variation in FA composition of the coral *Acropora tenuis* along water quality gradients within two regions of the GBR. In the Burdekin region, where sites spanned a strong water quality gradient, variation in FA percentage composition was influenced by water quality. However, no pattern in FA percentage composition with site was detected in the Whitsunday region, where sites spanned a much weaker water quality gradient. In both regions, seasonal factors (associated with wet versus dry seasons) contributed strongly to variation in FA composition. Similar patterns in the variation of FA health indicator ratios with water quality in both regions may suggest their use as a coral health indicator. However, differentiate between autotrophically-sourced and heterotrophically-sourced FA was not possible as corals with the greatest *Symbiodinium* density were also exposed to the highest particulate and nutrient loads. Variation in FA composition in conjunction with variation in water quality and seasonal factors suggests that FA have the potential to be candidates for biomarkers in coral health assessments.

Seasonal variation of n-3 and n-6 PUFA

The contributions of n-3 PUFA and n-6 PUFA to overall FA composition of *A. tenuis* varied strongly between seasons. Higher percentages of n-3 PUFA were found in dry seasons (June 2013 and October 2013), when turbidity, particulates and temperatures were generally lower than in the wet season (see ***Chapter 2***: Appendix A Suppl. Fig. 2.1; Suppl. Fig. 2.2). This could indicate that corals are consuming prey containing higher quantities of the preferred LC n-3 PUFA, or alternatively, that these health promoting FA (Glencross 2009) are preferentially retained at the expense of LC n-6 PUFA. The ~ 75 % increase in LC n-6 PUFA concentrations, compared to the ~ 60 % increase in LC n-3 PUFA, between wet and dry seasons is consistent with this latter interpretation. The maintenance of higher proportions

of n-3 PUFA could offer a physiological advantage to corals by providing membrane fluidity, which permits diffusion of solutes and electrolytes across membranes (Hazel and Williams 1990), in the cooler temperatures associated with winter dry seasons (Los et al. 2013). High n-3 PUFA concentrations also enhance electron flow in chloroplasts when light levels are reduced (Mock and Kroon 2002). It is therefore possible that the higher concentrations of this FA class were associated with optimal functioning of *Symbiodinium* symbionts under lower light conditions in dry seasons.

The higher concentrations of n-6 PUFA in wet seasons (February 2013 and 2014) influenced overall FA composition. The Austral wet season is commonly associated with high summer temperatures, high levels of river runoff and consequently higher levels of particulates and dissolved nutrients (***Chapter 2***; Thompson et al. 2014). The higher levels of 18:2n-6, 20:3n-6, 22:4n-6 and 20:4n-6 (ARA) could reflect heterotrophic nutritional sources, as n-6 PUFA, particularly ARA, are dominant in the food chain, from marine algae up to fish, in the region (Johns et al. 1979; Armstrong et al. 1994). High proportions of n-6 PUFA, which decrease membrane fluidity and increase inflammation (Sargent et al. 1990; Nettleton 1995), may be a response to higher summer temperatures, enabling corals to maintain the structure and integrity of cellular membranes.

Temperature is a strong driver of biochemical and metabolic processes, including FA synthesis (Renaud et al. 1995). High PUFA concentrations in the winter dry season suggest a response to colder water temperatures, given that cold-water plankton and fish communities have also been found to have high PUFA content (Lewis 1962; Bergé and Barnathan 2005). Additionally, unsaturation of FA decreases (leading to an increase in SFA) with extreme heat stress in algae (Renaud et al. 1995; Kneeland et al. 2013). Therefore, PUFA can provide a physiological advantage at the cellular level, as these FA have a lower melting point

compared to SFA, allowing for increased membrane fluidity and proper functioning (Los et al. 2013) in colder temperatures.

Fatty acid health indicator ratios

Similar patterns of variation in FA ratios across water quality gradients in the Burdekin and Whitsunday regions, specifically ratios of n-3 PUFA to n-6 PUFA, support use of FA composition as a coral health indicator. Healthy cell membrane composition is highly dependent on PUFA acquisition and maintenance. Elevated levels of n-6 PUFA, particularly ARA, increase the rigidity and inflammation of cellular membranes (Simopoulos 2008). Conversely, intrinsic properties of n-3 PUFA include anti-inflammation and increased fluidity of cell membranes (Stubbs 1992; Russo 2009; Swanson et al. 2012). Increased n-3 PUFA composition has implications for improved growth, survival and stress resistance (Bachok et al. 2006). Acquisition, synthesis and maintenance of n-3 PUFA are essential to balance n-6 PUFA inflammation, as low n-3:n-6 ratios are usually due to excess intake of n-6 FA (Simopoulos 2008). This suggests that corals in relatively good water quality (such as sites B3 and W3, farthest from the coast and source of river runoff) have healthier FA compositions compared to corals exposed to reduced water quality.

The ratio of n-3:n-6 in *A. tenuis* also varied dependent on the season. The n-3:n-6 ratios of other marine invertebrates also vary with season, with higher n-3:n-6 ratios in the spring. For example, the prawn, *Crangon crangon*, had an n-3:n-6 ratio of 15.16 ± 0.76 in spring, compared to 0.63 ± 0.003 in autumn (Mika et al. 2014), and the clam, *Ensis siliqua,* had a ratio of 5.21 ± 0.25 in spring compared to 3.66 ± 0.15 during the remaining seasons (Baptista et al. 2014). Higher n-3:n-6 ratios associated with the spring, or the dry season, further supports the hypothesis of selective n-3 PUFA retention as a mechanism for coping with reduced food availability or cooler temperatures.

The overall (holobiont) n-3:n-6 ratio of 0.87 ± 0.03 for *A. tenuis* is comparable to ratios found for other invertebrates with photosymbionts. Several studies have compared these ratios between host tissues/cell layers and photosymbionts (Treignier et al. 2008; Revel et al. 2016) to determine the respective FA contributions of biologically distinct components. When the coral holobiont was split into animal host tissues (*Turbinaria reniformis*) and photosynthetic *Symbiodinium* cells, the respective n-3:n-6 ratios were 0.25 and 1.50; in comparison, their heterotrophic zooplankton food source had a mean ratio of 4.80 (Treignier et al. 2008). Similarly, when the symbiotic sea anemone *Anemonia viridis* was split into epidermal tissue, gastrodermal tissue and *Symbiodinium* cells, the n-3:n-6 ratios were 2.3, 3.8 and 4.0, respectively (Revel et al. 2016). However, these ratios may not accurately depict the health of the holobiont, as variation in these ratios may occur due to total lipid and FA reserves of the symbiotic host, *Symbiodinium* density and potentially *Symbiodinium* type.

As FA health indicator ratios are highly dependent on nutritional sources and environmental conditions (Al-Moghrabi et al. 1993; Dalsgaard et al. 2003), these ratios can be used in energy budgets to assess the trophic level of a variety of taxa (Rosa et al. 2007; Seemann et al. 2013; Parrish et al. 2015), including the coral host, its endosymbionts, and the combined coral holobiont (Treignier et al. 2008; Tolosa et al. 2011). The EPA:DHA ratio can indicate the degree of carnivory and relative reliance on autotrophic versus heterotrophic nutritional sources because DHA is highly conserved through the food web (i.e. high EPA:DHA indicates a low trophic level and higher reliance on autotrophy; Dalsgaard et al. 2003). Therefore, corals at site B3 were at a lower trophic level and more reliant on autotrophy compared to corals at B1 and B2. The relative proportions of FA contributed by the animal host compared to the *Symbiodinium*, as well as different *Symbiodinium* clades could also influence the trophic levels. *Symbiodinium* associations within the Burdekin region differed significantly among sites; only clade C1-associations were found at B1, whereas a

combination of C1/C2 mixed- and C2-associations were found at B3 (***Chapter 2***). Furthermore, the FA compositions of *Symbiodinium* clades C1 and D change differently under thermal stress (> 28 °C; Kneeland et al. 2013), although differences in FA composition were not detected prior to thermal stress. However, the high EPA:DHA ratios of coral holobionts located at sites B3 and W3 suggest that these corals rely more heavily on autotrophic nutritional sources, as these sites had the lowest exposure to particulates and dissolved nutrients within their respective regions (Thompson et al. 2014a).

Fatty acid variation across water quality gradients

Spatial variation in FA percentage composition was evident among sites in the Burdekin region, but not as evident in the Whitsunday region (as presented in Fig. 3.2 and further supported by Appendix B Suppl. Fig. 3.2 and Suppl. Fig. 3.3). The closest Whitsunday site is located approximately 20 km from the river source, and sites W1 and W3 are less than 30 km apart, whereas the Burdekin sites span 65 km. The absence of differences in FA percentage composition within the Whitsunday region is likely due to the limited spatial scale and correspondingly limited variation in environmental conditions in this region (***Chapter 2***; Hinrichs et al., 2013). Coral health attributes, including skeletal density, ash-free dry weight and total lipid content were also not affected by this limited spatial scale (***Chapter 2***). The similar patterns of FA composition and more general biochemical attributes of ***A. tenuis*** among Whitsunday sites accord with the limited variation in environmental conditions in this region.

Correlations of fatty acids with coral health attributes

Individual FA are not indicators of species-specific nutritional sources (i.e. *Symbiodinium* versus *Artemia* food sources), but the presence and combination of FA can be indicative of taxonomic classes (Volkman et al. 1993; Bergé and Barnathan 2005). SFA and

MUFA, including 14:0, 16:0, 16:1n-7 and 18:1n-9, were positively correlated with *Symbiodinium* density. These FA can be biomarkers of symbionts, as they can be produced by *Symbiodinium* (Figueiredo et al., 2012; Latyshev et al., 1991; Papina et al., 2003; Patton and Burris, 1983; Treignier et al., 2008). However, it must be emphasised that these individual FA can also be obtained through heterotrophic food sources. Negative correlations of the essential FA, EPA, DHA and ARA, with *Symbiodinium* density could suggest less healthy FA profiles could be related to high *Symbiodinium* densities. These FA are essential for general physiological functioning of the holobiont, including maintenance of symbiont populations, metabolism, immune response and respiration (Stimson 1987; Ward 1995; Yamashiro et al. 1999; Hulbert 2003).

Conclusions

This study presented a comprehensive assessment of the FA composition of *A. tenuis* colonies along inshore water quality gradients of the GBR, identifying variation in n-3 and n-6 PUFA percentage composition associated with variation in both water quality and seasonal factors. FA composition was also spatially distinct over geographical distances at the regional scale (i.e. Burdekin versus Whitsunday regions). Ratios of essential FA respond to fluctuations in both water quality and seasonal factors associated with wet versus dry seasons, suggesting that they are good candidates for indicators of coral holobiont health. Although there are other coral health indicators that may be easier and cheaper to measure, FA play a vital role in coral metabolism and stress resistance and are necessary for physiological processes, such as immune responses and cellular integrity. FA profiles provide additional depth and insights into the functioning of underlying mechanisms. Therefore, FA may not be a practical tool for large-scale monitoring but can provide deep insights into coral holobiont health and its interaction with the environment in more focused studies. Future

research should aim to further discern the influence of autotrophic versus heterotrophic nutritional sources on FA profiles, as well as investigate how FA composition changes in the face of novel and/or stressful environmental conditions.

CHAPTER 4: Transplanting corals from a high to a low turbidity environment results in decreased colony area and structural biomass in *Acropora tenuis*

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4.1 Synopsis

The degradation of coral reefs, specifically the loss of structural biomass created by coral skeletons, is an important issue in coral reef science. In this study, the mean size of coral colonies transplanted from a high turbidity to a low turbidity environment declined dramatically after 4 months in the novel environment. Specifically, the mean size of coral colonies originating from the highly turbid Geoffrey Bay site (B1; Magnetic Island, Australia, ~ 8 km offshore) and transplanted to a more clear-water site at Pelorus Island (B3; Palm Islands, Australia, ~ 16 km offshore) declined ~ 70 % when compared to mean colony size at the beginning of the study. In contrast, control colonies (B1 corals remaining at B1) increased ~ 70 % in size, and both B3 controls (B3 corals remaining at B3) and transplants (B3 corals transplanted to B1) increased in size, albeit to a lesser extent. These results are consistent with higher predation on corals originating from a turbid environment when they are transplanted into a less turbid environment, resulting in substantial loss of skeletal structure. Selective predation on turbid-water corals suggests marked intraspecific differences in the physiological condition of coral colonies, strengthening the need for detailed investigations of the underlying causes, as well as the consequences of skeletal loss in an important branching species of coral, *Acropora tenuis*.

4.2 Introduction

Recent declines in coral cover along the Great Barrier Reef (GBR; De'ath et al. 2012) raise the need for further exploration of causes and consequences of partial colony mortality and loss of structural biomass in corals. Biotic interactions between corallivores and coral species are important factors affecting the growth, survival, and replenishment of coral populations (Neudecker 1979), and some corallivorous fish are known to remove coral biomass (e.g. Rotjan and Lewis 2008; Bonaldo et al. 2011). Along the GBR, the primary obligate consumers of coral biomass are butterflyfishes and the nominally herbivorous parrotfishes, although other corallivorous fish species from different families (e.g. Tetraodontidae, Monacanthidae) and phyla (e.g. *Acanthaster planci*, Drupella spp.) are also important (Cole et al. 2008; Bonaldo et al. 2011, 2012). Butterflyfishes principally feed on coral mucus or single coral polyp tissues (Cole et al. 2008); however, some species (e.g., *Chaetodon unimaculatus*) can remove skeletal material in addition to soft tissues (Motta 1980). Parrotfish species, conversely, remove large portions of coral colonies, which include both tissue and skeletal biomass (Bruckner and Bruckner 1998; Rotjan and Lewis 2008; Bonaldo et al. 2011). Among parrotfishes, scraping species remove only the outer tissue layer and marginal parts of skeletal material, while excavating species remove both tissue and large sections of skeletal material (Bonaldo et al. 2012).

Both the morphology and species of coral influence the functional type of corallivores targeting coral colonies; however, the fundamental basis of selective feeding is largely unknown (Pratchett 2007; Cole et al. 2008). Characteristics that dictate corallivore preferences include, but are not limited to, gross coral morphology (e.g. Hobbs 2013), presence of diseased or damaged tissues (e.g. Hoeksema et al. 2013), types of symbiont associations and their densities (e.g. Rotjan et al. 2006), and the extent of lipid or energy reserves (e.g. Rotjan and Lewis 2009).

On the GBR, corals in coastal or inner-shelf regions are subjected to many adverse influences, including elevated temperatures (Berkelmans 2002), high turbidity (Anthony 2006; Fabricius et al. 2013b), enhanced nutrients (De'ath and Fabricius 2010), and higher numbers of internal macroborers (Risk et al. 1995; Grand and Fabricius 2010). These conditions have induced a suite of phenotypic responses (Anthony and Fabricius 2000; Pisapia et al. 2012) and/or genotypic adaptations (Bay et al. 2009b, 2013), leading to intraspecific variation in the physiology of corals with increasing distance from the coast. Thus, the question arises, "Do these physiological differences affect interactions between corals and their corallivores?" This study is a preliminary exploration of the impact of transplantation on intraspecific variation in colony area and structural biomass of *Acropora tenuis*. Results strengthen the need for further investigations of the biological attributes of corals from inshore environments and the consequences of coral predation by corallivorous fishes for coral populations and reef ecosystems.

4.3 Methods

In a large reciprocal transplant study, 20 partial colonies of the coral *Acropora tenuis* (~ 10 cm^2) were collected from Geoffrey Bay (B1; Magnetic Island, Australia, ~ 8 km offshore) and 19 partial colonies were collected from the northwest, leeward corner of Pelorus Island (B3; Palm Island Group, Australia, ~ 16 km offshore; Fig. 4.1). All colonies collected were divided in half to produce genetically identical fragments for use as cross-transplants (corals moved from their site of origin to the transplant location) and back-transplants (corals moved from their source location back to their source location; cf. Barshis et al. 2010) in February 2013. Colonies for cross- and back-transplanting were collected from 2 - 4 m depth at both sites, held on board the research vessel to control for transplantation/handling stress, and haphazardly mounted onto a single wire-mesh rack

(following Berkelmans and van Oppen 2006), which was placed onto the reef at 2 m depth at each site. Corals at these two sites typically experience differences in turbidity levels (Thompson et al. 2011); the coral population at site B1 is closer to the coast and the mouth of the Burdekin River and typically experiences higher levels of turbidity.

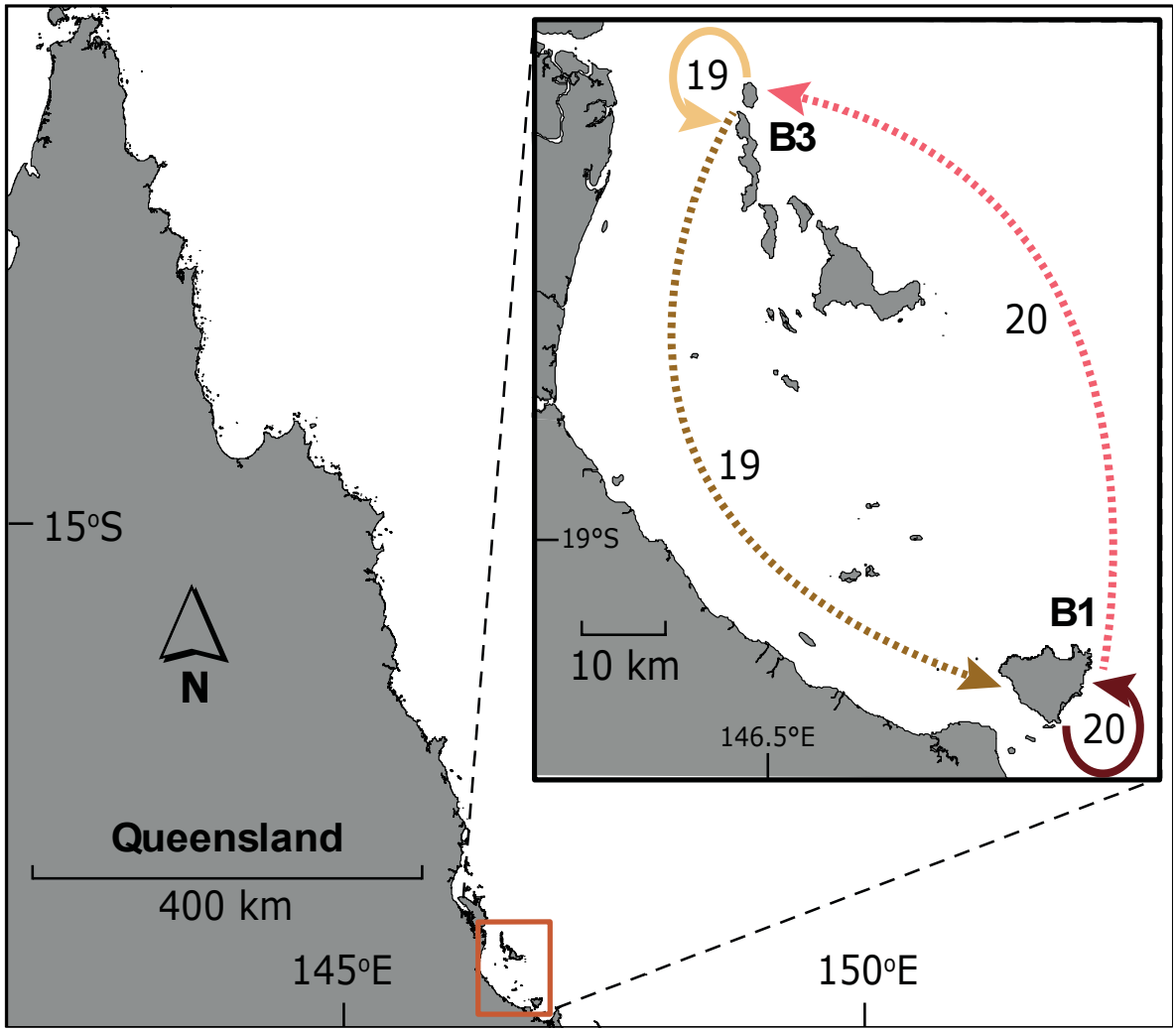


Fig. 4.1 Map of the coast of Queensland, Australia and the inshore sites on the Great Barrier Reef used for the reciprocal transplant study of *Acropora tenuis*. Colonies for back-transplanting (solid arrows) and cross-transplanting (dashed arrows) were from B1 (Geoffrey Bay) and B3 (Pelorus Island). Numbers indicate original deployment sample sizes.

All colonies were assessed for partial mortality and signs of stress (e.g. bleaching) after two weeks (mid-February 2013) and four months (June 2013) using *in situ* observations and photographic records (Fig. 4.2). The initial assessment at two weeks was performed to ensure that no mortality had occurred directly due to transplantation stress. Three B1 colonies and one B3 colony were subsequently lost from the B3 site (due to abiotic dislodgement or predation) and excluded from subsequent analyses. Loss of structural biomass was readily identified by missing branches in sequential photographs (Fig. 4.3). Changes in the two-dimensional area of colonies between monitoring periods were measured by tracing planar outlines of each colony in ImageJ (version 1.46r, National Institutes of Health, USA). *In situ* visual assessments suggested that little to no loss of structural biomass occurred from the base of colonies.

Colony area estimates from February and June were regressed against their initial area measurement (prior to transplantation) to account for differences in growth attributable to differences in initial colony size. The residuals of the regressions were compared using two-way analyses of variance (ANOVAs), with site of origin (B1 or B3) and transplant treatment (transplanted or control) as main effects for the February and June measurements. Tukey's HSD was applied post-hoc to reveal homogenous classes. All analyses were performed on square-root transformed data to meet assumptions of normality and homoscedasticity of variance. All analyses were performed using the software R 2.15.3 (R Core Team 2013).

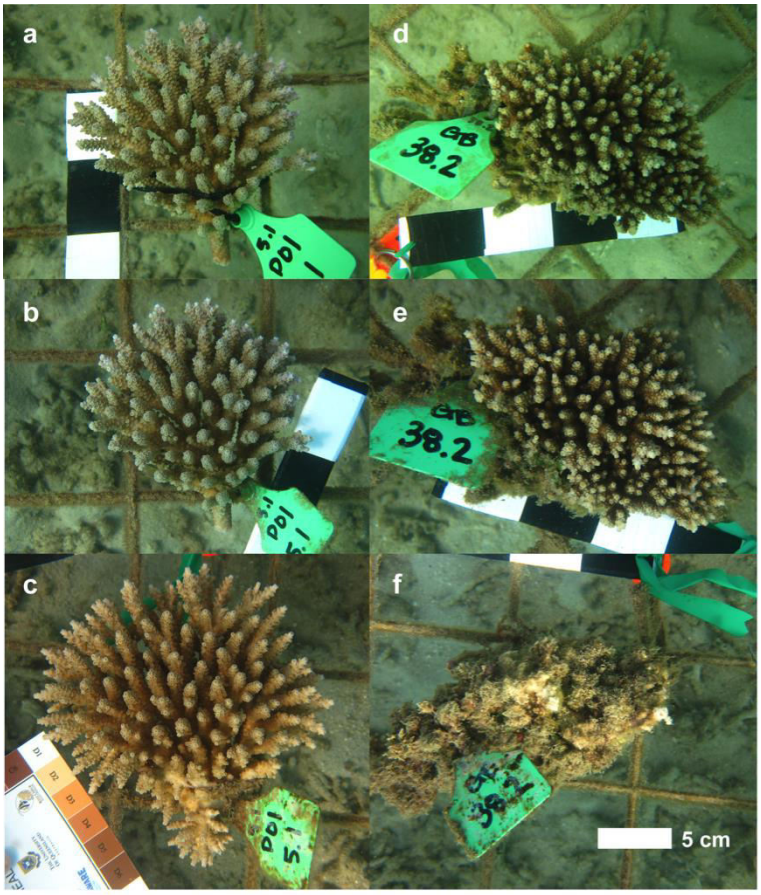


Fig. 4.2 Photographic time series for two colonies of *Acropora tenuis* sourced from Pelorus Island (B3; a, b, c) and Geoffrey Bay (B1; d, e, f) from initial deployment at Pelorus Island at the beginning of February 2013 (a, d), mid-February 2013 (b, e), and June 2013 (c, f). Loss of structural biomass through time is clearly illustrated for the B1 colony (cf. d vs f), whereas the B3 colony has increased in size over the 4 months (cf. a vs c). Scale bar is consistent for all photographs.

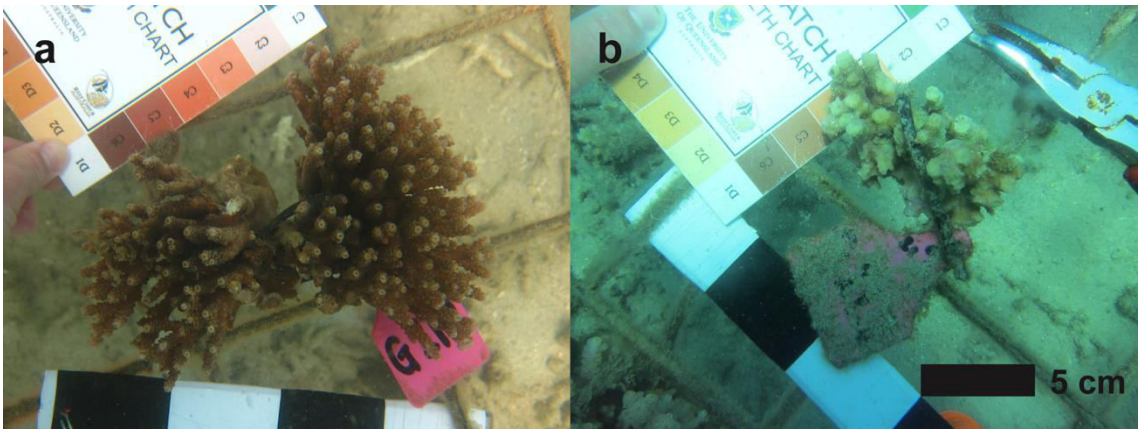


Fig. 4.3 Photographs illustrating loss of structural biomass in a colony of *Acropora tenuis* sourced from B1 and deployed at B3 in (a) February 2013, and (b) June 2013.

4.4 Results and discussion

This study revealed that changes in the mean planar area of experimental colonies of *Acropora tenuis* over a 4-month period (February to June 2013) differed significantly between transplant treatments (controls versus transplants: $F_{1,70} = 57.8$, $P < 0.001$). However, patterns in colony size change for controls versus transplants differed at the two sites of origin (Origin × Treatment: $F_{1,70} = 77.0$, $P < 0.001$). Site of origin alone had no significant effect on changes in colony area (Origin: $F_{1,70} = 3.1$, $P = 0.085$). Corals transplanted from the turbid B1 site to the clearer-water B3 site were the only ones whose planar area declined over time (by -128.73 ± 12.2 cm^2 mean ± SE); Tukey's HSD post-hoc tests confirmed that the change in planar area of this experimental group differed significantly from those of corals in all other treatments (Fig. 4.4). Interestingly, changes in colony area of control corals at the turbid B1 site were at least 4.7-fold greater than area changes for all other experimental groups. No statistically significant effects of transplant treatment or site of origin on area changes were detected for the initial 2-week period (beginning to mid-February 2013; Origin: $F_{1,70} = 0.2$, $P = 0.631$; Treatment: $F_{1,70} = 2.4$, $P = 0.120$; Origin × Treatment: $F_{1,70} = 0.6$, $P = 0.434$). Changes in colony size over the 4-month period suggest that transplantation of colonies from B1 to B3 triggered a significant loss in the structural biomass of these corals. Given that no changes occurred within the first two weeks after transplantation, initial stress does not appear to be responsible for the observed pattern. Instead, results suggest that extrinsic factors at Pelorus Island (B3 driving skeletal loss in corals transplanted from B1.

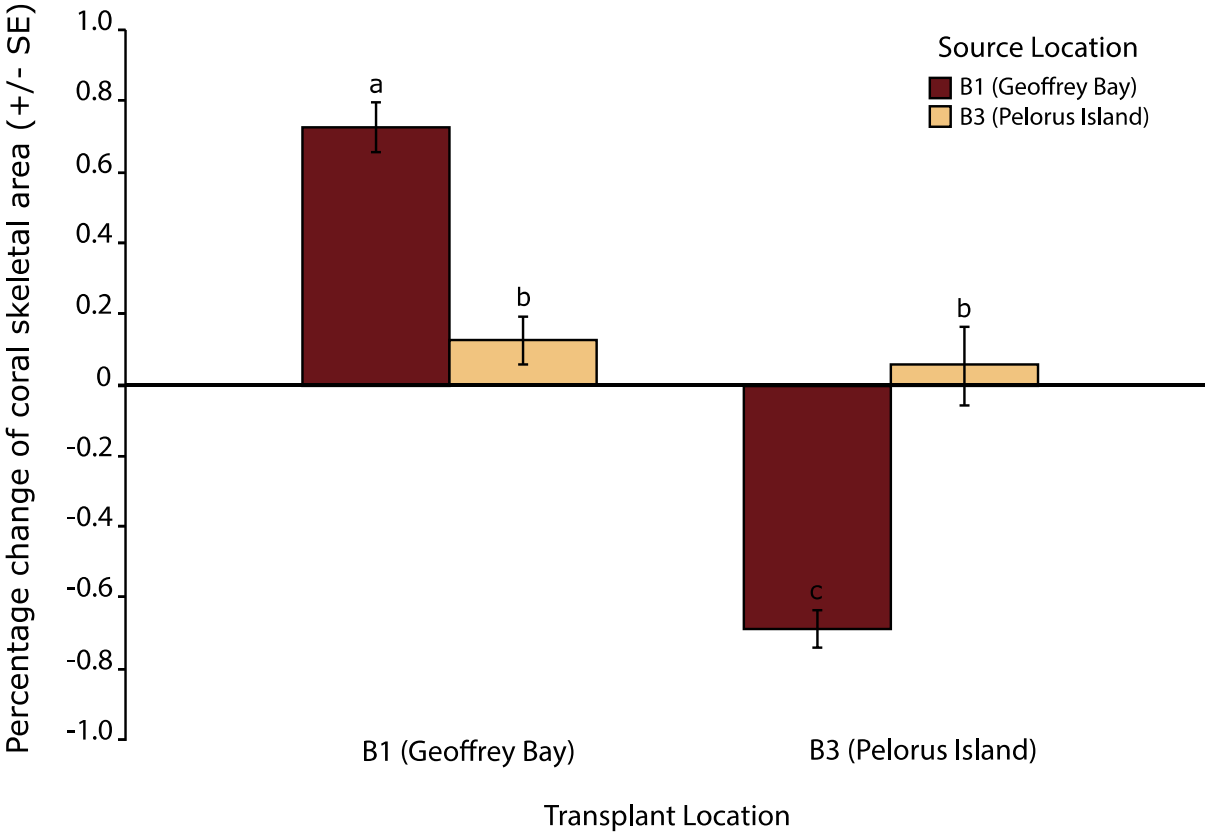


Fig. 4.4 Mean change in the planar area of coral colonies after 4 months, expressed as a percentage of initial colony area ± SE, for corals fragmented and reciprocally transplanted between a more turbid site (B1) and a less turbid site (B3). Letters indicate homogenous groups identified by Tukey's HSD.

These results raise two important questions: 1) What are the underlying factors driving loss of structural biomass in colonies from B1 (Geoffrey Bay) at B3 (Pelorus Island)? and 2) What are the possible consequences for coral populations, given predicted environmental changes in the future? There are several possible explanations for loss of structural biomass in turbid-water corals at a clearer-water site. Skeletal loss could be due to a transplant effect; however, since measures were taken to monitor and account for stress associated with transplantation, and no losses in colony area were detected within the first two weeks, this is unlikely. Human-induced damage (Hawkins and Roberts 1992) is another possible cause of skeletal loss; yet, this is also doubtful as human destruction is usually indiscriminate, whilst the observed patterns of colony area loss appear to be selective; B1 corals transplanted to B3 were affected exclusively. Coral disease, specifically skeletal eroding band (SEB), could be a potential cause for skeletal loss as it can colonise exposed coral skeleton; however this disease typically causes slow skeletal erosion and is not sufficient on its own to cause tissue mortality followed by substantial skeletal loss (Page and Willis 2008). Macroborers are also an unlikely cause of the substantial loss in structural biomass, given the short duration of the experiment, which does not conform with the timeframe of previous studies reporting substantial bioerosion (e.g., Risk et al. 1995; Tribollet et al. 2002). Similarly, corallivorous invertebrates, including the crown-of-thorns seastar and *Drupella* spp., target coral tissue, but they leave the skeletal structure largely intact (Cumming and McCorry 1988; Pratchett 2007). Thus, the most parsimonious explanation for the skeletal loss observed in this study is selective predation by corallivorous fishes (Neudecker 1979). Among fish, it is unlikely that butterflyfishes inflicted the damage observed, as these species usually cause little or no skeletal loss. Instead, the extent of skeletal loss suggests that larger piscine corallivores, specifically parrotfishes or

tetraodontiform species from B3 may be responsible for the observed pattern of selectively targeting transplanted corals from B1.

Factors underpinning selection of corals by large corallivorous fishes have been studied in the Caribbean, where overall nutritional content, reproductive structures, symbiont type and densities, high abundance of macroborers, skeletal hardness and chemical/physical defense mechanisms may underlie parrotfish foraging patterns (Littler et al. 1989; Gochfeld 2004; Rotjan and Lewis 2005, 2009; Rotjan et al. 2006; Rotjan and Dimond 2010). Higher nutritional gain may be a reason for the clear preference for corals from the more turbid B1 site. On the GBR, lipid stores within corals have been found to be 2-fold higher in *Acropora* on inshore reefs compared to offshore reefs (Anthony 2006). While environmental gradients are likely to be less pronounced between the two inshore locations in this study, B1 corals had higher total lipid content (13.6 ± 1.80 mg lipid g dw^{-1}) and ash-free dry weight (7.4 ± 0.20 %) compared to B3 corals (7.13 ± 1.13 mg lipid g dw^{-1} and 5.6 ± 0.31 %, respectively; ***Chapter 2***), making these colonies a more lucrative food source.

Higher symbiont densities or different symbiont types may fortify this effect, as corals from nearshore B1 had 3 times higher densities of *Symbiodinium* compared to those at the B3 site (***Chapter 2***). B1 corals are known to harbour different *Symbiodinium* types than B3 corals (C1-dominant at B1 and C2-dominant at B3; ***Chapter 2***; Abrego et al. 2009). In addition, the abundance of macroborers, which is known to be higher in turbid, inshore environments (Risk et al. 1995), may influence the nutritional content of B1 colonies. Thus, if large corallivorous fishes caused the loss of structural biomass, these fishes may have selected corals from B1 due to fundamental physiological differences, suggesting high intraspecific variation between colonies from two coastal environments.

The removal of dead and live coral biomass is a key functional process on coral reefs. This study reveals striking intraspecific differences in the loss of structural biomass in

Acropora tenuis following transplantation of colonies to a new environment. The extent and selectiveness of the damage suggest that corallivorous fishes may have driven the pattern by preferentially feeding on *A. tenuis* colonies originating from a turbid environment. Thus, corals exposed to a new environment, possibly including a different suite of predators, may be heavily targeted and subsequently experience significant reductions in their reproductive output or suffer complete mortality.

It is not yet known how projected changes in environmental conditions will affect the physiology of scleractinian corals or the behaviour of coral-associated fauna. Presumably, all coral species at different sites (e.g. longitudinally and latitudinally) will undergo physiological changes; yet, inshore corals may be most affected and hence may be required to acclimatise and/or adapt at a faster pace. These findings emphasise the need for detailed assessments of physiological variation in the biochemical properties of corals among different environments and the ecological consequences arising from such variation.

CHAPTER 5: Plasticity in gene expression and fatty acid profiles of *Acropora tenuis* reciprocally transplanted between two water quality regimes in the central Great Barrier Reef

5.1 Synopsis

To investigate plasticity in biochemical health attributes of corals, genetically identical fragments from two inshore populations of the coral *Acropora tenuis* were reciprocally transplanted between native and novel water quality (WQ) regimes for four months. Variation in global gene expression (GE) and fatty acid (FA) composition of surviving experimental fragments was also quantified. Major FA classes, with the exception of short-chain polyunsaturated fatty acids, decreased in concentration when corals were transplanted from comparatively good to moderate WQ environments, converging on values characterising native corals at the poor WQ site. Conversely, moderate WQ corals transplanted to the good WQ environment increased concentrations of these FA classes, demonstrating phenotypic plasticity in FA composition. In contrast, significant differences in GE profiles persisted between corals from the two source populations, regardless of location (novel versus native sites). For corals sourced from moderate WQ, profiles were enriched with higher relative expression of genes associated with translation, ribosome biogenesis and ribosome cellular components, compared to corals sourced from relatively good WQ. One cluster of co-expressed genes was positively correlated with multiple individual FA, and included genes involved in developmental processes and cellular pathways. However, there was also plasticity in the expression of genes relating to health and immunity in response to environmental change. Results indicate both a strong influence of source population on the expression of genes involved in fundamental biochemical pathways and plasticity in other genes relating to coral health in a novel environment. Phenotypic plasticity in FA

composition and gene expression demonstrate that *A. tenuis* has some capacity to respond to water quality stressors over a relatively short timeframe.

5.2 Introduction

On Australia's Great Barrier Reef (GBR), nearshore reefs are predicted to be the most affected by local environmental and anthropogenic stressors (Wooldridge 2009; Brodie et al. 2010), as these corals are subjected to higher temperatures (Berkelmans 2002), turbidity (Anthony, 2006; Fabricius et al., 2013) and dissolved nutrients (De'ath and Fabricius 2010) than corals on offshore reefs. To distinguish the effects of chronic versus acute exposure to these stressors, biomarkers are needed to monitor and characterise coral health. Biochemical attributes and gene expression profiles of corals hold promise for measuring how local environmental conditions influence the health of inshore populations, but further baseline work is needed to understand the potential for corals from different source populations to vary these attributes in response to variation in environmental conditions.

Organisms can optimise their survival and performance under local environmental regimes through physiological acclimatisation and/or genetic adaptation. Physiological acclimatisation describes the capacity of an organism to tune its biochemical attributes and physiological performance to a variety of environmental conditions within its lifetime and is also referred to as phenotypic plasticity (Coles and Brown 2003; Weis 2010; Brown and Cossins 2011; Sanford and Kelly 2011). Epigenetic modifications of DNA that affect transcriptional regulation, for example methylation or histone modification, can underpin phenotypic plasticity and can potentially be inherited (Feil and Fraga 2012), thereby blurring the distinction between acclimatisation and adaptation. Genetic adaptation is the result of natural selection, whereby over time, populations optimise fitness traits under local environmental conditions (e.g. Sanford and Kelly, 2011). Locally-adapted native genotypes, therefore, are predicted to have greater fitness than foreign genotypes under native conditions and this effect can be measured as a genotype-environment interaction (Kawecki and Ebert 2004; Brown and Cossins 2011). Physiological acclimatisation and/or genetic adaptation can

enable corals to survive in changing local environmental regimes, such as deteriorating water quality, and in response to global environmental changes under climate change (Donner et al. 2005).

Reciprocal transplant experiments can reveal the capacity of wild populations to respond to novel conditions. Being modular, coral colonies can be fragmented into identical genotypes. Environmental effects can then be measured on the same genotype, thus controlling for longer-term prior environmental history and genotype (Howells et al. 2013; Kenkel et al. 2015). These designs can distinguish between acclimatisation versus local adaptation as the mechanism underlying the phenotypic traits expressed, e.g., biochemical or physiological traits, by revealing either (1) differences in the performance of corals from different source populations regardless of environmental conditions (source effects), indicating no plasticity in phenotypic responses (Fig. 5.1a); or (2) plasticity in the performance of corals depending on the environment (environmental effects), leading to similarly expressed phenotypes at a given location through acclimatisation of foreign genotypes to local conditions (Fig. 5.1b); or (3) a source × environment interaction, indicating local adaptation (Fig. 5.1c; Coles and Brown 2003; Brown and Cossins 2011; Sanford and Kelly 2011). In reciprocal transplant studies involving adult organisms, it should be acknowledged that a strong source effect or a source × environment interaction could also arise as a consequence of long term acclimatisation or developmental canalisation, which would not be distinguishable from local adaptation with this design. A capacity for phenotypic plasticity in key biochemical and physiological traits is important in the life histories of corals facing environmental change.

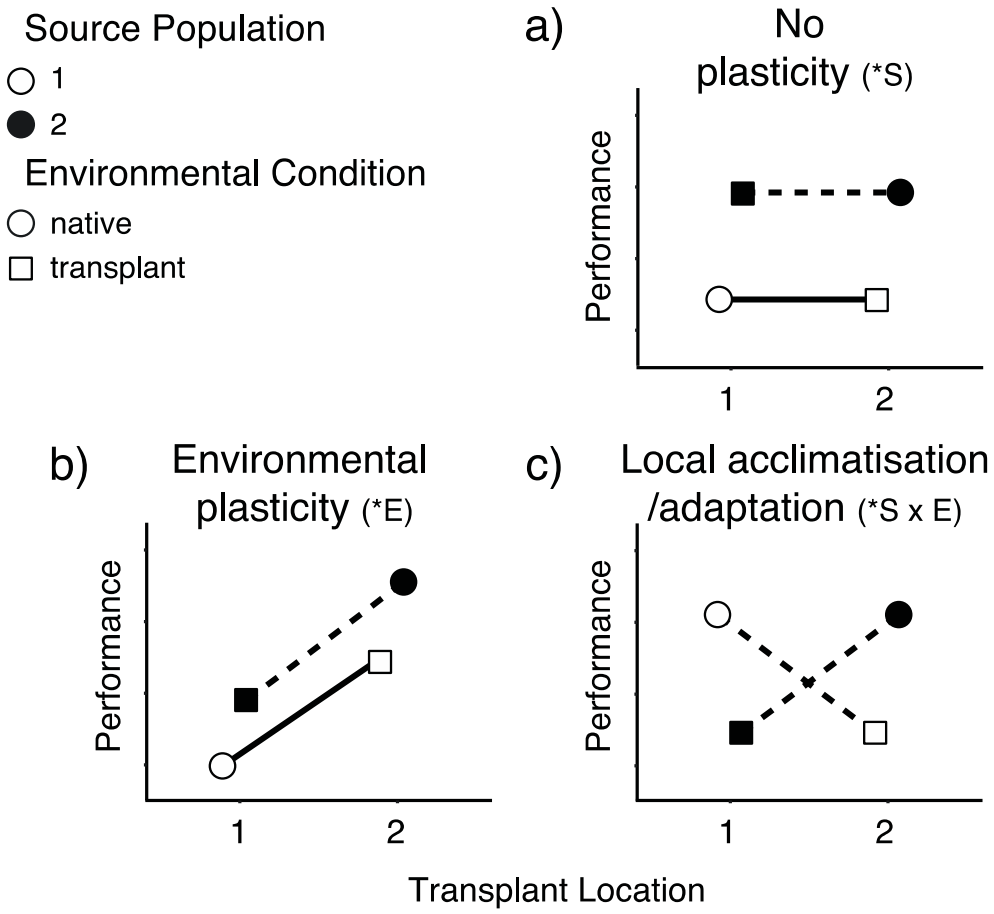


Fig. 5.1 Potential phenotypic responses revealed by a reciprocal transplant design: (a) fixed differences in the performance of corals from different source populations regardless of environmental exposure (source effect), indicating no plasticity in phenotypic responses; (b) plasticity in the performance of corals depending on the environment (environmental effect), leading to similarly expressed phenotypes at a given location and acclimatisation of foreign genotypes to local conditions; and (c) a source by environment interaction, indicating local adaptation arising from genetic effects or potentially through developmental canalisation.

Analyses of gene expression (GE) can provide insights into biological processes, molecular functions and cellular components that support healthy functioning of the coral holobiont (Bay et al. 2009a, 2013; Moya et al. 2012; Kenkel et al. 2014; Rocker et al. 2015; Wright et al. 2015). Global GE assesses a multivariate, molecular phenotype with no *a priori* information required and can be used to describe the physiological state of, or physiological processes occurring within, that organism (Dixon et al. 2015). These data can also be used to describe temporal changes in expression occurring within an organism (Barshis et al. 2013) through processes such as up-regulation or front-loading of genes. Global GE is a useful tool for assessing the molecular drivers of biochemical and physiological changes induced by environmental stressors in corals. Detection of changes in gene expression and functional pathways can help to interpret the status and trends in coral health in response to stress.

The aim of this study was to determine the extent to which coral populations can acclimatise to different environmental regimes (specifically good water quality and moderate water quality) when genotypes are reciprocally exposed to novel and native water quality environments over a four-month period. To partition the effects of source population genetics (and/or canalisation) and environmentally-induced phenotypic plasticity, variation in global GE and relative fatty acid (FA) composition (i.e. percentages and concentrations) of surviving fragments of experimental colonies was quantified. Analyses of differential gene expression (using DESeq) and a weighted gene correlation network analysis (WGCNA), which correlated variation in FA composition and global GE profiles, were used to explore differential gene expression patterns and the potential molecular mechanisms underpinning variation in biochemical and population attributes and growth. The genetic and biochemical responses of inshore corals to variation in water quality environments can aid in understanding the ability of corals to acclimatise to future deteriorated or ameliorated conditions.

5.3 Methods

Study sites and sampling design

This study was conducted in conjunction with the reciprocal transplant study described in *Chapter 4*, which assessed total change in area and loss of structural biomass for colonies of *Acropora tenuis* transplanted between Geoffrey Bay on Magnetic Island (B1), a moderate water quality environment, and Pelorus Island (B3), a good water quality environment (water quality environments as defined by Thompson et al. (2014a)). Briefly, twenty and nineteen partial colonies of the coral *A. tenuis* were collected from B1 and B3, respectively. All colonies collected were halved to produce fragments approximately 10 × 10 cm^2. One genetically identical fragment was left at the native site (source population) and the second was moved to the novel site (transplant location; see Fig. 4.1). All fragments spent approximately equal time on a vessel to control for handling stress (see Section 4.3 for further details).

The experiment commenced in early February 2013 and was inspected after two weeks (mid-February 2013) to quantify stress and mortality from experimental handling and transplantation. Final sampling occurred after 4 months, in June 2013, for both FA composition (i.e. percentage of total FA and absolute concentrations) and GE analysis. Mortality occurred, likely from selective predation on corals transplanted from B1 to B3 (see ***Chapter 4***). Small samples (< 2 cm long branches) were collected for genetic analyses from ten B1-to-B3 transplants in June 2013; samples from five of these transplants provided sufficient material for biochemical analyses. For all other treatments, one branch 2 - 6 cm long was collected from the middle of each colony at each site, at each sampling time point, and used for both FA and GE analyses. Samples were immediately snap-frozen in liquid nitrogen (-80 °C) for further analyses.

Quantification of fatty acid concentration

Methods for the lipid and FA analyses undertaken in this study are described in *Chapters 2* and *3*. Briefly, single replicate branch samples were subsampled to quantify ash-free dry weight and total lipid and FA composition following Fitt et al. (2000) and Conlan et al. (2014), respectively. Branches were crushed using a pneumatic press, ground to a fine powder on liquid nitrogen and freeze-dried for 48 hours for total lipid and subsequent FA analyses.

Samples were further processed for total lipid analyses (mg lipid g dw^{-1}) using methods described in ***Chapter 2,*** followed by quantification of FA composition as described in ***Chapter 3***. FA concentrations were standardised to weight of total lipids and expressed as mg FA g $lipid^{-1}$ for quantitative comparisons. As in ***Chapter 3,*** individual FA concentrations, sum of the concentration of FA classes, percent composition of FA, and ratios of FA health indicators were calculated to explore source and transplant effects on FA composition among water quality environments.

Global coral gene expression

Tag-based RNA-Seq libraries were prepared following Meyer et al., (2011), with modifications for sequencing on the Illumina HiSeq 2500 platform. This methodology allows for deep sequencing and quantitative analyses of short cDNA reads in organisms without a reference genome (Meyer et al. 2011). The tag-based approach utilises small quantities of initial RNA, efficiently uses sequencing coverage and only requires an assembled transcriptome as a reference database (Meyer et al. 2011). Furthermore, this tag-based RNA-Seq method outperforms traditional RNA-sequencing for a fraction of the cost (Lohman et al. 2016).

Total RNA was extracted from ten genotypes from B1 and 11 genotypes from B3 at each site. All genotypes were sampled after four months of exposure to novel and native environmental conditions. Total RNA was extracted from the 42 samples with an Aurum™ Total RNA Mini Kit (Bio-Rad Laboratories, Hercules, California) following the manufacturer's protocol, with the modification that diluted DNase I was incubated on the filter membrane at room temperature for one hour. The concentration and purity of RNA were determined in each sample using Nanodrop 2000 (ThermoScientific) and a 1 % agarose gel to confirm two distinct ribosomal RNA bands. Samples were diluted to 100 ng μL^{-1} in Aurum™ Elution Solution (Bio-Rad, Hercules, California) for further analysis.

Sample preparation followed protocols from Meyer et al. (2011) and updates on the Matz website (http://www.bio.utexas.edu/research/matz_lab/matzlab/Methods.html, released for unrestricted use prior to this manuscript). Briefly, one mg of RNA per sample was fragmented at 95 °C for 12 minutes. Extent of RNA fragmentation was determined by a smear in the ribosomal RNA band region from 1.0 uL (~ 100 ng) of product on a 1 % agarose gel (30 minutes at 100 V). Oligonucleotide 3ILL-30TV (10 µM) primer was incorporated by incubating the fragmented RNA product and primer at 65 °C for three minutes, followed by reverse transcription to single strand cDNA in 10.0 µL reactions containing 1 X SMARTScribe Reverse Transcriptase (Clontech, Mountain View, California) and S-ILL-swMW 10 µM RNA oligonucleotide incubated at 42 °C for one hour and 65 °C for 15 minutes. Samples of first strand cDNA then underwent amplification to enrich gene concentrations in 50.0 µL reactions containing 1 X Titanium Taq Polymerase (Clontech, Mountain View, California), 10 µM 5ILL oligonucleotide, 10 µM 3ILL-30TV oligonucleotide and 100.0 ng first strand cDNA product. The amplification profile consisted of five minutes at 95 °C and 16 cycles of 40 seconds at 95 °C, two minutes at 60 °C, and one minute at 72 °C. The cDNA products were treated with PCR Kleen Spin Columns (Bio-Rad

Laboratories, Hercules, California) following the manufacturer's protocol to remove unincorporated dNTPs, primers and short primer-dimers.

Clean cDNA products were diluted to 5.0 ng μL^{-1} in 10mM tris HCl pH 8 prior to barcode labelling for sample identification. Sample barcoding mix was prepared by combining 10 X PCR buffer, 10 μM TrueSeqMpx2n Illumina Universal oligo, 1 μM barcode oligo, 1 X Titanium Taq Polymerase (Clontech, Mountain View, California), and 50 ng cDNA template. The cycling profile was customised to include a five-minute hot start at 95 °C, four cycles of 40 seconds at 95 °C, two minutes of 63 °C and one minute of 72 °C. Barcoded cDNA products were run on a 2 % agarose gel in 1 X TBE buffer, with SYBR Green I nucleic acid gel staining dye adding according to manufacturer's protocol for size selection (400 to 500 base pairs).

Bioinformatic analyses

A total of 42 libraries were sequenced on three lanes of the Illumina HiSeq 2500 (SE 1 × 50 base pairs) at the Genome Sequencing and Analysis Facility at the University of Texas at Austin. Samples were spread across lanes such that all combinations of source and transplant treatments were represented in each lane. On average, 9.8 million sequences were generated per library for a total of 391 million raw reads. Of these, reads without the 5'-Illumina leader sequence were discarded, and this leader was trimmed from remaining reads. The *fastx_toolkit* (http://hannonlab.cshl.edu/fastx_toolkit) was then used to trim the reads after a homopolymer run of 'A' ≥ 8 bases was encountered, retain reads with minimum sequence length of 20 bases, and quality filter them, which required a PHRED quality of at least 20 over 90 % of the sequence. 3.6 million reads per sample on average remained after quality filtering. Filtered reads were mapped to the *A. tenuis* reference transcriptome (http:// www.bio.utexas.edu/research/matz_lab/matzlab/Data.html, released for unrestricted use prior

to this manuscript) using Bowtie 2 (Langmead and Salzberg 2012). Overall, 61.7 million reads were mapped for all 42 samples, with 1.5 million mapped reads per sample on average. Read counts were assembled by isogroup (i.e. groups of sequences putatively originating from the same gene, or with sufficiently high sequence similarity to justify the assumption that they serve the same function) using a custom perl script (https://github.com/z0on/tag-based_RNAseq). This script discarded any PCR duplicates, which were defined as reads mapping to the same starting position in the reference and aligning with 100 % identity along the length of the shorter read. Reads mapping to multiple isogroups were also disregarded. In total, 1.3 million unique reads per sample, on average, were successfully mapped to 78,000 isogroups (Appendix C Suppl. Table 5.1). Isogroups with counts < 10 in more than 90 % of the samples (defined as low coverage reads; Langfelder and Horvath 2008) were removed, resulting in 25,000 isogroups remaining for statistical analysis (i.e. removal of 53,000 low coverage isogroups).

Statistical analyses

All analyses were performed using R 2.15.3 (R Core Team 2015). To test for differences in means of FA concentrations of source and transplant corals from the two populations, generalised linear mixed effect models (GLMMs) were used in the R package 'MASS' (Venables and Ripley 2002). The model was run with explanatory fixed effects of source and transplant locations and a random effect of genotype. Corals back-transplanted to their source site (B3) were set as 'intercept' in GLMM analyses due to low sample size (and large error margins) of B1 colonies transplanted to B3. GLMMs were performed on total lipid, FA classes and individual FA concentrations using Gamma error distributions to control for normality and homogeneity of variance. FA health indicator ratios were tested with Gaussian error distributions, as assumptions of normality and homogeneity of variance

were met. All model fits were assessed through examination of the distribution of Pearson residuals and fitted residuals in diagnostic plots.

Variations in FA percentage composition of corals among source populations and transplant locations were characterised with multivariate principal component analyses (PCA) using the package 'vegan' (Oksanen et al. 2015). Analyses of variance (ANOVAs) were performed on the four principal components considered important (i.e. when standard deviations were greater than 1.0), with two, fully crossed fixed factors – source population and transplant location.

Gene expression patterns of *A. tenuis* were *regularised logarithm* (rlog) transformed, in order to shrink together values of different samples for genes with low counts, while $\log_2$ transforming genes with high counts, using the package 'DESeq2' (Anders and Huber 2010). GE expression patterns were analysed using the 'WGCNA' package in R, following methods in in Langfelder and Horvath (2008). 'WGCNA' allows for analyses of large, high dimensional data sets, such as global gene expression, and correlates gene expression data with multiple traits of the samples (i.e. coral phenotypic and health attributes; Langfelder and Horvath 2008; Wright et al. 2015). Furthermore, 'categorical' traits or traits that are defined as experimental conditions (i.e. source populations, transplant location, etc.) can also be included and assessed through 'WGNCA', providing an alternative to traditional DESeq analyses. One outlying sample (P15.2; coral sourced from B3 and transplanted to B1) was detected from a sample network with a standardised connectivity score of < -2.5 and removed from further analyses (Appendix C Suppl. Fig. 5.1). Remaining samples were not considered outliers and hence retained in further analyses. A signed co-expression network was constructed using Pearson correlations of all genes across all treatments. Similarities in expression were transformed into connection strengths using a soft threshold power of 13, based on scale-free topology fit index. Linkage hierarchical clustering and a topological

overlap matrix were used to identify groups of genes (or network modules) with highly positive correlations of relative expression levels. Modules with > 85 % similar expression profiles and < 30 genes were merged. Coral source and transplant locations, coral colony area change (from ***Chapter 4***), individual FA concentrations and FA health indicator ratios were correlated to the uniquely identified co-expression modules. Gene ontology (GO) enrichment was performed on modules with significant correlations to coral attributes. If no significant GO terms were detected, differential expression of individual genes within the modules was examined directly.

5.4 Results

Fatty acid composition of source and transplant colonies of Acropora tenuis

A PCA of the FA percent compositions of reciprocally transplanted colonies of *Acropora tenuis* identified four important principal components that best represented variation in 17 individual FA and explained 51.5, 13.6, 11.1 and 6.0 % of the variance, respectively (Fig. 5.2; Table 5.1; Appendix C Suppl. Table 5.2; Suppl. Fig. 5.2). Source populations separated along PC1, with B1-sourced corals generally having negative PC1 scores and B3-sourced corals generally having positive PC1 scores (Fig. 5.2). Overall, B1-sourced corals were defined by higher percentages of FA 14:0, 16:0, 16:1n-7, 18:1n-9, 18:2n-6, 18:3n-6, 20:3n-6, 18:4n-3 and 22:6n-3 (negative PC1 values; Fig. 5.2). In contrast, B3-sourced corals were defined by higher percentages of FA 17:0, 18:0, 21:0, 20:1n-9, 20:5n-3, 22:5n-3, 20:4n-6 and 22:4n-6 (positive PC1 values; Fig. 5.2). Although significant variance was found to attribute to PC2 (13.6 %; Table 5.1), no significant effects, of source, transplant, nor their interaction, on FA percentage composition were found along PC2 (Appendix C Suppl. Table 5.2; Suppl. Fig. 5.2). PC3 separated variation attributable to significant source and transplant effects (Fig. 5.2; Appendix C Suppl. Table 5.2). Generally, coral colonies

transplanted to the B1 site had negative PC 3 scores, whereas coral colonies transplanted to the B3 site had positive PC3 scores. FA that characterised corals transplanted to the more turbid B1 environment were 18:0, 21:0, 18:1n-9, 20:1n-9, 18:3n-6, 20:3n-6, 22:4n-6, 18:4n-3, 22:5n-3 and 22:6n-3 (negative PC3 values; Fig. 5.2). Coral colonies transplanted to the clear water B3 environment were defined by 14:0, 16:0, 17:0, 16:1n-7, 18:2n-6, 20:4n-6 and 20:5n-3 (positive PC3 values; Fig. 5.2).

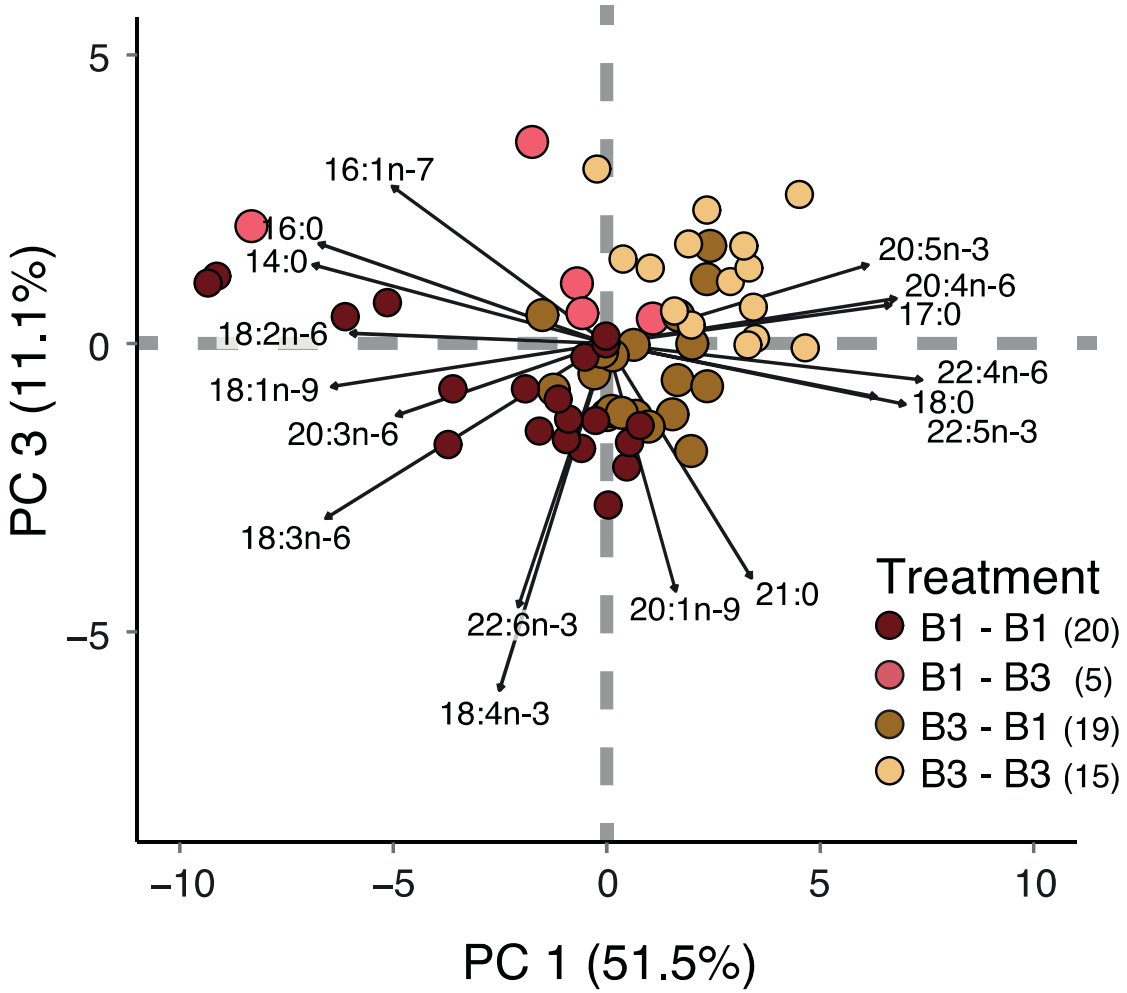


Fig. 5.2 Biplot of principal component analysis of 17 FA measured in coral colonies reciprocally transplanted between Geoffrey Bay (B1) and Pelorus Island (B3), located in the Burdekin region, in June 2014. PC1 and PC3 are plotted as indicators of source and transplant effects, respectively. FA are expressed as percentage composition of total FA. Lettering for treatments denote 'source population – transplant location'. Sample sizes for FA analyses are in parentheses.

Table 5.1 Standard deviation and proportion of variance explained in a principal component analysis (PCA) of the percentage composition of 17 FA quantified in *Acropora tenuis* reciprocally transplanted between B1 and B3 within the Burdekin region. * denotes important principal components defined by > 1.0 standard deviation.

Importance of components				
	PC1	*PC2*	*PC3*	*PC4*
Standard deviation	2.96 *	1.52*	1.38*	1.01*
Proportion of variance	0.515	0.136	0.111	0.060
Cumulative proportion	0.515	0.651	0.762	0.823

Significant effects of transplantation to a novel environment were found, despite the large standard errors (SE) associated with mean FA concentrations of the B1 to B3 transplants. SE's in this treatment are large because of the small number of samples retrieved as consequence of selective predation on this group of corals (see ***Chapter 4***; Fig. 5.3; Table 5.2; Appendix C Suppl. Fig. 5.3; Suppl. Fig. 5.4; Suppl. Fig. 5.5; Suppl. Table 5.3). In general, coral transplants adjusted their FA concentrations to local concentrations, with most FA classes significantly higher at B3 than B1 (Fig 5.3a-f). In contrast, short-chain polyunsaturated fatty acids (SC PUFA), which can be broken down into short-chain omega-3 polyunsaturated fatty acids (SC n-3 PUFA) and short-chain omega-6 polyunsaturated fatty acids (SC n-6 PUFA), displayed source by transplant effects that were significant in two comparisons (Table 5.2). In these latter two cases, mean concentrations of SC PUFA and SC n-6 PUFA were greater at B3 for corals sourced from moderate water quality (B1-sourced corals), but greater at B1 for more clear-water corals (B3-sourced corals; Fig. 5.3g, i but not 5.3h).

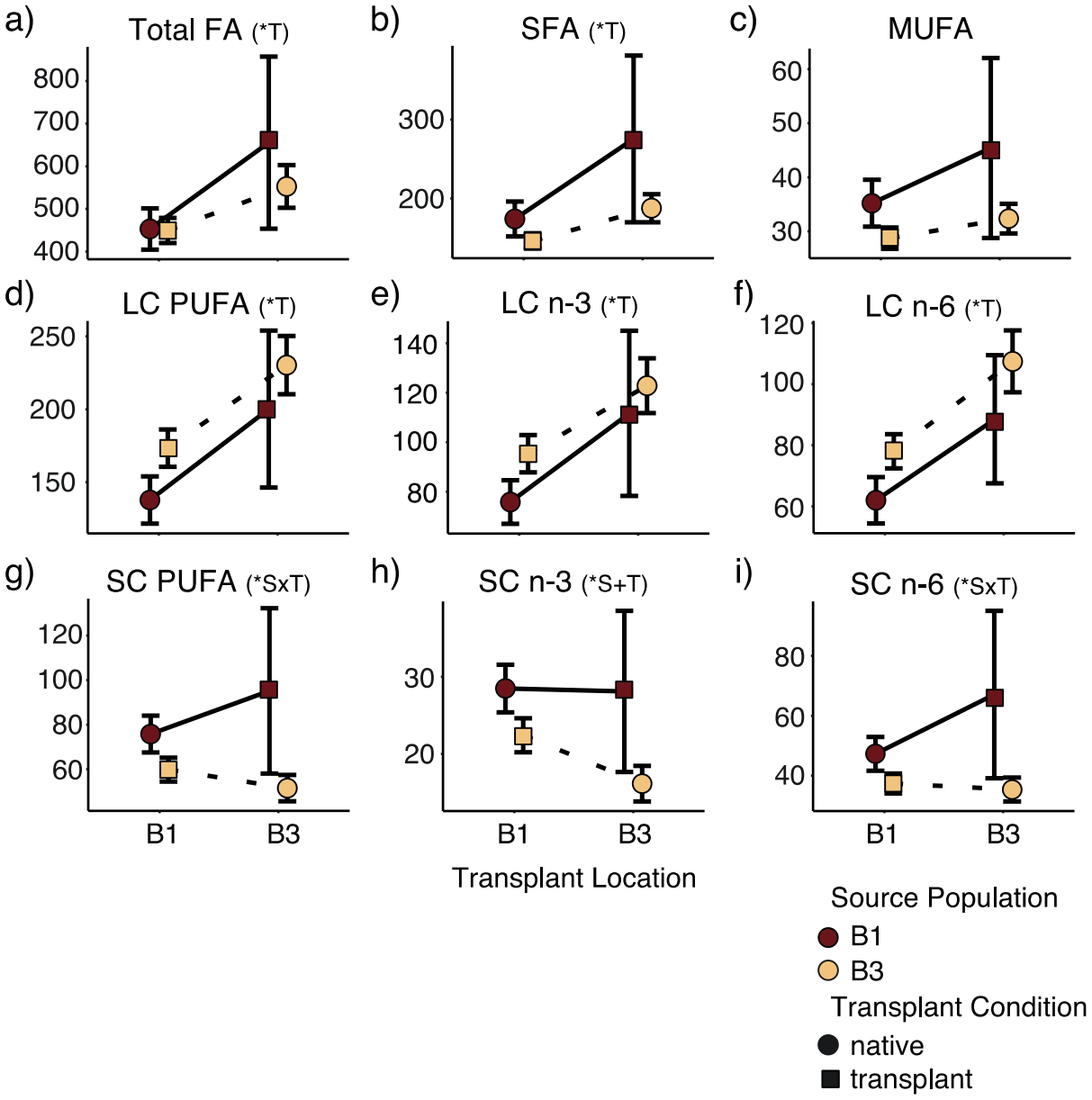


Fig. 5.3 Mean concentrations (± SE) of total FA and eight FA classes for corals reciprocally transplanted between B1 (Geoffrey Bay) and B3 (Pelorus Island) in June 2014. Source population is indicated by the colour of the symbols: B1 (dark red) and B3 (light yellow). Transplant treatment is indicated by the shape of symbols: native (O) and transplant (□). Concentrations of FA classes are standardised to mg FA g lipid^{-1}. Sample sizes for FA analyses: native B1 (n = 20), transplanted B1 (n = 5), native B3 (n = 15) and transplanted B3 (n = 19).

Table 5.2 Generalised linear mixed model (GLMMs) analyses of FA class concentrations (mg FA g lipid^{-1}; June 2014 samples) to determine the effects of reciprocally transplanting colonies of *Acropora tenuis* between Burdekin sites B1 and B3, with genotype considered a random effect. FA classes use a Gamma error distribution and the "intercept" parameter corresponds to the predicted response variable for native coral colonies at B3. *** denotes significance at α = 0.001. ** denotes significance at α = 0.01. * denotes significance at α = 0.05.

Total Fatty Acids					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.0020	0.0002	8.185	<0.001	***
Source (B1)	-0.0005	0.0004	-1.180	0.238	
Transplant (B1)	0.0004	0.0002	2.307	0.021	*
Source*Transplant (B1)	0.0005	0.0004	1.592	0.112	
Saturated Fatty Acids					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.0059	0.0008	7.477	<0.001	***
Source (B1)	-0.0022	0.0013	-1.676	0.094	
Transplant (B1)	0.0016	0.0005	2.783	0.005	**
Source*Transplant (B1)	0.0020	0.0011	1.854	0.064	
Monounsaturated Fatty Acids					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.0336	0.0042	8.074	<0.001	***
Source (B1)	-0.0109	0.0067	-1.630	0.103	
Transplant (B1)	0.0047	0.0029	1.570	0.117	
Source*Transplant (B1)	0.0095	0.0055	1.736	0.825	
Long Chain Polyunsaturated Fatty Acids					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.0048	0.0007	7.149	<0.001	***
Source (B1)	0.0002	0.0012	0.157	0.875	
Transplant (B1)	0.0015	0.0005	2.922	0.003	**
Source*Transplant (B1)	0.0016	0.0012	1.355	0.175	
Long Chain Omega-3 Fatty Acids					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.0090	0.0013	7.039	<0.001	***
Source (B1)	-0.0001	0.0023	-0.048	0.962	
Transplant (B1)	0.0025	0.0010	2.629	0.009	**
Source*Transplant (B1)	0.0033	0.0022	1.501	0.133	
Long Chain Omega-6 Fatty Acids					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.0103	0.0015	6.916	<0.001	***
Source (B1)	0.0010	0.0028	0.354	0.723	
Transplant (B1)	0.0036	0.0012	3.051	0.002	**
Source*Transplant (B1)	0.0030	0.0027	1.105	0.269	
Short Chain Polyunsaturated Fatty Acids					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.0207	0.0024	8.626	<0.001	***
Source (B1)	-0.0094	0.0036	-2.605	0.009	**
Transplant (B1)	-0.0023	0.0022	-1.060	0.289	
Source*Transplant (B1)	0.0070	0.0034	2.064	0.039	*
Short Chain Omega-3 Fatty Acids					

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.0647	0.0074	8.694	<0.001	***
Source (B1)	-0.0276	0.0111	-2.499	0.013	*
Transplant (B1)	-0.0156	0.0073	-2.150	0.032	*
Source*Transplant (B1)	0.0203	0.0108	1.882	0.060	
Short Chain Omega-6 Fatty Acids					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.0311	0.0038	8.282	<0.001	***
Source (B1)	-0.0142	0.0058	-2.474	0.013	*
Transplant (B1)	-0.0012	0.0032	-0.393	0.695	
Source*Transplant (B1)	0.0111	0.0051	2.163	0.031	*

Correlations between gene expression modules and coral attributes

According to DESeq analyses, 5,873 genes had a significant origin effect, no genes had a significant transplant effect, and 75 genes had an interactive origin × transplant effect (Fig. 5.4). This analysis found comparable qualitative patterns to 'WGCNA' analysis. Seventeen unique groups of genes or network modules were identified in linkage hierarchical clustering (modules correspond to the 17 colours listed vertically in Fig. 5.5). Of these, 12 modules were correlated with a range of coral attributes and experimental parameters, specifically source population or transplant location, principal components of FA analyses, colony growth (total change in area), and concentrations of individual FA (Fig. 5.5; Appendix C Suppl. Fig. 5.6).

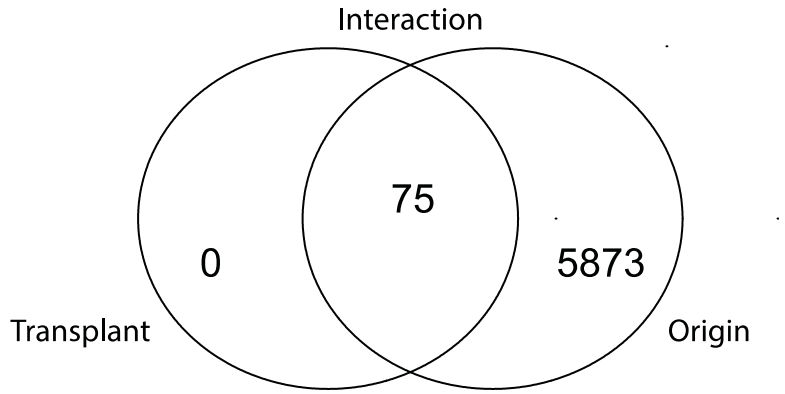


Fig. 5.4 Venn diagram showing the number of differentially expressed genes detected by 'DESeq2' analyses to determine origin and transplant effects based on coral colonies reciprocally transplanted between B1 and B3 from the Burdekin region in June 2014.

Of the total 24,848 genes assigned to the 17 unique modules (Fig. 5.5), 9,113 genes were annotated (36.7 %). Modules ranged from being 21.1 % up to 55.3 % annotated. Eigengenes (defined as the first principal component of a given module or a representative of the gene expression profile within a module; Langfelder and Horvath 2008) of the black module (the seventh largest module containing 1365 genes, 29.3 % of which were annotated) were strongly correlated with source population. GO (gene ontology) enrichment analysis of biological processes and cellular components indicated that the gene enrichment of this module was associated with ribosomal cellular components, macromolecule biosynthetic processing, translation, and ribosome biogenesis (Fig. 5.6). Genes in the black module were expressed 11.7 % (0.12-fold) higher, on average, in corals sourced from B1 compared to B3 (Fig. 5.7). The pink module (63 genes) was also correlated with source population and FA 14:0, 16:0, 18:1n-9 and 18:3n-6 (Fig. 5.5; Appendix C Suppl. Fig. 5.7). Average GE associated with the pink module was 46.0 % (0.46-fold) higher in corals sourced from B1, regardless of location (native versus novel; Fig. 5.8; Appendix C Suppl. Fig. 5.8).

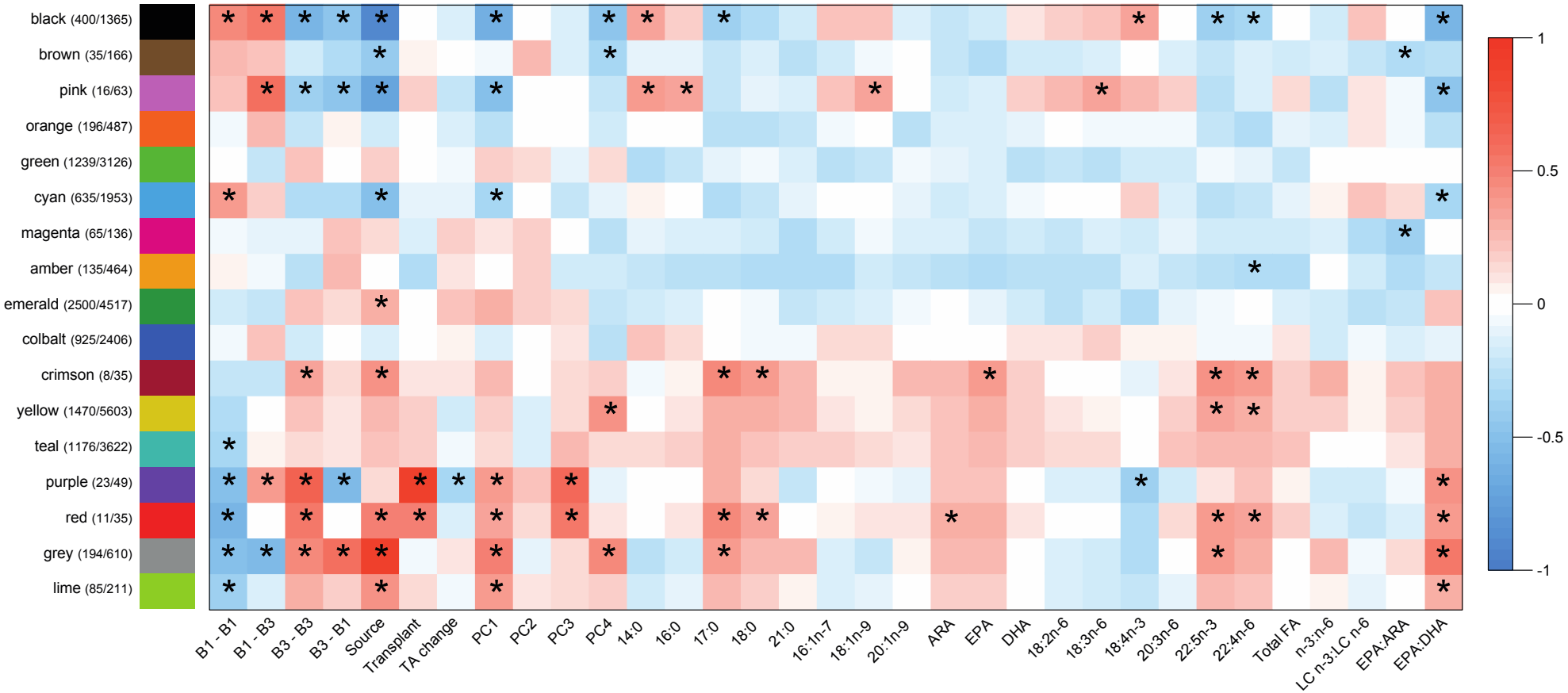


Fig. 5.5 Gene module – coral attribute matrix showing correlations between 17 unique gene modules (colours listed vertically) and 33 coral health attributes or experimental parameters (listed horizontally). Unique modules were determined by WGCNA analyses. Number of annotated genes and total number of genes in each module are indicated by numbers following module colour, respectively. * denotes a significant correlation at $p < 0.05$; heatmap colours indicate the magnitude and direction of the relationship. Coral and experimental attributes include four treatments (lettering denotes 'source population – transplant location'), source and transplant effects, total area change, principal components from PCA of FA percentage composition, individual and total FA concentrations, and coral health indicator ratios.

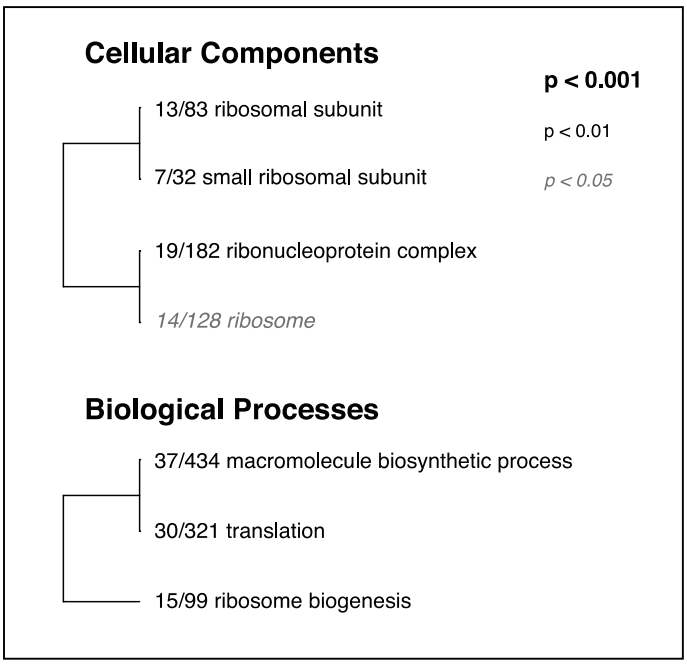


Fig. 5.6 Gene ontology (GO) for categories within the black module, which was significantly correlated with source effects. For reciprocally transplanted coral samples, gene enrichment was associated with cellular components and biological processes. Font type and boldness indicate the significance of the term. The fraction preceding the GO term indicates the number of genes annotated with the category that passed an unadjusted p-value threshold of 0.05. The trees indicate sharing of genes among GO categories. Terms on the same branch represent subsets.

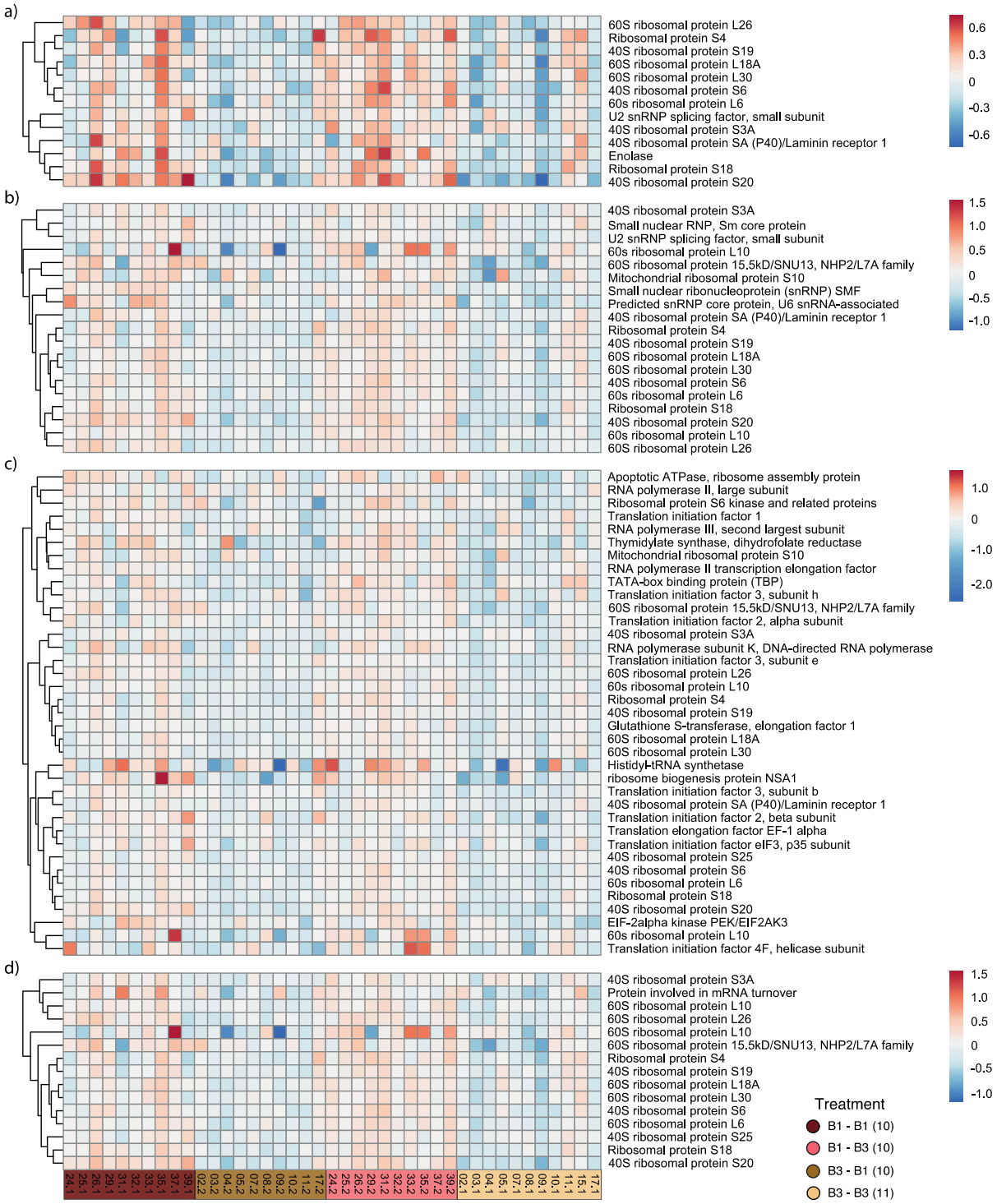


Fig. 5.7 Heatmaps depicting the relative expression of co-expressed genes within the black module (significantly correlated with source effects) that were enriched for cellular components and biological processes: (a) ribosomal and small ribosomal subunits; (b) ribonucleoprotein complex and ribosome; (c) macromolecule biosynthetic process and translation; and (d) ribosome biogenesis. The trees are hierarchical clustering of genes based on Pearson's correlation of their expressions across samples. Rows are genes; columns are samples ordered by treatment. Treatment is indicated by 'source population – transplant location'; sample sizes for GE analyses are in parentheses.

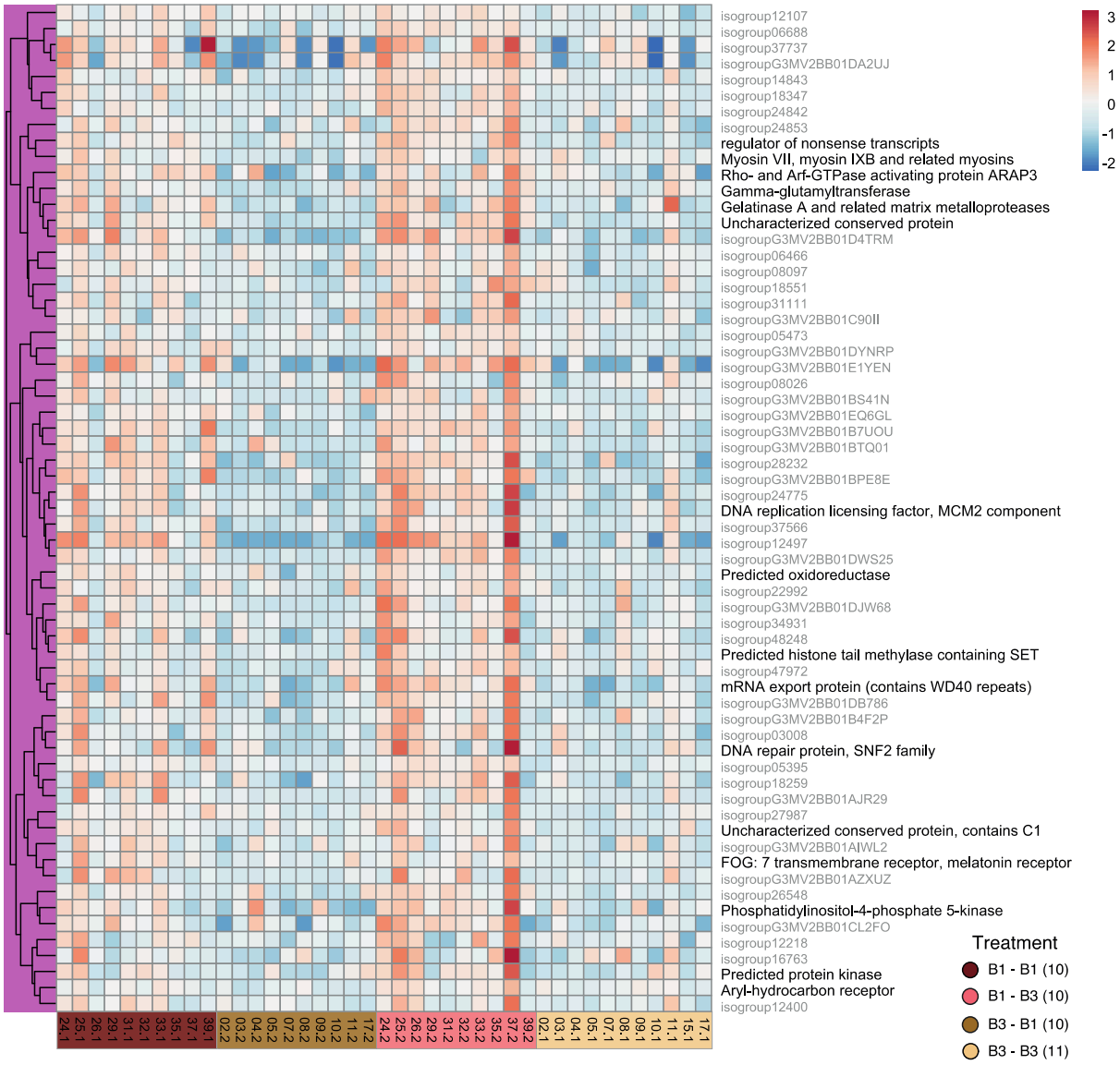


Fig. 5.8 Gene expression heatmap of differentially expressed genes within the pink module (significantly correlated with source effects and individual FA 14:0, 16:0, 18:1n-9 and 18:3n-6). Trees are hierarchical clustering of genes based on Pearson's correlation of their expression across samples. Columns are samples ordered by treatment. Treatment is indicated by 'source population – transplant location' and sample sizes for GE analyses are in parentheses.

Eigengenes of the purple module (49 genes; 46.9 % annotated) were strongly correlated with transplant location and had higher expression in colonies at B3 compared to colonies at B1 (Fig. 5.9a). Additionally, the purple module was the only module to be significantly correlated with total change in area of coral colonies (Fig. 5.5). Average GE decreased by 12.3 % (0.12-fold) in corals when exposed to B1 conditions compared to B3 conditions (Fig. 5.9a; Appendix C Suppl. Fig. 5.9). This module included genes related to mitochondrial functioning (i.e. NADH ubiquinone oxidoreductase, UDP-glucose 4-epimerase, and succinate dehydrogenase flavoprotein subunit), as well as chitinase and trypsin.

The red module (35 genes) was strongly correlated with both source population and transplant location (Fig. 5.5) and was 31.4 % annotated. Expression was lower in B1 natives and higher in B3 natives, whereas both B1 and B3 transplants had individuals with both higher and lower expression in a novel environment compared to corals in their native environments (Fig. 5.9b). Corals sourced from B1 had 35.2 % (0.35-fold) lower average expression in their source environment when compared to B3 corals in their source environment (Fig. 5.9b; Appendix C Suppl. Fig. 5.10). GE was 11.9 % (0.12-fold) higher than the local optimum when corals were transplanted from B3 to B1. Comparatively, GE was 10.0 % (0.10-fold) lower than the local optimum when corals were transplanted from B1 to B3. Genes within this module were related to tissue and muscle structure (collagen, immunoglobulin C-2 type and myosin heavy chain and transcription factor of HNF3 family), or associated with immunity (C-type lectin/mannose receptor, transcription factor of HNF3 family and immunoglobulin C-2 type).

Fig. 5.9 Gene expression heatmaps of differentially co-expressed genes within the (a) purple module (significantly correlated with transplant environment), and (b) red module (significantly correlated with source and transplant effects). The trees are hierarchical clustering of genes based on Pearson's correlation of their expression across samples. Columns are samples ordered by treatment. Treatment is indicated by 'source population – transplant location' and sample size for GE analyses is in parentheses.

5.5 Discussion

Substantial variations in gene transcripts and fatty acid (FA) concentrations were detected in colonies of the coral *Acropora tenuis* reciprocally transplanted between two water quality regimes in the Burdekin region, i.e., between a turbid, moderate water quality environment (B1) and a comparatively good water quality environment (B3; see ***Chapter 2*** for further characterisation of the two water quality regimes). Analyses indicate that variations in these biochemical and molecular attributes are a function of both genetic differences associated with source populations and phenotypic responses when exposed to a new environment at novel sites, although the relative importance of these influences varied among attributes. Source population effects were stronger for transcriptomic profiles; nine gene expression (GE) modules were correlated with source population, whereas only two gene modules were correlated with transplant location. In contrast, changes in FA concentrations and overall FA composition in novel environments indicate that variation in these biochemical attributes is strongly driven by environmental conditions. The strong influence of source population on gene expression may limit the capacity of these two coral populations to acclimatise and/or adapt to future conditions. However, restrictions imposed by population-level genotypic differences may be at least partially mitigated by the capacity of corals to vary their FA metabolism in response to the surrounding environment.

Source effects on gene expression of Acropora tenuis

Effects of source populations occur when colonies transplanted to a novel environment express phenotypic responses similar to those at their native site and/or different to those of native corals in the novel environment, indicating a limited capacity of the transplants to respond to environmental change (Weis 2010). For example, genes associated with ribosomal structures, translation and biogenesis were uniformly expressed at lower

levels in corals sourced from B3 (good water quality site), regardless of whether they were located in their native or the novel environment. These gene ontology (GO) categories describe basic biological functions that may be ubiquitously expressed and lack dynamic regulation (Dixon et al. 2014). Having the differential expression of these underlying biological functions defined by the source population, with limited capacity for phenotypic plasticity, could potentially underpin the slower growth rates of corals from the good water quality B3 site (see ***Chapter 2***). However, down-regulation of ribosomal- and translation-related genes has been found in heat-stressed larvae of *Acropora millepora* (Meyer et al. 2011; Dixon et al. 2015), indicating that regulation of genes defining basic biological functioning can be responsive to environmental variation at an early life stage when conditions are extreme. These findings suggest that the expression of ribosomal-related genes may become fixed under local environmental conditions during the early life stages of corals examined here, or that expression is stable unless corals are exposed to more extreme environmental conditions.

The strong source effects on gene expression detected here are consistent with local adaptation leading to population genetic divergence. Gene flow may be limited or restricted among populations of broadcast-spawning corals across geographic distances like those between the two reefs studied here (~ 60 km). Limited gene flow has been found at regional scales (e.g. < 100 km; Baums et al., 2005) and at shorter distances (e.g. < 35 km) for reefs separated in a cross-shelf direction (Mackenzie et al., 2004). Interestingly, even coral populations in close proximity (separated by < 5 km) can have divergent population structures related to nuclear ribosomal genes of the coral host (Barshis et al., 2010), providing further support for findings from this study that suggest source effects associated with population-level genetic differences are limiting the dynamic responses of corals transplanted to novel water quality regimes. However, the magnitude of differential regulation between

populations in this study was relatively small (~ 10 % or 0.1-fold) compared to multiple-fold changes reported elsewhere (e.g. Barshis et al., 2013; Kenkel et al., 2014).

Expression of genes within the pink module was significantly affected by the source population. Higher expression of these genes, which are involved in developmental processes, cellular pathways and immunity (e.g. protein kinase, histone methyltransferase, and aryl hydrocarbon receptor), in corals sourced from the moderate WQ site (B1) than in corals from the good WQ site (B3) may be a response to more stressful environmental conditions at B1. Positive correlations between the expression of these genes and FA concentrations (14:0, 16:0, 18:1n-9 and 18:3n-6) suggest that gene and FA functions may be linked. FA and other lipids have been implicated in the activation and regulation of protein kinases and growth factors (Merrill and Schroeder, 1993). Furthermore, 18:3n-6 and its metabolites can affect the expression of genes associated with immune functions and apoptosis (Kapoor and Huang 2006), such as the aryl hydrocarbon receptor (AhR). Activation of AhR is suggested to be important for immunological responses, as well as for the inhibition of inflammation (Li et al. 2011). 18:3n-6 is an omega-6 polyunsaturated fatty acid (n-6 PUFA), which decreases membrane fluidity and increase cellular inflammation (Sargent et al. 1990; Nettleton 1995). At the moderate water quality site (B1), genes associated with immunological responses, including AhR, may be activated to counteract the negative effects of n-6 PUFA.

Effect of transplant location on coral biochemical attributes and gene expression

The reciprocal transplant design identified a number of traits that are phenotypically plastic. After four months, FA concentrations of corals transplanted to a novel environment were similar to those of natives at each site, for both transplanted populations. Total FA concentrations increased by ~ 20 % and long chain polyunsaturated fatty acids (LC PUFA),

which include essential FA, increased by ~ 30 % when B1 corals were transplanted to B3. Plasticity in FA concentrations enables corals to optimise their energy stores, cellular membrane characteristics and biological functioning (Tchernov et al. 2004; Francis et al. 2014). The higher concentrations of LC PUFA at B3 may be a hallmark of a healthier population, as these FA provide a higher level of protection against environmental stressors by maintaining cellular membrane fluidity and by protecting the photosynthetic machinery of symbionts (Los et al. 2013). Furthermore, 20:5n-3 (EPA) and 20:4n-6 (ARA), which are FA proposed to be involved in immune responses (Kaur et al., 2011), decreased in all corals when transplanted to B1 (moderate WQ environment). Reefs closer to shore are considered to be more stressful environments (Fabricius et al. 2005; Kenkel et al. 2015), with reduced water quality decreasing coral fitness. Therefore, it is possible that corals exposed to deteriorated water quality are catabolising n-3 PUFA (EPA & DHA). In terms of FA consumption, production and maintenance, corals at B3 (the site further from shore with good water quality) are healthier compared to corals at the more inshore B1 site with reduced water quality.

Interestingly, colony morphology differed between the two source populations, likely as a consequence of differences in colony extension rates and skeletal density. Corals from B1 were significantly less dense and extended faster than corals from B3 (see ***Chapter 2***). However, comparisons of changes in these biochemical attributes between transplants from the two sites were unable to be conducted, as corals from B1 were affected by selective predation when transplanted to B3 (see ***Chapter 4***; Rocker and Brandl 2014); instead mean change in coral colony area was compared between the transplant groups. The purple module was the only gene module that was correlated with change in colony area, and one of two modules correlated with transplant site, suggesting that this gene module was the only one to be affected by the predation event. Genes in the purple module had higher relative

expression, by an average of 12.5 %, within transplants outgrown at B3 compared to transplants outgrown at B1. Corals native to B3 had 15.4 % (0.15-fold) higher expression compared to corals native to B1. Two genes, chitinase and trypsin, had higher expression within corals at B3. Both these genes are inherent to digestive processes (Rawlings and Barrett 1994; Dahiya et al. 2006) and have implications for defense and symbiotic interactions within corals (Wood-Charlson et al. 2006; Harvell et al. 2007). Higher expression of these genes could indicate higher levels of control over *Symbiodinum* populations within coral hosts at the good WQ site (B3), as well as greater functional capacity to break down nutritional sources. Genes related to mitochondrial functioning (i.e. NADH ubiquinone oxidoreductase, UDP-glucose 4-epimerase, and succinate dehydrogenase flavoprotein subunit), which are suggested to have a role in stress tolerance (Dixon et al. 2015), also had higher expression in corals outgrown at B3. Overall, transplant effects detected in this study suggest higher molecular and biochemical performance, leading to increased fitness, in corals exposed to cleaner water quality, regardless of origin.

Interactive and additive effects on gene expression and fatty acid composition

SC PUFA, including SC n-6 PUFA, and a single module (red) were significantly correlated with both source population and transplant location. Of the 35 genes in the red module, only 11 were annotated. GE was 12 % higher than the local optimum in corals transplanted from B3 to B1 and 10 % lower than the local optimum in corals transplanted from B1 to B3. Annotations were related to tissue and muscle structure (i.e. collagens, immunoglobulin C-2 type, myosin heavy chain and transcription factor of HNF3 family) and immunity (i.e. C-type lectin/mannose receptor, transcription factor of HNF3 family, immunoglobulin C-2 type). Collagens are the main structural protein in connective tissues within animal bodies (Exposito et al. 2008). Corals both originating from, and transplanted

to, a good water quality environment exhibited higher expression of collagens and other genes annotated as having a role in tissue and muscle structure. Higher expression of these genes at B3 could be interpreted as higher investment in the structural integrity of coral host tissues when water quality regimes are comparatively good. Contrary to these results, other studies have reported that collagen was expressed at higher levels in inshore-sourced corals compared to offshore corals (Kenkel et al. 2013) and in corals from variable environments (tide pools with highly variable temperatures; Barshis et al. 2013). However, another study found that when environmental conditions are stressful enough to cause bleaching, collagens are compromised (Moya et al. 2012), suggesting that investment in genes associated with tissue and muscular structures is strongly influenced by environmental conditions.

C-type lectin/mannose receptor and immunoglobulin C-2 type genes, which have putative roles in immunity and symbiosis, were more highly expressed in corals sourced from, and transplanted to, a cleaner water environment (B3). Lectins are commonly down-regulated in corals exposed to acute heat stress (Barshis et al. 2013; Kenkel et al. 2014) and immunoglobins are down-regulated in bleached corals (DeSalvo et al. 2008), suggesting that these genes are also be involved in pathogen and symbiont control (Wood-Charlson et al. 2006; Jimbo et al. 2010; Kvennefors et al. 2010). Immunoglobins, which were front-loaded in heat-tolerant corals in tide pools (Barshis et al. 2013), had 15.8 % lower expression in B3 corals transplanted to B1 compared to the local optimum and 20.4 % lower expression in B1 corals transplanted to B3 than the local optimum. This relatively higher expression suggests corals exposed to and sourced from a cleaner water quality environment (B3) may be front-loading immunity-related genes allowing for a higher stress tolerance if environmental conditions deteriorate.

Conclusions

Biochemical and genetic responses of the coral *A. tenuis* transplanted between two sites with different water quality regimes were a function of both their source population and the transplant environment. GE profiles demonstrated strong source population effects in genes relating to basic biological functions, consistent with either population-level genetic divergence or canalisation in early development. Although local adaptation is beneficial when environments are stable, differential regulation of GE in local source populations may reduce the capacity of corals to respond to a changing environment. Plasticity in FA concentrations and specific genes relating to improved health and immunity highlight alternative pathways for corals to respond to future changes in environmental conditions. These findings suggest hope for future corals, if anthropogenic water quality stressors can be reduced.

CHAPTER 6: General Discussion

In this PhD thesis, I compare a range of biochemical and molecular attributes of the coral *Acropora tenuis* when exposed to differing water quality regimes, to reveal new insights into environmental and genetic drivers of coral health and condition and identify biomarkers of coral condition related to water quality. This research demonstrates that a common coral species on inshore reefs can grow rapidly under water quality conditions characterised by high concentrations of particulate and dissolved nutrients. However, a negative correlation between rate of linear extension and skeletal density, both of which are commonly associated with good health, highlights the need to carefully define coral health attributes when used to assess overall coral holobiont health.

A 1.5-year monitoring scheme and a reciprocal transplant study revealed that inshore populations of the coral *A. tenuis* respond to local environmental conditions through variation in linear extension rates, *Symbiodinium* type and density, skeletal density and organic tissue components, including FA composition. Yet, global gene expression of transplanted corals also maintained a unique signature of their site of origin. Such genetic differentiation between inshore populations is consistent with local adaptation. Genetic differentiation can also arise through developmental canalisation and other epigenetic longer-term acclimatisation mechanisms. By integrating data on coral health attributes, biochemical composition and functional genomics, this research improves current understanding of how coral phenotypes cope with variation in water quality and their ability to respond to seasonal and spatial variation in environmental conditions through acclimatisation and/or adaptation.

6.1 Defining and understanding coral health and condition

The status of coral reefs has typically been assessed by measuring coral cover and diversity, with reefs generally being defined as healthy when coral cover is high and

degraded when coral cover is low (Bruno and Selig 2007; Sweatman et al. 2011; De'ath et al. 2012). However, such measures provide little insight into reasons for declines, mechanisms of recovery and limited warning of impending changes on coral colony health. Other attributes of coral reef health range from community and ecosystem measures (e.g. larval supply, recruitment, taxonomic richness) through to colony level measures (e.g. gene expression, lipid content, linear extension and mortality), with the applicability of these measures depending on the objective of the research or monitoring program (Cooper et al. 2009).

The research presented here indicates that attributes of coral colonies provide important insights into sources of growth and variation of coral condition, and hence may play an important role in recovery of inshore corals affected by anthropogenic stressors. Consequently, a suite of physiological, biochemical and gene expression attributes should be measured, as some attributes respond in a counter-intuitive manner. For example, it is commonly assumed that high extension rates indicate limited or no physiological stress, while decreased extension rates can suggest stress related to reef degradation (Edinger et al. 2000). High skeletal extension rates may be associated with negative states of other attributes (Madin et al. 2016). High extension rates were linked with decreased skeletal densities, which suggests that fast growing colonies may have increased susceptibility to physical disturbances linked to water quality environments characterised by high levels of turbidity and nutrients. Hence, relying on a single measure of coral colony health should be avoided. This study enhances the understanding of water quality effects on coral holobiont health and can be used to develop health indicators whose status and trend can be monitored during extreme and benign events across the GBR. Future studies should assess how multiple physiological and biochemical attributes of the coral holobiont differ among coral species to develop indicators of general relevance to reef community and ecosystem health.

In ***Chapter 2***, a number of biochemical attributes of corals were found to vary with water quality environments, highlighting the potential to develop a range of biomarkers of coral colony health. Positive correlations between linear extension and *Symbiodinium* density, and also between lipid and organic content, suggest that these attributes hold promise as biomarkers for measuring the impact of water quality on coral health. The less dense skeletons of corals at sites closest to river run-off suggest that skeletal density is a good biomarker of reduced water quality (***Chapter 2***; Madin et al. 2016). Moreover, high extension rates have been linked to elevated respiration rates due to greater densities of *Symbiodinium* populations in enhanced nutrient and/or higher temperature environments (Wooldridge 2014). Higher phosphate levels in inshore environments may explain the lower skeletal densities (***Chapter 2***), as phosphate is known as a crystal poison (Simkiss 1964) and may lead to lower rates of in-filling during light-enhanced calcification (Lough and Barnes 1992; Browne 2012; Browne et al. 2012; Manzello et al. 2015). Regardless of the environmental driver, compromised structural integrity increases susceptibility of coral colonies to physical damage by breakage, bio-eroders, cyclones and/or destructive fishing practices. Results of other studies have also suggested that exposure of corals to deteriorated water quality results in lower skeletal density and higher susceptibility to physical damage (Edinger et al. 2000; Holmes et al. 2000; Fabricius 2005; Madin et al. 2016). The negative correlation found between skeletal density and linear extension highlights a potential mechanism leading to differential growth of coral colonies across environments.

A properly functioning relationship between the coral host and its photosymbiont *Symbiodinium* is essential for optimal health of the coral holobiont; thus, it is important to include measures of *Symbiodinium* performance in the suite of indicators measured. Both the coral host and its endosymbiont *Symbiodinium* benefit from this relationship, but the integrity of the partnership is highly dependent on surrounding environmental conditions (Wooldridge

2010; Lesser et al. 2013). *Symbiodinium* densities in coral tissues are generally higher under high nutrient and low irradiance conditions (Smith and Hoegh-Guldberg 1989; Hinrichs et al. 2013c), potentially leading to *Symbiodinium* populations that exceed the optimum density for maximum autotrophic functioning (Hoogenboom et al. 2010; Wooldridge 2012). Thresholds proposed for optimal functioning were defined as Photosynthesis:Respiration (P:R) ratios between ~ 1.25 to 1.75, which correlates with *Symbiodinium* densities between 1.0 – 2.5 x 10^6 cell mg protein^{-1} (Hoogenboom et al. 2010). *Symbiodinium* densities found in corals across the Burdekin inshore region decreased with increasing distance from the coast and source of runoff. While symbiont densities were in a healthy range, I detected differences in the organic content, lipid content and FA composition (***Chapter 2*** and ***Chapter 3***), indicating autotrophic indices based on *Symbiodinium* densities are a poor indicator of coral health and condition. However, when interpreted in conjunction with other health attributes a more detailed description of coral health can be achieved.

Symbiodinium communities may contribute substantially to organic and lipid reserves within coral tissues, as *Symbiodinium* density was positively correlated with both ash-free dry weight and total lipid content (***Chapter 2***). However, deteriorated environmental conditions, including increased nutrient loads and decreased irradiance (associated with increased turbidity), can lead to increased heterotrophy, which can also impact the nutritional status of corals (Anthony and Fabricius 2000; Thornhill et al. 2011). Autotrophic and heterotrophic impacts can potentially be measured in the FA composition of corals (Al-Moghrabi et al. 1993; Dalsgaard et al. 2003). However, the higher organic and lipid concentrations in corals at the more nutrient rich, turbid site detected in this research did not allow for partitioning between autotrophy (from higher *Symbiodinium* densities) and predicted greater potential for heterotrophic feeding. Future studies need to apply tools to separate organic contributions of the coral host and its *Symbiodinium*.

Variation in FA composition and FA health indicator ratios of coral colonies in different water quality regimes suggests that FA composition can be used to detect differences in the maintenance of FA profiles driven by the surrounding environmental conditions (***Chapter 3*** and ***Chapter 5***). Differences in the biochemical composition and condition of coral tissues underlie differences in physiological performance and functioning, and thus, the capacity of corals to grow and survive under inshore water quality regimes. Key components of physiological performance include maintenance of structural integrity of cellular membranes, storage of energy reserves, and require an appropriate balance among essential long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) and long chain omega-6 polyunsaturated fatty acids (LC n-6 PUFA; Sargent et al. 1990; Russo 2009; Richier et al. 2010). A healthy n-3:n-6 ratio is generally defined as 1:1 (Simopoulos 2002).

Variation in FA health indicator ratios for ***A. tenuis*** varied among sites and seasons (***Chapter 3***). Corals exposed to relatively good water quality along the gradients (B3 and W3, farthest from the coast and sources of runoff) had the highest n-3:n-6 ratios, suggesting that exposure to good water quality induces healthier overall FA composition. However, higher health indicator ratios at sites with deteriorated water quality (closest to the coast and sources of runoff within each region) compared to mid-gradient sites, suggests these corals may be supplementing autotrophically-sourced FA with heterotrophically-sourced FA to increase or maintain optimal levels of health. Low n-3:n-6 ratios are usually due to excess intake of n-6 PUFA (Simopoulos 2008), which can counteract the benefits of n-3 PUFA, indicating either selective retention or selective acquisition of n-3 PUFA as a potential mechanism for coping with reduced food availability and/or cooler temperatures during winter months. FA health indicator ratios suggest that deteriorated environmental conditions can, at times, ameliorate the otherwise negative effects of reduced water quality on coral health by providing additional or supplemental sources of nutrition (***Chapter 2*** and ***Chapter 3***). Future work

should further develop and ground-truth FA health indicators as these ratios may be an indispensable lipidomic approach for coral health biomarkers.

6.2 Responses of coral health attributes to different water quality environments

Understanding how coral colony health and condition respond to changes in water quality (WQ) is important for evaluating the potential impacts of management actions to control and optimise WQ in reef systems. Corals from the environment with lower turbidity and nutrient enrichment had lower skeletal extension rates, *Symbiodinium* densities, and organic and lipid content, but higher skeletal densities. Intriguingly, these differences also appear detectable by large corallivorous fishes, which selectively preyed upon turbid-water corals transplanted to the more off-shore site with improved water quality (***Chapter 4***; Rocker and Brandl 2014). FA concentrations of transplanted corals approached that of native corals at both transplant locations and confirmed relatively rapid acclimatisation in response to environmental variation (***Chapter 5***). Plasticity in FA composition and concentrations enables corals to optimise energy stores, cellular membrane characteristics and biological functioning (Tchernov et al. 2004; Francis et al. 2014). Importantly, FA profiles were healthier (i.e. higher in n-3 PUFA) in populations of *A. tenuis* on reefs further from the coast and river sources (***Chapter 3*** and ***Chapter 5***). I hypothesise that to cope with stress associated with deteriorated water quality, corals from (and transplanted to) the moderate water quality site were catabolising n-3 PUFA (EPA and DHA), leading to the FA ratio patterns observed.

Limits to the plasticity of coral phenotypes are suggested by consistent differences in GE profiles between source populations, regardless of their exposure to native or foreign environments (***Chapter 5***). In particular, the expression of genes associated with basic biological functions was limited in response to transplantation into a novel water quality regime. Variation in the expression of genes relating to ribosomal translation have been

detected in response to acute stress (Meyer et al. 2011; Dixon et al. 2015), potentially through mechanisms of metabolic arrest (Bay et al. 2009b). The expression level of genes relating to basic biological functions may become set as a consequence of either local adaptation or canalisation at an early life-history stage. Genes associated with cellular pathways and immunity correlated with individual FA suggesting alternative pathways for corals to respond to changes in environmental conditions. Furthermore, the expression of other genes related to mitochondrial processes, defense and symbiotic interactions varied with the water quality environment (***Chapter 5***). In summary, ***A. tenuis*** has demonstrated some capacity to respond to both improved and deteriorated environmental conditions; however, this capacity is restricted by genotypic differences associated with the source population. This highlights the need to mitigate anthropogenically-induced water quality stress if there is to be hope for future coral reefs.

6.3 Future directions and conclusions

As climate change and warming ocean temperatures progressively threaten the survival of reef corals, a priority for future studies should be to explore the interactive effects with water quality on the health, bleaching susceptibility, stress tolerance and potential recovery of multiple species of corals. This thesis provides a comprehensive assessment of coral condition and health across water quality gradients of a single coral species and evaluates the utility of these as indicators of baseline coral health and predictive of resilience to disturbance. By combining long term monitoring and reciprocal transplant studies of multiple coral species (to determine appropriate model species) and physiological measures, such as photosynthesis, respiration and calcification rates, with the biochemical and genomic measures employed in this thesis, locally acclimatised or adapted responses of coral species can be evaluated.

Defining variability in biochemical measures (e.g. FA composition) is essential for determining the *in situ* dynamics of coral health in response to local environmental conditions (***Chapter 3*** and ***Chapter 5***). However, further studies are necessary to understand the dynamics of FA within and between the coral host and its endosymbiotic *Symbiodinium*. Specifically, future studies should investigate the acquisition and partitioning of photosynthetic and heterotrophic sources of nutrition within the coral holobiont, determine the potential of host-symbiont resource sharing and examine the FA composition and potential transfers of different *Symbiodinium* clades.

The major findings from this thesis will assist in the understanding, management, and protection of inshore corals, where the threat of local stressors is most pertinent. This thesis provides documentation of long-term effects of water quality on coral colony condition and physiology that underpin biological and ecological processes, including survival, growth, susceptibility to disturbance and mortality. A suite of attributes indicating the status of skeletal integrity and biochemical composition, as well as integrating lipidomic and genomic markers, would improve assessment of coral health and condition across time and space and provide advanced warning of coral susceptibility to environmental stressors and physical damage. Additionally, this research demonstrates that essential FA and health indicator ratios show promise as indicators of coral holobiont health and condition. Autotrophic and heterotrophic indices should also be incorporated as indicators of increased coral vulnerability to environmental stressors.

By monitoring these coral attributes in combination with changes in coral cover and diversity, a comprehensive understanding of the drivers and mechanisms behind changes in coral cover can be established. Further research is needed to establish stress tolerance thresholds of corals in response to environmental stressors (e.g. heat stress, storm damage) before the full value to management programs can be assessed. With this knowledge it would

be possible to provide water quality corrected bleaching thresholds to be used in forecast products (e.g., NOAA, BoM) and a coral condition index for reporting the status and trend alongside coral reef (cover and diversity) and water quality indices in report cards and ecosystem assessment reports. It is clear that this task would be relatively expensive and time consuming, however, of potential high value to integrated monitoring programs of coral reef ecosystems. This integrated approach will enhance our understanding of the impacts of local and global stressors on the overall health of inshore corals and underpin management actions to enhance the resilience of the reef in an uncertain future.

LITERATURE CITED

Abramovitch-Gottlib L, Dahan D, Golan Y, Vago R (2005) Effect of light regimes on the microstructure of the reef-building coral *Fungia simplex*. Mater. Sci. Eng. C 25:81–85

Abrego D (2008) Temporal and environmental influences on the early establishment and maintenance of coral-*Symbiodinium* symbioses. PhD Thesis, James Cook University

Abrego D, van Oppen MJH, Willis BL (2009) Highly infectious symbiont dominates initial uptake in coral juveniles. Mol. Ecol. 18:3518–3531

Ackman RG (2002) The gas chromatograph in practical analyses of common and uncommon fatty acids for the 21st century. Anal. Chim. Acta 465:175–192

Al-Kandari NM, Jolliffe IT (2005) Variable selection and interpretation in correlation principal components. Environmetrics 16:659–672

Al-Moghrabi S, Allemand D, Couret JM, Jaubert J (1995) Fatty acids of the scleractinian coral *Galaxea fascicularis*: effect of light and feeding. J. Comp. Physiol. B 165:183–192

Al-Moghrabi S, Allemand D, Jaubert J (1993) Valine uptake by the scleractinian coral *Galaxea fascicularis*: characterization and effect of light and nutritional status. J. Comp. Physiol. B 163:355–362

Anders S, Huber W (2010) Differential expression analysis for sequence count data. Genome Biol. 11:R106

Anthony KRN (2006) Enhanced energy status of corals on coastal, high-turbidity reefs. Mar. Ecol. Prog. Ser. 319:111–116

Anthony KRN, Connolly SR (2004) Environmental limits to growth: physiological niche boundaries of corals along turbidity-light gradients. Oecologia 141:373–384

Anthony KRN, Connolly SR, Hoegh-Guldberg O (2007) Bleaching, energetics, and coral mortality risk: effects of temperature, light, and sediment regime. Limnol. Oceanogr. 52:716–726

Anthony KRN, Connolly SR, Willis BL (2002) Comparative analysis of energy allocation to tissue and skeletal growth in corals. Limnol. Oceanogr. 47:1417–1429

Anthony KRN, Fabricius KE (2000) Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. J. Exp. Mar. Bio. Ecol. 252:221–253

Anthony KRN, Hoegh-Guldberg O (2003) Variation in coral photosynthesis, respiration and growth characteristics in contrasting light microhabitats: an analogue to plants in forest gaps and understoreys? Funct. Ecol. 17:246–259

Anthony KRN, Ridd PV, Orpin AR, Larcombe P, Lough JM (2004) Temporal variation of light availability in coastal benthic habitats: effects of clouds, turbidity and tides. Limnol. Oceanogr. 49:2201–2211

ANZECC (2000) Australian and New Zeland guidelines for fresh and marine water quality. Australian and New Zealand Environment and Conservation Council, Canberra, Australia, pp 319

Armstrong SG, Wyllie SG, Leach DN (1994) Effects of season and location of catch on the fatty acid compositions of some Australian fish species. Food Chem. 51:295–305

Ayas D, Ozogul Y, Yazgan H (2013) The effects of season on fat and fatty acids contents of shrimp and prawn species. Eur. J. Lipid Sci. Technol. 115:356–362

Bachok Z, Mfilinge P, Tsuchiya M (2006) Characterization of fatty acid composition in healthy and bleached corals from Okinawa, Japan. Coral Reefs 25:545–554

Bainbridge ZT, Wolanski E, Álvarez-Romero JG, Lewis SE, Brodie JE (2012) Fine sediment and nutrient dynamics related to particle size and floc formation in a Burdekin River flood plume, Australia. Mar. Pollut. Bull. 65:236–248

Baird AH, Cumbo VR, Leggat W, Rodriguez-Lanetty M (2007) Fidelity and flexibility in coral symbioses. Mar. Ecol. Prog. Ser. 347:307–309

Baptista M, Repolho T, Maulvault AL, Lopes VM, Narciso L, Marques A, Bandarra N, Rosa R (2014) Temporal dynamics of amino and fatty acid composition in the razor clam *Ensis siliqua* (Mollusca: Bivalvia). Helgol. Mar. Res. 68:465–482

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR (2013) Genomic basis for coral resilience to climate change. Proc. Natl. Acad. Sci. 110:1387–1392

Barshis DJ, Stillman JH, Gates RD, Toonen RJ, Smith LW, Birkeland C (2010) Protein expression and genetic structure of the coral *Porites lobata* in an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity? Mol. Ecol. 19:1705–1720

Bates D, Maechler M, Bolker BM, Walker S (2015) Fitting linear mixed-effects models using {lme4}. J. Stat. Softw. 67:1–48

Baums IB, Miller MW, Hellberg ME (2005) Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. Mol. Ecol. 14:1377–1390

Bay LK, Guérécheau A, Andreakis N, Ulstrup KE, Matz MV (2013) Gene expression signatures of energetic acclimatisation in the reef building coral *Acropora millepora*. PLoS One 8:e61736

Bay LK, Nielsen HB, Jarmer H, Seneca FO, van Oppen MJH (2009a) Transcriptomic variation in a coral reveals pathways of clonal organisation. Mar. Genomics 2:119–125

Bay LK, Ulstrup KE, Nielsen HB, Jarmer H, Goffard N, Willis BL, Miller DJ, van Oppen MJH (2009b) Microarray analysis reveals transcriptional plasticity in the reef building coral *Acropora millepora*. Mol. Ecol. 18:3062–3075

Ben-David-Zaslow R, Benayahu Y (1999) Temporal variation in lipid, protein and carbohydrate content in the Red Sea soft coral *Heteroxenia fuscescens*. J. Mar. Biol. Assoc. United Kingdom 79:1001–1006

Benjamini Y, Yekutieli D (2001) The control of the false discovery rate in multiple testing under dependency. Ann. Stat. 29:1165–1188

Bergé J-P, Barnathan G (2005) Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. Mar. Biotechnol. 1:51–111

Berkelmans R (2002) Time-integrated thermal bleaching thresholds of reefs and their variation on the Great Barrier Reef. Mar. Ecol. Prog. Ser. 229:73–82

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

Bonaldo RM, Krajewski JP, Bellwood DR (2011) Relative impact of parrotfish grazing scars on massive *Porites* corals at Lizard Island, Great Barrier Reef. Mar. Ecol. Prog. Ser. 423:223–233

Bonaldo RM, Welsh JQ, Bellwood DR (2012) Spatial and temporal variation in coral predation by parrotfishes on the GBR: evidence from an inshore reef. Coral Reefs 31:263–272

Brodie JE, Devlin MJ, Haynes D, Waterhouse J (2010) Assessment of the eutrophication status of the Great Barrier Reef lagoon (Australia). Biogeochemistry 106:281–302

Brodie JE, McKergow LA, Prosser IP, Furnas MJ, Hughes AO, Hunter H (2003) Sources of sediment and nutrient exports to the Great Barrier Reef World Heritage Area Report No. 03/11. Australian Centre for Tropical Freshwater Research, James Cook University Townsville, Australia, pp 192

Brohan P, Kennedy JJ, Harris I, Tett SFB, Jones PD (2006) Uncertainty estimates in regional and global observed temperature changes: a new data set from 1850. J. Geophys. Res. Atmos. 111:1–21

Brown BE, Cossins AR (2011) The potential for temperature acclimatisation of reef corals in

the face of climate change. In: Dubinsky Z, Stambler N (eds) Coral Reefs: An Ecosystem in Transition. Springer Netherlands, Dordrecht, pp 421–433

Browne NK (2012) Spatial and temporal variations in coral growth on an inshore turbid reef subjected to multiple disturbances. Mar. Environ. Res. 77:71–83

Browne NK, Smithers SG, Perry CT (2012) Spatial and temporal variations in turbidity on two inshore turbid reefs on the Great Barrier Reef, Australia. Coral Reefs 32:195–210

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

Bruckner AW, Bruckner RJ (1998) Destruction of coral by *Sparisoma viride*. Coral Reefs 17:350

Bruno JF, Selig ER (2007) Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. PLoS One 2:e711

Bureau DP, Kaushik SJ, Cho CY (2002) Bioenergetics. In: Halver JE, Hardy RW (eds) Fish Nutrition. Academic Press San Diego, CA, pp 1–59

Burke L, Reytar K, Spalding M, Perry A (2011) Reefs at risk revisited. World Resources Institute Washington, DC, pp 130

Carilli JE, Norris RD, Black BA, Walsh SM, McField M (2009) Local stressors reduce coral resilience to bleaching. PLoS One 4: e6324

Cole AJ, Pratchett MS, Jones GP (2008) Diversity and functional importance of coral-feeding fishes on tropical coral reefs. Fish Fish. 9:286–307

Coles SL, Brown BE (2003) Coral bleaching-capacity for acclimatization and adaptation. Adv. Mar. Biol. 46:183–223

Conlan JA, Jones PL, Turchini GM, Hall MR, Francis DS (2014) Changes in the nutritional composition of captive early-mid stage *Panulirus ornatus* phyllosoma over ecdysis and larval development. Aquaculture 434:159–170

Connell JH, Hughes TP, Wallace CC (1997) A 30-Year study of coral abundance, recruitment, and disturbance at several scales in space and time. Ecol. Monogr. 67:461–488

Cooper TF, Gilmour JP, Fabricius KE (2009) Bioindicators of changes in water quality on coral reefs: review and recommendations for monitoring programmes. Coral Reefs 28:589–606

Cooper TF, Uthicke S, Humphrey C, Fabricius KE (2007) Gradients in water column nutrients, sediment parameters, irradiance and coral reef development in the Whitsunday

Region, central Great Barrier Reef. Estuar. Coast. Shelf Sci. 74:458–470

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

Cunning RL, Baker AC (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nat. Clim. Chang. 3:259–262

Cunning RL, Baker AC (2014) Not just who, but how many: the importance of partner abundance in reef coral symbioses. Front. Microbiol. 5:1–10

Dahiya N, Tewari R, Hoondal GS (2006) Biotechnological aspects of chitinolytic enzymes: a review. Appl. Microbiol. Biotechnol. 71:773–782

Dalsgaard J, St John M, Kattner G, Müller-Navarra DC, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv. Mar. Biol. 46:225–340

De'ath G (2007) The spatial, temporal and structural composition of water quality of the Great Barrier Reef, and indicators of water quality and mapping risk. Australian Insititute of Marine Science Townsville, Australia, pp 67

De'ath G, Fabricius KE (2010) Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. Ecol. Appl. 20:840–850

De'ath G, Fabricius KE, Sweatman H, Puotinen ML (2012) The 27-year decline of coral cover on the Great Barrier Reef and its causes. Proc. Natl. Acad. Sci. 109: 17995–17999

DeSalvo MK, Voolstra CR, Sunagawa S, Schwarz JA, Stillman JH, Coffroth MA, Szmant AM, Medina M (2008) Differential gene expression during thermal stress and bleaching in the Caribbean coral *Montastraea faveolata*. Mol. Ecol. 17:3952–3971

Dethier MN, Sosik E, Galloway AWE, Duggins DO, Simenstad CA (2013) Addressing assumptions: variation in stable isotopes and fatty acids of marine macrophytes can confound conclusions of food web studies. Mar. Ecol. Prog. Ser. 478:1–14

Dewick PM (1997) The acetate pathway: fatty acids and polyketides. In: Medicinal Natural Products. John Wiley & Sons Publishing West Sussex, UK, pp 35–90

Dixon GB, Bay LK, Matz MV (2014) Bimodal signatures of germline methylation are linked with gene expression plasticity in the coral *Acropora millepora*. BMC Genomics 15:1109

Dixon GB, Davies SW, Aglyamova GV, Meyer E, Bay LK, Matz MV (2015) Genomic determinants of coral heat tolerance across latitudes. Science 348:1460–1462

Done TJ (1982) Patterns in the distribution of coral communities across the central Great Barrier Reef. Coral Reefs 1:95–107

Donner SD, Skirving WJ, Little CM, Oppenheimer M, Hoegh-Guldberg O (2005) Global

assessment of coral bleaching and required rates of adaptation under climate change. Glob. Chang. Biol. 11:2251–2265

Drewry JJ, Higham W, Mitchell C (2009) Water quality objectives and targets in the Mackay Whitsunday region to protect water quality to the Great Barrier Reef. 18th World IMACS/MODSIM Congress, Cairns, Aust. 3308–3314

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

Edinger EN, Limmon GV, Jompa J, Widjatmoko W, Heikoop JM, Risk MJ (2000) Normal coral growth rates on dying reefs: are coral growth rates good indicators of reef health? Mar. Pollut. Bull. 40:404–425

Edmunds PJ, Davies PS (1986) An energy budget for *Porites porites* (Scleractinia). Mar. Biol. 92:339–347

Edmunds PJ, Gates RD (2008) Acclimatization in tropical reef corals. Mar. Ecol. Prog. Ser. 361:307–310

Erftemeijer PLA, Riegl B, Hoeksema BW, Todd PA (2012) Environmental impacts of dredging and other sediment disturbances on corals: a review. Mar. Pollut. Bull. 64:1737–1765

Exposito JY, Larroux C, Cluzel C, Valcourt U, Lethias C, Degnan BM (2008) Demosponge and sea anemone fibrillar collagen diversity reveals the early emergence of A/C clades and the maintenance of the modular structure of type V/XI collagens from sponge to human. J. Biol. Chem. 283:28226–28235

Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Mar. Pollut. Bull. 50:125–146

Fabricius KE, Cooper TF, Humphrey C, Uthicke S, De'ath G, Davidson J, LeGrand H, Thompson AA, Schaffelke B (2012) A bioindicator system for water quality on inshore coral reefs of the Great Barrier Reef. Mar. Pollut. Bull. 65:320–332

Fabricius KE, Cséke S, Humphrey C, De'ath G (2013a) Does trophic status enhance or reduce the thermal tolerance of scleractinian corals? A review, experiment and conceptual framework. PLoS One 8:e54399

Fabricius KE, De'ath G, Humphrey C, Zagorskis I, Schaffelke B (2013b) Intra-annual variation in turbidity in response to terrestrial runoff on near-shore coral reefs of the Great Barrier Reef. Estuar. Coast. Shelf Sci. 116:57–65

Fabricius KE, De'ath G, McCook L, Turak E, Williams DM (2005) Changes in algal, coral

and fish assemblages along water quality gradients on the inshore Great Barrier Reef. Mar. Pollut. Bull. 51:384–398

Fabricius KE, De'ath G, Puotinen ML, Done TJ, Cooper TF, Burgess SC (2008) Disturbance gradients on inshore and offshore coral reefs caused by a severe tropical cyclone. Limnol. Oceanogr. 53:690–704

Fabricius KE, Logan M, Weeks S, Brodie JE (2014) The effects of river run-off on water clarity across the central Great Barrier Reef. Mar. Pollut. Bull. 84:191–200

Feil R, Fraga MF (2012) Epigenetics and the environment: emerging patterns and implications. Nat. Rev. Genet. 13:97–109

Ferrier-Pagès C, Witting J, Tambutté E, Sebens KP (2003) Effect of natural zooplankton feeding on the tissue and skeletal growth of the scleractinian coral *Stylophora pistillata*. Coral Reefs 22:229–240

Ferrier MD (1991) Net uptake of dissolved free amino acids by four scleractinian corals. Coral Reefs 10:183–187

Figueiredo J, Baird AH, Cohen MF, Flot JF, Kamiki T, Meziane T, Tsuchiya M, Yamasaki H (2012) Ontogenetic change in the lipid and fatty acid composition of scleractinian coral larvae. Coral Reefs 31:613–619

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

Fitt WK, Spero HJ, Halas J, White MW, Porter JW (1993) Recovery of the coral *Montastrea annularis* in the Florida Keys after the 1987 Caribbean "Bleaching event". Coral Reefs 12:57–64

Flores F, Hoogenboom MO, Smith LD, Cooper TF, Abrego D, Negri AP (2012) Chronic exposure of corals to fine sediments: lethal and sub-lethal impacts. PLoS One 7:e37795

Francis DS, Salmon ML, Kenway MJ, Hall MR (2014) Palinurid lobster aquaculture: nutritional progress and considerations for successful larval rearing. Rev. Aquac. 6:180–203

Funk CD (2001) Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 294:1871–1875

GBRMPA (2010) Water quality guidelines for the Great Barrier Reef Marine Park: revised edition 2010. Great Barrier Reef Marine Park Authority Townsville, Australia, pp 100

Gladfelter EH (1982) Skeletal development in *Acropora cervicornis*: I. Patterns of calcium carbonate accretion in the axial corallite. Coral Reefs 1:45–51

Gladfelter EH (1983) Skeletal development in *Acropora cervicornis*: II. diel patterns of calcium carbonate accretion. Coral Reefs 2:91–100

Glencross BD (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquac. 1:71–124

Gochfeld DJ (2004) Predation-induced morphological and behavioral defenses in a hard coral: implications for foraging behavior of coral-feeding butterflyfishes. Mar. Ecol. Prog. Ser. 267:145–158

Golbuu Y, van Woesik R, Richmond RH, Harrison P, Fabricius KE (2011) River discharge reduces reef coral diversity in Palau. Mar. Pollut. Bull. 62:824–31

Granados-Cifuentes C, Bellantuono AJ, Ridgway T, Hoegh-Guldberg O, Rodriguez-Lanetty M (2013) High natural gene expression variation in the reef-building coral *Acropora millepora*: potential for acclimative and adaptive plasticity. BMC Genomics 14: e50685

Grand HM, Fabricius KE (2010) Relationship of internal macrobioeroder densities in living massive *Porites* to turbidity and chlorophyll on the Australian Great Barrier Reef. Coral Reefs 30:97–107

Grottoli AG, Rodrigues LJ, Juarez C (2004) Lipids and stable carbon isotopes in two species of Hawaiian corals, *Porites compressa* and *Montipora verrucosa*, following a bleaching event. Mar. Biol. 145:621–631

Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440:1186–1189

Guil-Guerrero JL (2007) Stearidonic acid (18:4n-3): metabolism, nutritional importance, medical uses and natural sources. Eur. J. Lipid Sci. Technol. 109:1226–1236

Haapkylä J, Unsworth RKF, Flavell M, Bourne DG, Schaffelke B, Willis BL (2011) Seasonal rainfall and runoff promote coral disease on an inshore reef. PLoS One 6: e16893

Harland AD, Davies PS, Fixter LM (1992) Lipid content of some Carribbean corals in relation to depth and light. Mar. Biol. 113:357–361

Harland AD, Fixter LM, Davies PS, Anderson RA (1991) Distribution of lipids between the zooxanthellae and animal compartment in the symbiotic sea anemone *Anemonia viridis*: wax esters, triglycerides and fatty acids. Mar. Biol. 110:13–19

Harland AD, Navarro JC, Davies PS, Fixter LM (1993) Lipids of some Caribbean and Red Sea corals: total lipid, wax esters, triglycerides and fatty acids. Mar. Biol. 117:113–117

Harvell CD, Jordan-Dahlgren E, Merkel S, Rosenberg E, Raymundo L, Smith EG, Weil E, Willis BL (2007) Coral disease, environmental drivers, and the balance between coral and microbial associates. Oceanography 20:172–195

Hawkins JP, Roberts CM (1992) Effects of recreational SCUBA diving on fore-reef slope communities of coral reefs. Biol. Conserv. 62:171–178

Hazel JR, Williams EE (1990) The role of alterations in membrane lipid composition in enabling physiological adaptation of organisms to their physical environment. Prog. Lipid Res. 29:167–227

Hinrichs S, Patten NL, Allcock RJN, Saunders SM, Strickland D, Waite AM (2013a) Seasonal variations in energy levels and metabolic processes of two dominant *Acropora* species (*A. spicifera and A. digitifera*) at Ningaloo Reef. Coral Reefs 32:623–635

Hinrichs S, Patten NL, Feng M, Strickland D, Waite AM (2013b) Which environmental factors predict seasonal variation in the coral health of *Acropora digitifera* and *Acropora spicifera* at Ningaloo Reef? PLoS One 8:e60830

Hinrichs S, Patten NL, Waite AM (2013c) Temporal variations in metabolic and autotrophic indices for *Acropora digitifera* and *Acropora spicifera* - implications for monitoring projects. PLoS One 8:e63693

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

Holmes KE, Edinger EN, Hariyadi, Limmon GV, Risk MJ (2000) Bioerosion of live massive corals and branching coral rubble on Indonesian coral reefs. Mar. Pollut. Bull. 40:606–617

Hoogenboom MO, Beraud E, Ferrier-Pagès C (2010) Relationship between symbiont density and photosynthetic carbon acquisition in the temperate coral *Cladocora caespitosa*. Coral Reefs 29:21–29

Hoogenboom MO, Connolly SR, Anthony KRN (2011) Biotic and abiotic correlates of tissue quality for common scleractinian corals. Mar. Ecol. Prog. Ser. 438:119–128

Hopley D, Smithers SG, Parnell KE (2007) The Geomorphology of the Great Barrier Reef; Development, Diversity and Change. Cambridge University Press New York, NY, pp 532

Houlbrèque F, Ferrier-Pagès C (2009) Heterotrophy in tropical scleractinian corals. Biol. Rev. 84:1–17

Howells EJ, Berkelmans R, van Oppen MJH, Willis BL, Bay LK (2013) Historical thermal regimes define limits to coral acclimatization. Ecology 94:1078–1088

Hughes TP (1987) Skeletal density and growth form of corals. Mar. Ecol. Prog. Ser. 35:259–

266

Hulbert AJ (2003) Life, death and membrane bilayers. J. Exp. Biol. 206:2303–2311

Imbs AB, Dang LPT, Rybin VG, Svetashev VI (2015) Fatty acid, lipid class, and phospholipid molecular species composition of the soft coral *Xenia* sp. (Nha Trang Bay,the South China Sea, Vietnam). Lipids 50:575–589

Imbs AB, Yakovleva IM, Latyshev NA, Pham LQ (2010) Biosynthesis of polyunsaturated fatty acids in zooxanthellae and polyps of corals. Russ. J. Mar. Biol. 36:452–457

Jimbo M, Yamashita H, Koike K, Sakai R, Kamiya H (2010) Effects of lectin in the scleractinian coral *Ctenactis echinata* on symbiotic zooxanthellae. Fish. Sci. 76:355–363

Johns RB, Nichols PD, Perry GJ (1979) Fatty acid composition of ten marine algae from Australian waters. Phytochemistry 18:799–802

Jokiel PL (2011) The reef coral two compartment proton flux model: a new approach relating tissue-level physiological processes to gross corallum morphology. J. Exp. Mar. Bio. Ecol. 409:1–12

Jones RJ, Bessell-Browne P, Fisher R, Klonowski W, Slivkoff M (2016) Assessing the impacts of sediments from dredging on corals. Mar. Pollut. Bull. 102:9–29

Joseph JD (1979) Lipid composition of marine and estuarine invertebrates: Porifera and Cnidaria. Prog. Lipid Res. 18:1–30

Kapoor R, Huang YS (2006) Gamma linolenic acid: an anti-inflammatory omega-6 fatty acid. Curr. Pharm. Biotechnol. 7:531–534

Kaur G, Cameron-Smith D, Garg M, Sinclair AJ (2011) Docosapentaenoic acid (22:5n-3): a review of its biological effects. Prog. Lipid Res. 50:28–34

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol. Lett. 7:1225–1241

Kellogg RB, Patton JS (1983) Lipid droplets, medium of energy exchange in the symbiotic anemone *Condylactis gigantea*: a model coral polyp. Mar. Biol. 75:137–149

Kenkel CD, Meyer E, Matz MV (2013) Gene expression under chronic heat stress in populations of the mustard hill coral (*Porites astreoides*) from different thermal environments. Mol. Ecol. 22:4322–4334

Kenkel CD, Setta SP, Matz MV (2015) Fine-scale environmental specialization of reef-building corals might be limiting reef recovery in the Florida Keys. Ecology 96:3197–3212

Kenkel CD, Sheridan C, Leal MC, Bhagooli R, Castillo KD, Kurata N, McGinty ES, Goulet TL, Matz MV (2014) Diagnostic gene expression biomarkers of coral thermal stress. Mol. Ecol. Resour. 14:667–678

Kneeland J, Hughen K, Cervino J, Hauff B, Eglinton T (2013) Lipid biomarkers in *Symbiodinium* dinoflagellates: new indicators of thermal stress. Coral Reefs 32:923–934

Koop K, Booth DJ, Broadbent A, Brodie JE, Bucher D, Capone D, Coll J, Dennison W, Erdmann M, Harrison P, Hutchings PA, Jones GB, Larkum AWD, Neil JO, Steven A, Tentori E, Ward S, Williamson JE, Yellowlees D (2001) ENCORE: The effect of nutrient enrichment on coral reefs. Synthesis of results and conclusions. Mar. Pollut. Bull. 42:91–120

Kuhnert PM, Henderson BL, Lewis SE, Bainbridge ZT, Wilkinson SN, Brodie JE (2012) Quantifying total suspended sediment export from the Burdekin River catchment using the loads regression estimator tool. Water Resour. Res. 48:1–18

Kvennefors ECE, Leggat W, Kerr CC, Ainsworth TD, Hoegh-Guldberg O, Barnes AC (2010) Analysis of evolutionarily conserved innate immune components in coral links immunity and symbiosis. Dev. Comp. Immunol. 34:1219–1229

Langfelder P, Horvath S (2008) WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9:559

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat. Methods 9:357–359

Latyshev NA, Naumenko N, Svetashev V, Latypov YY (1991) Fatty acids of reef-building corals. Mar. Ecol. Prog. Ser. 76:295–301

Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129: 271–280

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

Leuzinger S, Anthony KRN, Willis BL (2003) Reproductive energy investment in corals: scaling with module size. Oecologia 136:524–531

Lewis RW (1962) Temperature and pressure effects on the fatty acids of some marine ectotherms. Comp. Biochem. Physiol. 6:75–89

Li Y, Innocentin S, Withers DR, Roberts NA, Gallagher AR, Grigorieva EF, Wilhelm C, Veldhoen M (2011) Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell 147:629–640

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

Littler MM, Taylor PR, Littler DS (1989) Complex interactions in the control of coral zonation on a Caribbean reef flat. Oecologia 80:331–340

Lohman BK, Weber JN, Bolnick DI (2016) Evaluation of TaqSeq, a reliable low-cost alternative for RNAseq. BioRxiv pre-print:1–16

Los DA, Mironov KS, Allakhverdiev SI (2013) Regulatory role of membrane fluidity in gene expression and physiological functions. Photosynth. Res. 116:489–509

Lough JM, Barnes DJ (1992) Comparisons of skeletal density variations in *Porites* from the central Great Barrier Reef. J. Exp. Mar. Bio. Ecol. 155:1–25

Loya Y, Sakai K, Nakano Y, van Woesik R (2001) Coral bleaching: the winners and the losers. Ecol. Lett. 4:122–131

Mackenzie JB, Munday PL, Willis BL, Miller DJ, van Oppen MJH (2004) Unexpected patterns of genetic structuring among locations but not colour morphs in *Acropora nasuta* (Cnidaria; Scleractinia). Mol. Ecol. 13:9–20

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

Madin JS, Hughes TP, Connolly SR (2012) Calcification, storm damage and population resilience of tabular corals under climate change. PLoS One 7:e46637

Maina J, de Moel H, Zinke J, Madin JS, McClanahan TR, Vermaat JE (2013) Human deforestation outweighs future climate change impacts of sedimentation on coral reefs. Nat. Commun. 4:1–7

Manzello DP, Enochs IC, Kolodziej G, Carlton R (2015) Recent decade of growth and calcification of *Orbicella faveolata* in the Florida Keys: an inshore-offshore comparison. Mar. Ecol. Prog. Ser. 521:81–89

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

Meissner KJ, Lippmann T, Sen Gupta A (2012) Large-scale stress factors affecting coral reefs: open ocean sea surface temperature and surface seawater aragonite saturation over the next 400 years. Coral Reefs 31:309–319

Merrill Jr AH, Schroeder JJ (1993) Lipid modulation of cell function. Annu. Rev. Nutr. 13:539–59

Meyer E, Aglyamova GV, Matz MV (2011) Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. Mol. Ecol. 20:3599–3616

Meyers PA (1979) Polyunsaturated fatty acids in coral: indicators of nutritional sources. Mar. Biol. Lett. 1:69–75

Meyers PA, Porter JW, Chad RL (1978) Depth analysis of fatty acids in two Caribbean reef corals. Mar. Biol. 49:197–202

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

Mika A, Gołębiowski M, Skorkowski E, Stepnowski P (2014) Lipids of adult brown shrimp, *Crangon crangon*: seasonal variations in fatty acids class composition. J. Mar. Biol. Assoc. United Kingdom 94:1–8

Mitchell C, Brodie JE, White I (2005) Sediments, nutrients and pesticide residues in event flow conditions in streams of the Mackay Whitsunday Region, Australia. Mar. Pollut. Bull. 51:23–36

Mock T, Kroon BMA (2002) Photosynthetic energy conversion under extreme conditions - II: the significance of lipids under light limited growth in Antarctic sea ice diatoms. Phytochemistry 61:53–60

Monroig Ó, Tocher DR, Navarro JC (2013) Biosynthesis of polyunsaturated fatty acids in marine invertebrates: recent advances in molecular mechanisms. Mar. Drugs 11:3998–4018

Moya A, Huisman L, Ball EE, Hayward DC, Grasso LC, Chua CM, Woo HN, Gattuso J-P, Forêt S, Miller DJ (2012) Whole transcriptome analysis of the coral *Acropora millepora* reveals complex responses to CO_2 -driven acidification during the initiation of calcification. Mol. Ecol. 21:2440–2454

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

Mydlarz LD, McGinty ES, Harvell CD (2010) What are the physiological and immunological responses of coral to climate warming and disease? J. Exp. Biol. 213:934–945

Nettleton JA (1995) Introduction to fatty acids. In: Omega-3 Fatty Acids and Health. Chapman & Hall New York, NY, pp 1–63

Neudecker S (1979) Effect of grazing and browsing fishes on the zonation of corals in Guam. Ecology 60:666–672

Nomura M, Kamogawa H, Susanto E, Kawagoe C, Yasui H, Saga N, Hosokawa M, Miyashita K (2013) Seasonal variations of total lipids, fatty acid composition, and fucoxanthin contents of *Sargassum horneri* (Turner) and *Cystoseira hakodatensis* (Yendo) from the northern seashore of Japan. J. Appl. Phycol. 25:1159–1169

Oksanen J, Guillaume Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin

PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2016) vegan: community ecology package. R package version 2.4-0

Oku H, Yamashiro H, Onaga K, Sakai K, Iwasaki H (2003) Seasonal changes in the content and composition of lipids in the coral *Goniastrea aspera*. Coral Reefs 22:83–85

Oliver JK, Chalker BE, Dunlap WC (1983) Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. I. long-term growth responses of *Acropora formosa* (Dana 1846). J. Exp. Mar. Bio. Ecol. 73:11–35

van Oppen MJH, Mahiny AJ, Done TJ (2005) Geographic distribution of zooxanthella types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. Coral Reefs 24:482–487

van Oppen MJH, Palstra FP, Piquet AMT, Miller DJ (2001) Patterns of coral-dinoflagellate associations in *Acropora*: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. Proc. R. Soc. B 268:1759–1767

Orpin AR, Ridd PV (2012) Exposure of inshore corals to suspended sediments due to wave-resuspension and river plumes in the central Great Barrier Reef: a reappraisal. Cont. Shelf Res. 47:55–67

Page CA, Willis BL (2008) Epidemiology of skeletal eroding band on the Great Barrier Reef and the role of injury in the initiation of this widespread coral disease. Coral Reefs 27:257–272

Papina M, Meziane T, van Woesik R (2003) Symbiotic zooxanthellae provide the host-coral *Monitpora digitata* with polyunsaturated fatty acids. Comp. Biochem. Physiol. Part B Comp. Biochem. 135:533–537

Parrish CC, Pethybridge HR, Young JW, Nichols PD (2015) Spatial variation in fatty acid trophic markers in albacore tuna from the southwestern Pacific Ocean - a potential "tropicalization" signal. Deep. Res. Part II Top. Stud. Oceanogr. 113:199–207

Patton JS, Burris JE (1983) Lipid synthesis and extrusion by freshly isolated zooxanthellae (symbiotic algae). Mar. Biol. 75:131–136

Perry CT, Smithers SG, Gulliver P, Browne NK (2012) Evidence of very rapid reef accretion and reef growth under high turbidity and terrigenous sedimentation. Geology 40:719–722

Philipp E, Fabricius KE (2003) Photophysiological stress in scleractinian corals in response to short-term sedimentation. J. Exp. Mar. Bio. Ecol. 287:57–78

Pigliucci M, Murren CJ, Schlichting CD (2006) Phenotypic plasticity and evolution by genetic assimilation. J. Exp. Biol. 209:2362–2367

Pisapia C, Hennige SJ, Haapkylä J, Matteucci R, Smith DJ (2012) Morphological changes in polyp structure of massive coral species in clear and turbid waters. Bull. Mar. Sci. 88:183–191

Pollock FJ, Lamb JB, Field SN, Heron SF, Schaffelke B, Shedrawi G, Bourne DG, Willis BL (2014) Sediment and turbidity associated with offshore dredging increase coral disease prevalence on nearby reefs. PLoS One 9:e102498

Porter JW, Fitt WK, Spero HJ, Rogers CS, White MW (1989) Bleaching in reef corals: physiological and stable isotopic responses. Proc. Natl. Acad. Sci. 86:9342–9346

Pratchett MS (2007) Dietary selection by coral-feeding butterflyfishes (*Chaetodontidae*) on the Great Barrier Reef, Australia. Raffles Bull. Zool. 14:171–176

Pratchett MS, Anderson KD, Hoogenboom MO, Widman E, Baird AH, Pandolfi JM, Edmunds PJ, Lough JM (2015) Spatial, temporal, and taxonomic variation in coral growth-implications for the structure and function of coral reef ecosystems. Oceanogr. Mar. Biol. An Annu. Rev. 53:215–296

Queensland Government (2015) Great Barrier Reef Report Card 2014: Reef water quality protection plan. Department of Premier and Cabinet Brisbane, Australia, pp 8

Rawlings ND, Barrett AJ (1994) Families of serine peptidases. Methods in Enzymology. 244:19–61

Renaud SM, Zhou HC, Parry DL, Thinh L, Woo KC (1995) Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, *Nitzschia paleacea*, and commercial species *Isochrysis* sp. (clone T.ISO). J. Appl. Phycol. 7:595–602

Revel J, Massi L, Mehiri M, Boutoute M, Mayzaud P, Capron L, Sabourault C (2016) Differential distribution of lipids in epidermis, gastrodermis and hosted *Symbiodinium* in the sea anemone *Anemonia viridis*. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 191:140–151

Richier S, Sabourault C, Ferrier-Pagès C, Merle P-L, Furla P, Allemand D (2010) Cnidarian-dinoflagellate symbiosis-mediated adaptation to environmental perturbations. In: Seckbach J, Grube M (eds) Symbiosis and Stress. Springer New York, NY, pp 145–175

Risk MJ, Sammarco PW, Edinger EN (1995) Bioerosion in *Acropora* across the continental shelf of the Great Barrier Reef. Coral Reefs 14:79–86

Rocker MM, Brandl SJ (2014) Transplantation of corals into a new environment results in substantial skeletal loss in *Acropora tenuis*. Mar. Biodivers. 45:321–326

Rocker MM, Noonan SHC, Humphrey C, Moya A, Willis BL, Bay LK (2015) Expression of

calcification and metabolism-related genes in response to elevated pCO2 and temperature in the reef-building coral *Acropora millepora*. Mar. Genomics 24:6–11

Rocker MM, Willis BL, Bay LK (2012) Thermal stress-related gene expression in corals with different *Symbiodinium* types. Proc. 12th Int. Coral Reef Symp. Cairns, Aust.

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

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar. Ecol. Prog. Ser. 243:1–10

Rosa R, Calado R, Narciso L, Nunes ML (2007) Embryogenesis of decapod crustaceans with different life history traits, feeding ecologies and habitats: a fatty acid approach. Mar. Biol. 151:935–947

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

Rosenfeld M, Bresler V, Abelson A (1999) Sediment as a possible source of food for corals. Ecol. Lett. 2:345–348

Rotjan RD, Dimond JL (2010) Discriminating causes from consequences of persistent parrotfish corallivory. J. Exp. Mar. Bio. Ecol. 390:188–195

Rotjan RD, Dimond JL, Thornhill DJ, Leichter JJ, Helmuth B, Kemp DW, Lewis SM (2006) Chronic parrotfish grazing impedes coral recovery after bleaching. Coral Reefs 25:361–368

Rotjan RD, Lewis SM (2005) Selective predation by parrotfishes on the reef coral *Porites astreoides*. Mar. Ecol. Prog. Ser. 305:193–201

Rotjan RD, Lewis SM (2008) Impact of coral predators on tropical reefs. Mar. Ecol. Prog. Ser. 367:73–91

Rotjan RD, Lewis SM (2009) Predators selectively graze reproductive structures in a clonal marine organism. Mar. Biol. 156:569–577

Russo GL (2009) Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemisty to clinical implications in cardiovascular prevention. Biochem. Pharmacol. 77:937–946

Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. Ann. Rev. Mar. Sci. 3:509–535

Sargent JR, Bell MV, Hendersen RJ, Tocher DR (1990) Polyunsaturated fatty acids in marine and terrestrial food webs. In: Truchot JP, Lahlou B (eds) Animal Nutrition and Tranport Processes. Karger Basel, Switzerland, pp 11–23

Saunders SM, Radford B, Bourke SA, Thiele Z, Bech T, Mardon J (2005) A rapid method for

determining lipid fraction ratios of hard corals under varying sediment and light regimes. Environ. Chem. 2:331–336

Schaffelke B, Carleton J, Skuza M, Zagorskis I, Furnas MJ (2012) Water quality in the inshore Great Barrier Reef lagoon: implications for long-term monitoring and management. Mar. Pollut. Bull. 65:249–260

Seemann J, Carballo-Bolaños R, Berry KL, González CT, Richter C, Leinfelder RR (2012) Importance of heterotrophic adaptations of corals to maintain energy reserves. Proc. 12th Int. Coral Reef Symp. Cairns, Aust.

Seemann J, González CT, Carballo-Bolaños R, Berry KL, Heiss GA, Struck U, Leinfelder RR (2014) Assessing the ecological effects of human impacts on coral reefs in Bocas del Toro, Panama. Environ. Monit. Assess. 186:1747–1763

Seemann J, Sawall Y, Auel H, Richter C (2013) The use of lipids and fatty acids to measure the trophic plasticity of the coral *Stylophora subseriata*. Lipids 48:275–286

Siebeck UE, Marshall NJ, Klüter A, Hoegh-Guldberg O (2006) Monitoring coral bleaching using a colour reference card. Coral Reefs 25:453–460

Simkiss K (1964) Phosphates as crystal poisons of calcification. Biol. Rev. Camb. Philos. Soc. 39:487–505

Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmacother. 56:365–379

Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp. Biol. Med. 233:674–688

Singmann H, Bolker B, Westfall J, Aust F (2016) afex: analysis of factorial experiments. R package version 0.16-1

Smith GJ, Hoegh-Guldberg O (1989) Influence of the population density of zooxanthellae and supply of ammonium on the biomass and metabolic characteristics of the reef corals *Seriatopora hystrix* and *Stylophora pistillata*. Mar. Ecol. Prog. Ser. 57:173–186

Smith LW, Barshis DJ, Birkeland C (2007) Phenotypic plasticity for skeletal growth, density and calcification of *Porites lobata* in response to habitat type. Coral Reefs 26:559–567

Sofonia J (2006) Sediment responses of corals from inshore reefs. Masters Thesis, James Cook University

Stambler N, Popper N, Dubinsky Z, Stimson JS (1991) Effects of nutrient enrichment and water motion on the coral *Pocillopora damicornis*. Pacific Sci. 45:299–307

Stephens GC (1962) Uptake of organic material by aquatic invertebrates. I. uptake of glucose by the solitary coral, *Fungia scutaria*. Biol. Bull. 123:648–659

Stimson JS (1987) Location, quantity and rate of change in quantity of lipids in tissue of Hawaiian hermatypic corals. Bull. Mar. Sci. 41:889–904

Stimson JS, Kinzie III RA (1991) The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damincornis* (Linnaeus) under nitrogen-enrichment and control conditions. J. Exp. Mar. Bio. Ecol. 153:63–74

Strahl J, Francis DS, Doyle J, Humphrey C, Fabricius KE (2016) Biochemical responses to ocean acidification contrast between tropical corals with high and low abundances at volcanic carbon dioxide seeps. ICES J. Mar. Sci. 73:897–909

Stubbs CD (1992) The structure and function of docosahexaenoic acid in membranes. The 3rd International Congress on Essential Fatty Acids and Eicosanoids, Adelaide, Aust. 116–121

Swanson D, Block R, Mousa S (2012) Omega-3 fatty acids EPA and DHA: health benefits throughout life. Adv. Nutr. 3:1–7

Sweatman H, Delean S, Syms C (2011) Assessing loss of coral cover on Australia's Great Barrier Reef over two decades, with implications for longer-term trends. Coral Reefs 30:521–531

Szmant-Froelich A, Pilson MEQ (1980) The effects of feeding frequency and symbiosis with zooxanthellae on the biochemical composition of *Astrangia danae* (Milne, Edwards & Haime 1849). J. Exp. Mar. Bio. Ecol. 48:85–97

Tchernov D, Gorbunov MY, de Vargas C, Yadav SN, Milligan AJ, Haggblom M, Falkowski PG (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. Proc. Natl. Acad. Sci. 101:13531–13535

Thompson AA, Costello P, Davidson J, Logan M, Schaffelke B, Uthicke S, Takahashi M (2011) Reef Rescue Marine Monitoring Program: Final report of AIMS activites 2011 inshore coral reef monitoring. Australian Insititute of Marine Science Townsville, Australia, pp 128

Thompson AA, Costello P, Davidson J, Schaffelke B, Uthicke S, Liddy M (2013) Reef Rescue Marine Monitoring Program: Report of AIMS activities - inshore coral reef monitoring 2012. Australian Insititute of Marine Science Townsville, Australia, pp 120

Thompson AA, Lonborg C, Costello P, Davidson J, Logan M, Furnas MJ, Gunn K, Liddy M, Skuza M, Uthicke S, Wright M, Zagorskis I, Schaffelke B (2014a) Marine Monitoring Program: annual report of AIMS activities 2013-2014 - inshore water quality and coral reef monitoring. Australian Insititute of Marine Science Townsville, Australia, pp 146

Thompson AA, Schroeder T, Brando VE, Schaffelke B (2014b) Coral community responses

to declining water quality: Whitsunday Islands, Great Barrier Reef, Australia. Coral Reefs 33:923–938

Thornhill DJ, Rotjan RD, Todd BD, Chilcoat GC, Iglesias-Prieto R, Kemp DW, LaJeunesse TC, Reynolds JM, Schmidt GW, Shannon T, Warner ME, Fitt WK (2011) A connection between colony biomass and death in Caribbean reef-building corals. PLoS One 6:e29535

Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11:107–184

Tolosa I, Treignier C, Grover R, Ferrier-Pagès C (2011) Impact of feeding and short-term temperature stress on the content and isotopic signature of fatty acids, sterols, and alcohols in the scleractinian coral *Turbinaria reniformis*. Coral Reefs 30:763–774

Tonk L, Sampayo EM, Weeks S, Magno-Canto M, Hoegh-Guldberg O (2013) Host-specific interactions with environmental factors shape the distribution of *Symbiodinium* across the Great Barrier Reef. PLoS One 8:e68533

Treignier C, Grover R, Ferrier-Pagès C, Tolosa I (2008) Effect of light and feeding on the fatty acid and sterol composition of zooxanthellae and host tissue isolated from the scleractinian coral *Turbinaria reniformis*. Limnol. Oceanogr. 53:2702–2710

Tribollet AE, Decherf G, Hutchings PA, Peyrot-Clausade M (2002) Large-scale spatial variability in bioerosion of experimental coral substrates on the Great Barrier Reef (Australia): importance of microborers. Coral Reefs 21:424–432

Ulstrup KE, Hill R, van Oppen MJH, Larkum AWD, Ralph PJ (2008) Seasonal variation in the photo-physiology of homogeneous and heterogeneous *Symbiodinium* consortia in two scleractinian corals. Mar. Ecol. Prog. Ser. 361:139–150

Ulstrup KE, van Oppen MJH (2003) Geographic and habitat partitioning of genetically distinct zooxanthellae (*Symbiodinium*) in *Acropora* corals on the Great Barrier Reef. Mol. Ecol. 12:3477–3484

Vago R, Gill E, Collingwood JC (1997) Laser measurements of coral growth. Nature 386:130–131

Venables WN, Ripley BD (2002) Modern Applied Statisticas with S. Springer New York, NY, pp 495

Veron JEN, Stafford-Smith M (2000) Corals of the World. Australian Institute of Marine Science Townsville, Australia, pp 463

Volkman JK (1999) Australasian research on marine natural products: chemistry, bioactivity and ecology. Mar. Freshw. Res. 50:761–779

Volkman JK, Barrett SM, Dunstan GA, Jeffrey SW (1993) Geochemical significance of the occurrence of dinosterol and other 4-methyl sterols in a marine diatom. Org. Geochem. 20:7–15

Ward S (1995) Two patterns of energy allocation for growth, reproduction and lipid storage in the scleractinian coral *Pocillopora damicornis*. Coral Reefs 14:87–90

Weber M, de Beer D, Lott C, Polerecky L, Kohls K, Abed RMM, Ferdelman TG, Fabricius KE (2012) Mechanisms of damage to corals exposed to sedimentation. Proc. Natl. Acad. Sci. 109:1558–1567

Weis VM (2010) The susceptibility and resilience of corals to thermal stress: adaptation, acclimatization or both? Mol. Ecol. 19:1515–1517

Wiedenmann J, Angelo CD, Smith EG, Hunt AN, Legiret F-E, Postle AD, Achterberg EP (2012) Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nat. Clim. Chang. 3:160–164

van Woesik R, Sakai K, Ganase A, Loya Y (2011) Revisiting the winners and the losers a decade after coral bleaching. Mar. Ecol. Prog. Ser. 434:67–76

van Woesik R, Tomascik T, Blake S (1999) Coral assemblages and physico-chemical cahracteristics of the Whitsundays Islands: evidence of recent community changes. Mar. Freshw. Res. 50:427–440

Wood-Charlson EM, Hollingsworth LL, Krupp DA, Weis VM (2006) Lectin/glycan interactions play a role in recognition in a coral/dinoflagellate symbiosis. Cell. Microbiol. 8:1985–1993

Wooldridge SA (2009) Water quality and coral bleaching thresholds: formalising the linkage for the inshore reefs of the Great Barrier Reef, Australia. Mar. Pollut. Bull. 58:745–751

Wooldridge SA (2010) Is the coral-algae symbiosis really "mutually beneficial" for the partners? BioEssays 32:615–25

Wooldridge SA (2012) A hypothesis linking sub-optimal seawater pCO_2 conditions for cnidarian-*Symbiodinium* symbioses with the exceedence of the interglacial threshold (>260 ppmv). Biogeosciences 9:1709–1723

Wooldridge SA (2014) Formalising a mechanistic linkage between heterotrophic feeding and thermal bleaching resistance. Coral Reefs 33:1131–1136

Wooldridge SA, Done TJ (2009) Improved water quality can ameliorate effects of climate change on corals. Ecol. Appl. 19:1492–1499

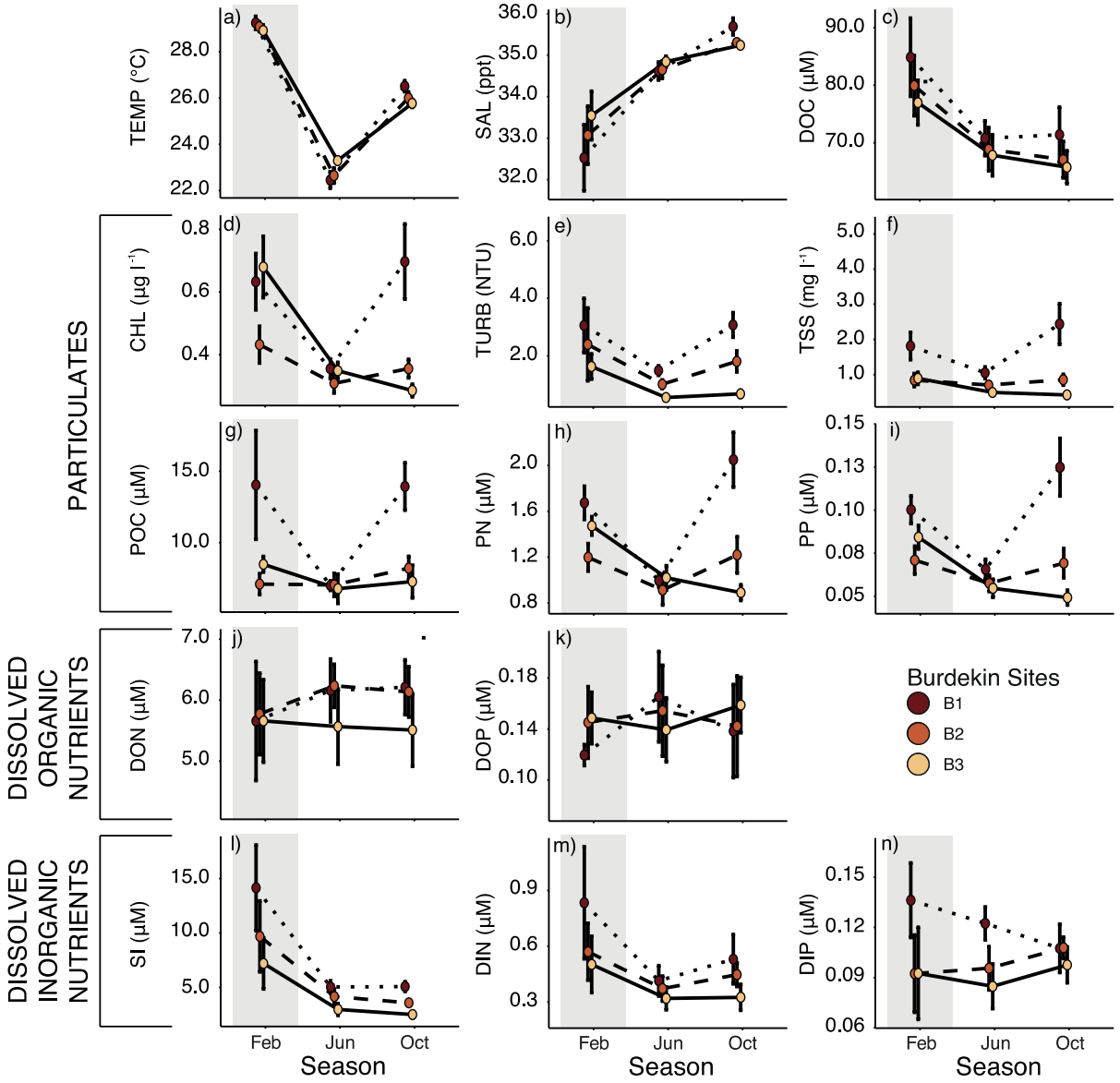
Wooldridge SA, Hinrichs S, Done TJ, Brodie JE (*in review*) Excess seawater nutrients, enlarged algal symbiont densities and bleaching sensitive reef locations: 1. Identifying

thresholds of concern for the Great Barrier Reef, Australia

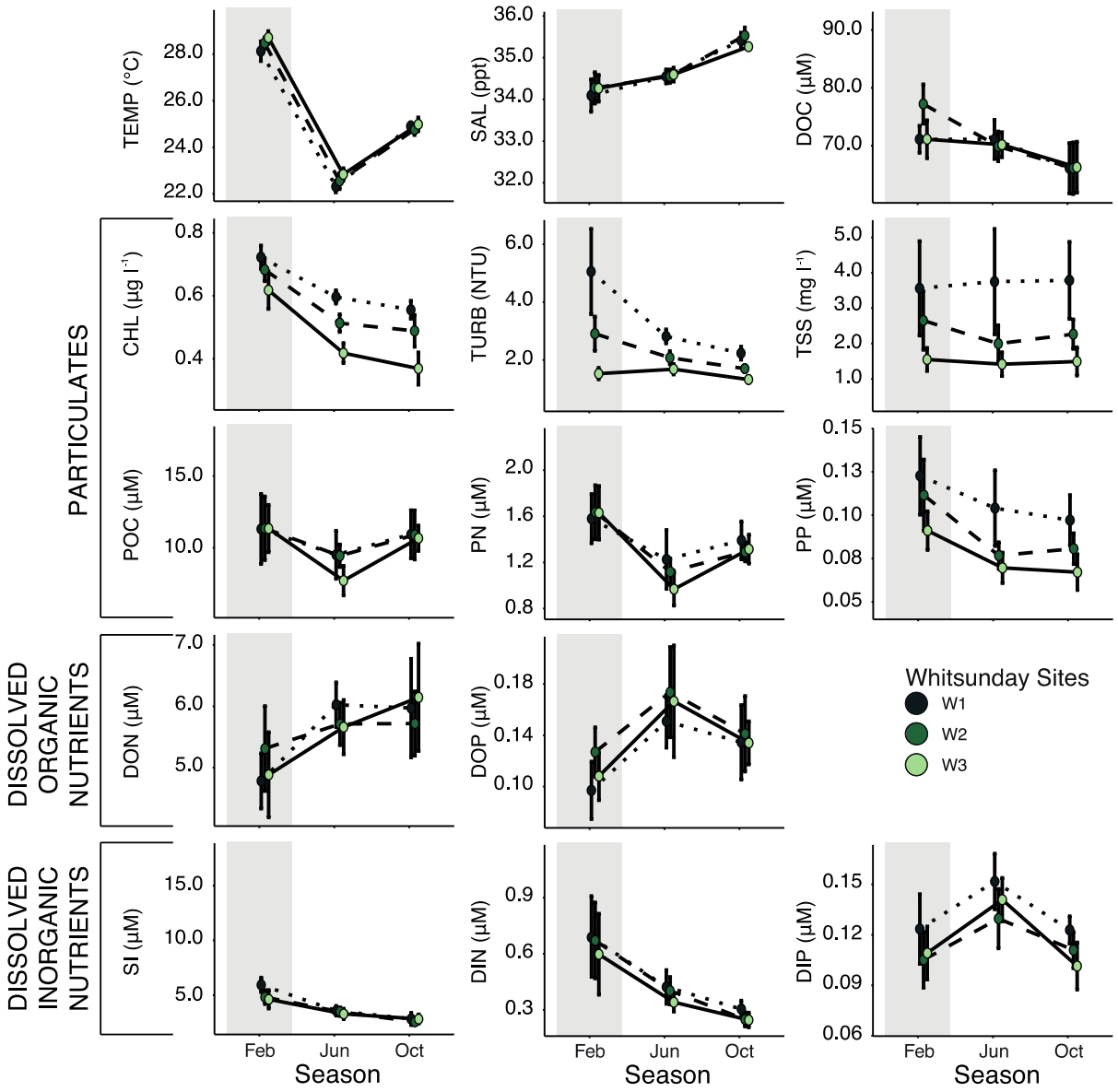
Wright RM, Aglyamova GV, Meyer E, Matz MV (2015) Gene expression associated with white syndromes in a reef building coral, *Acropora hyacinthus*. BMC Genomics 16:371

Yamashiro H, Oku H, Higa H, Chinen I, Sakai K (1999) Composition of lipids, fatty acids and sterols in Okinawan corals. Comp. Biochem. Physiol. Part B Comp. Biochem. 122:397–407

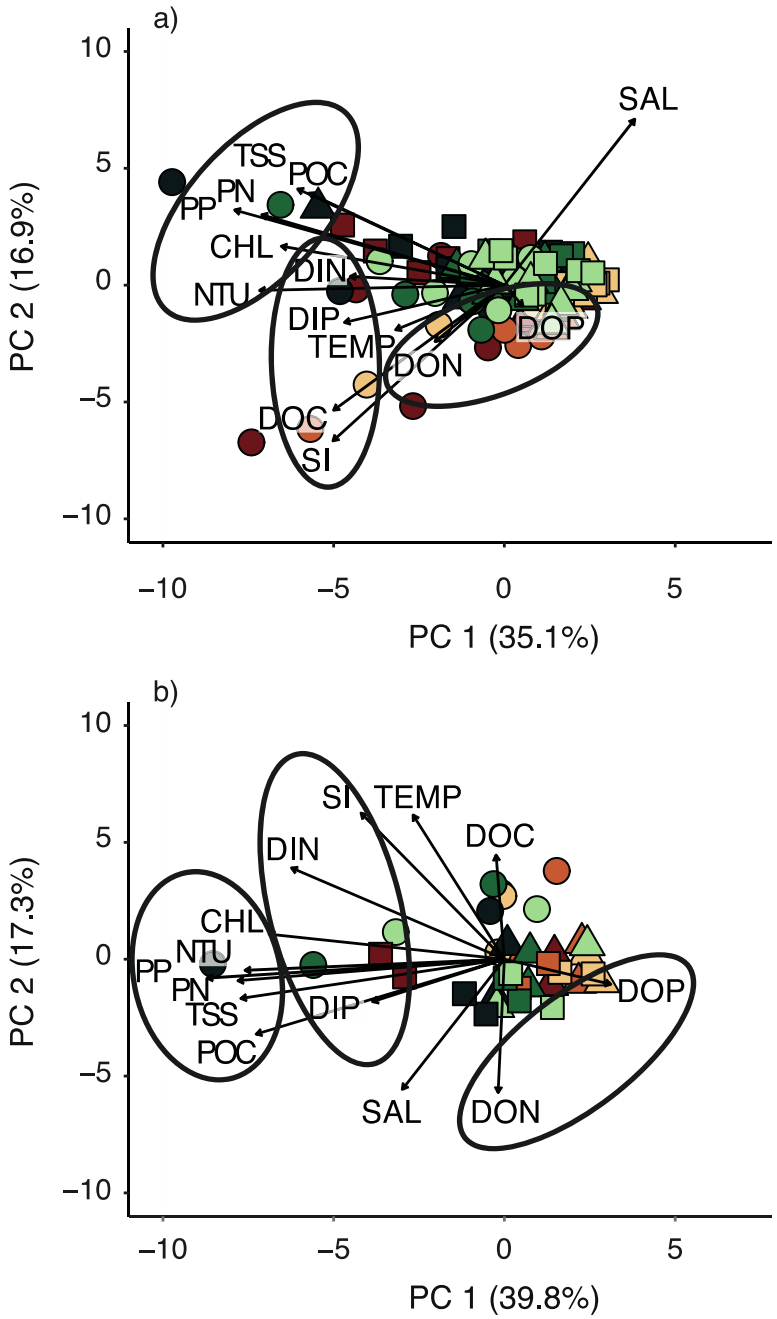
Yamashiro H, Oku H, Onaga K (2005) Effect of bleaching on lipid content and composition of Okinawan corals. Fish. Sci. 71:448–453



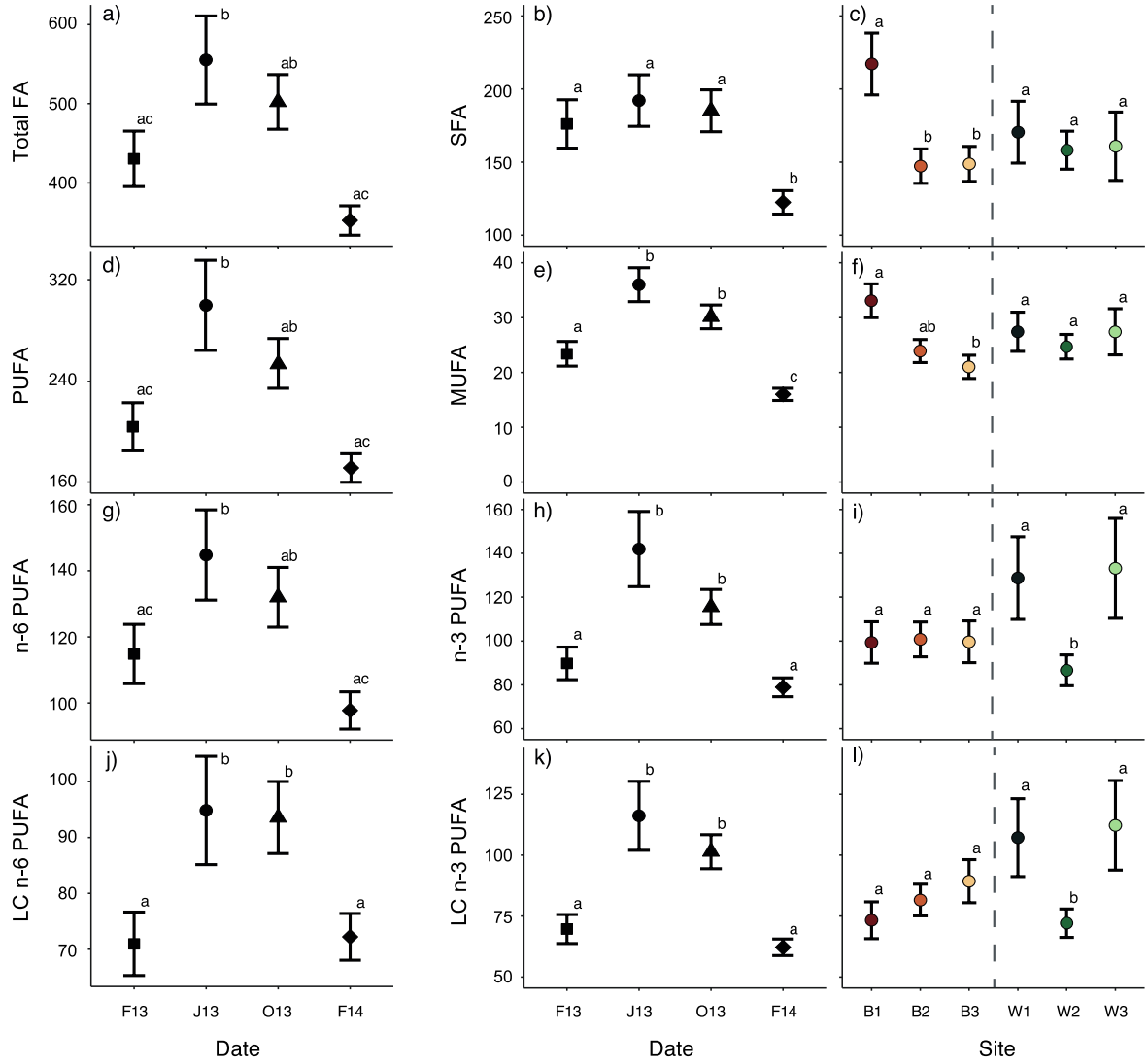
Supplementary Fig. 2.1 Variations in environmental water quality parameters at three inshore sites within the Burdekin region. Data points are means ± SE of samples collected in February, June, and October from 2008 until 2014. Environmental water quality parameters include: (a) Temperature (°C); (b) Salinity (ppt); (c) Dissolved Organic Carbon (µM); (d) Chlorophyll a (µg l^{-1}); (e) Turbidity (NTU); (f) Total Suspended Solids (mg l^{-1}); (g) Particulate Organic Carbon (µM); (h) Particulate Nitrogen (µM); (i) Particulate Phosphorus (µM); (j) Dissolved Organic Nitrogen (µM); (k) Dissolved Organic Phosphorus (µM); (l) Silicate (µM); (m) Dissolved Inorganic Nitrogen (µM); (n) Dissolved Inorganic Phosphorus (µM). Wet season is indicated by light grey panels.



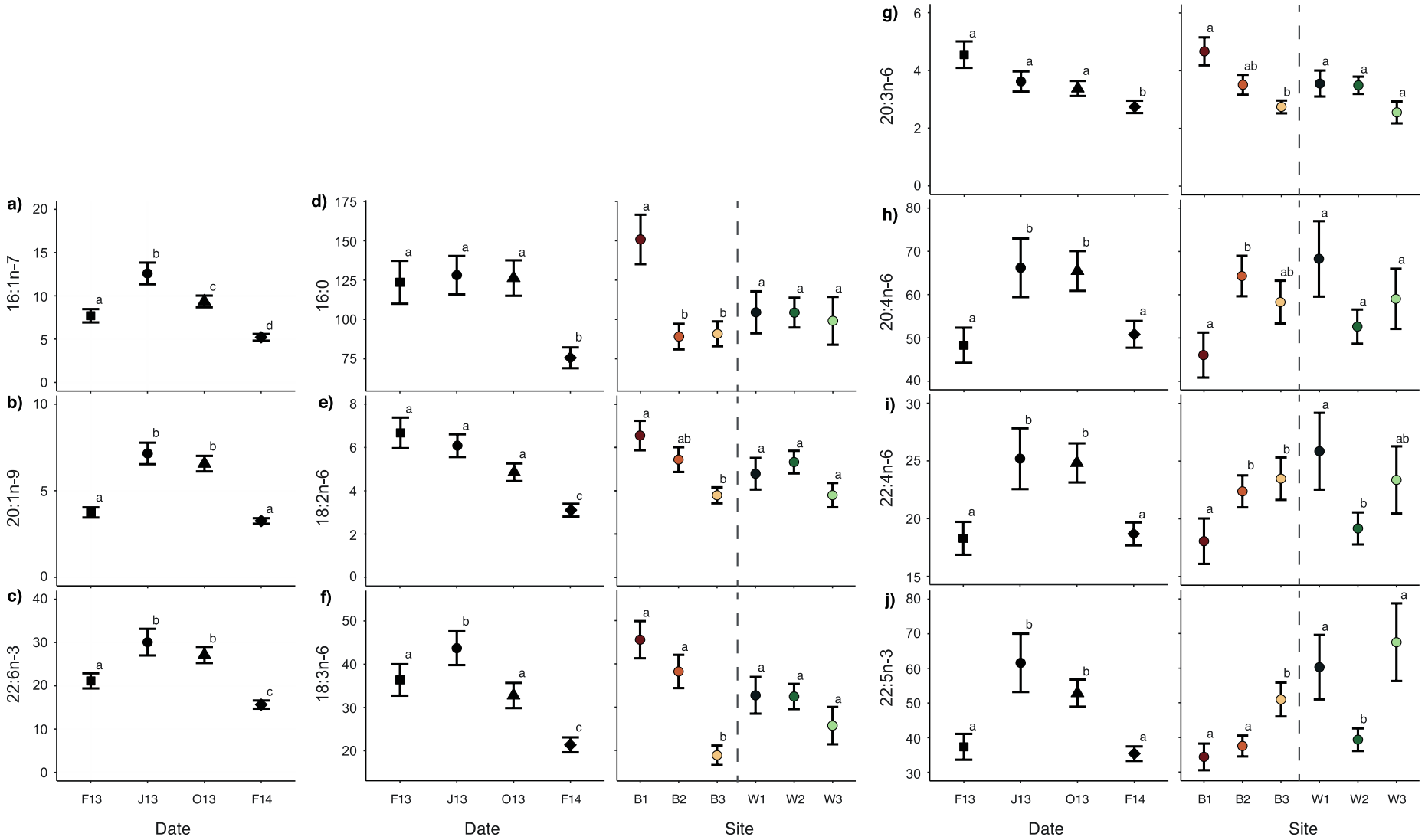
Supplementary Fig. 2.2 Variations in environmental water quality parameters at three inshore sites within the Whitsunday region. Data points are means ± SE of samples collected in February, June, and October from 2008 until 2014. Environmental water quality parameters include: (a) Temperature (°C); (b) Salinity (ppt); (c) Dissolved Organic Carbon (µM); (d) Chlorophyll a (µg l^{-1}); (e) Turbidity (NTU); (f) Total Suspended Solids (mg l^{-1}); (g) Particulate Organic Carbon (µM); (h) Particulate Nitrogen (µM); (i) Particulate Phosphorus (µM); (j) Dissolved Organic Nitrogen (µM); (k) Dissolved Organic Phosphorus (µM); (l) Silicate (µM); (m) Dissolved Inorganic Nitrogen (µM); (n) Dissolved Inorganic Phosphorus (µM). Wet season is indicated by light grey panels.



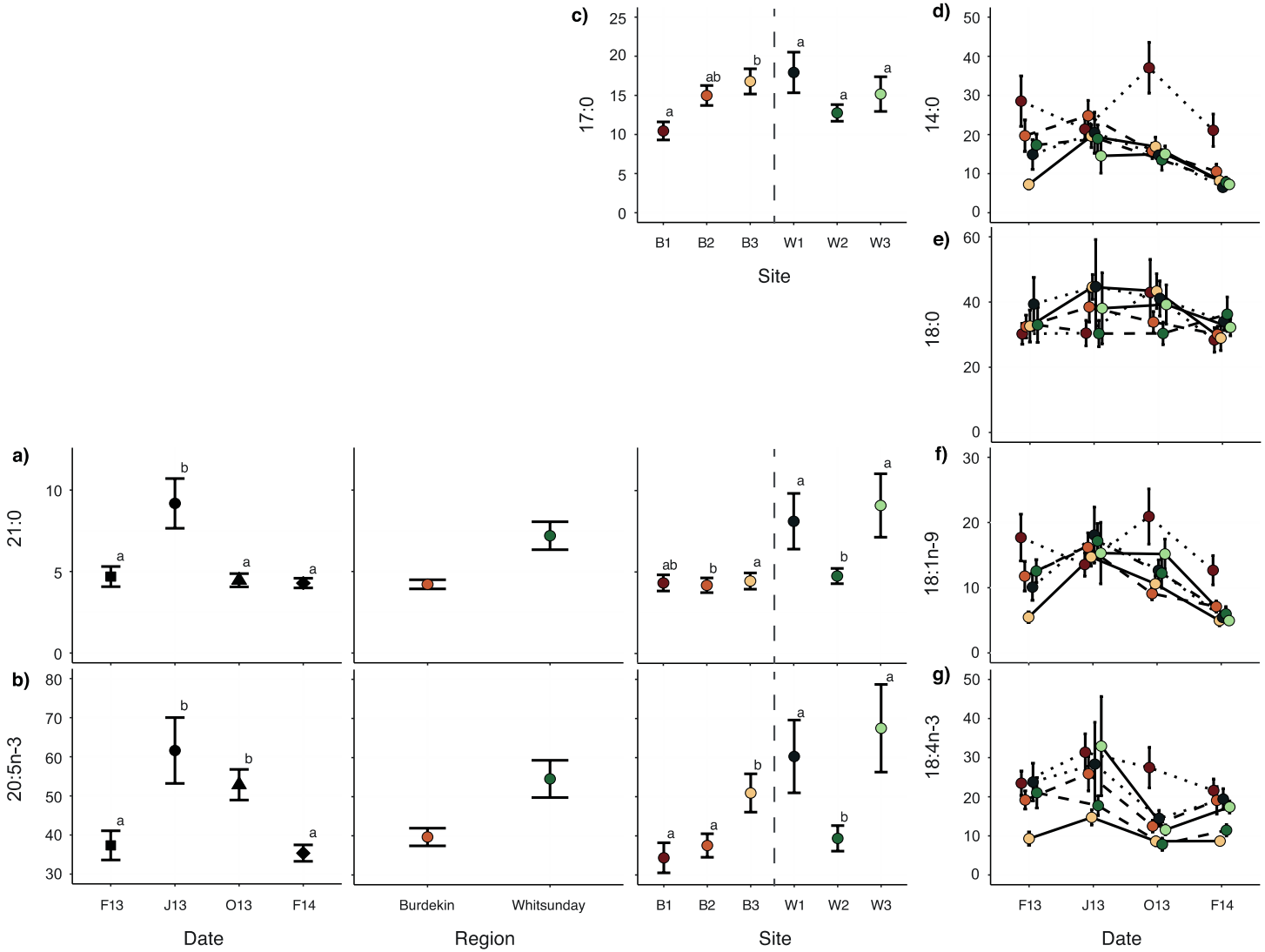
Supplementary Fig. 2.3 Principal component analysis biplots of water quality variables measured at six inshore sites from the Burdekin and the Whitsunday regions. Data were individual measures for each season (February, June and October) and across (a) six years (February 2008 until June 2014) and (b) 2.5 years when coral health attributes were measured (October 2012 until June 2014). Clustered variables were circled in bold black lines to become composite environmental parameters: particulates (PART), dissolved organic nutrients (DO) and dissolved inorganic nutrients (DI). Environmental parameters considered individually are temperature (TEMP), salinity (SAL) and dissolved organic carbon (DOC).



Supplementary Fig. 3.1 Relative abundance of FA classes (standardised to mg FA g lipid^{-1}) of *Acropora tenuis* colonies among variables determined significant by GLMMs in the Burdekin (orange) and Whitsunday (green) regions. FA classes with significant date effects (left column) include: (a) Total FA, (d) PUFA, (g) n-6 PUFA, and (j) LC n-6 PUFA. FA classes with significant date and site effects (right two columns) include: (b, c) SFA, (e, f) MUFA, (h, i) n-3 PUFA, and (k, l) LC n-3 PUFA. Coral colonies were sampled from February 2013 to February 2014.



Supplementary Fig. 3.2a Relative abundance of individual FA (standardised to mg FA g lipid^{-1}) of *Acropora tenuis* among variables determined significant by GLMMs. Individual FA with significant date effects (left column; a-c) and significant date and site effects (right columns; d-j). Coral holobionts were sampled from February 2013 to February 2014.



Supplementary Fig. 3.2b Relative abundance of individual FA (standardised to mg FA g lipid^{-1}) of *Acropora tenuis* among variables determined significant by GLMMs. Individual FA with significant date, region and site effects (left columns; a-b), significant site only effects (c), and significant interactive effects (right column; d-g). Coral holobionts were sampled from February 2013 to February 2014.

Supplementary Table 3.1 Effects of date, region and site (within region) on FA content of Burdekin and Whitsunday corals. Generalised linear mixed model (GLMM) outputs for FA classes and individual FA with a Gamma error distribution. *** denotes significance at α = 0.001. ** denotes significance at α = 0.01. * denotes significance at α = 0.05.

Fatty Acid Classes					
Total Fatty Acids					
	df	Deviance	F value	P	
Date	3	7.743	7.049	<0.001	***
Region	1	0.010	0.026	0.872	
Date*Region	3	0.367	0.334	0.801	
Region(Site)	4	1.604	1.095	0.360	
Saturated Fatty Acids					
	df	Deviance	F value	P	
Date	3	8.185	7.065	<0.001	***
Region	1	0.208	0.540	0.463	
Date*Region	3	0.846	0.731	0.535	
Region(Site)	4	4.584	2.968	0.020	*
Monounsaturated Fatty Acids					
	df	Deviance	F value	P	
Date	3	23.81	22.09	<0.001	***
Region	1	0.017	0.048	0.827	
Date*Region	3	1.543	1.431	0.234	
Region(Site)	4	4.649	3.234	0.013	*
Polyunsaturated Fatty Acids					
	df	Deviance	F value	P	
Date	3	9.009	7.951	<0.001	***
Region	1	0.147	0.390	0.533	
Date*Region	3	0.674	0.595	0.619	
Region(Site)	4	2.207	1.461	0.215	
Omega-3 Fatty Acids					
	df	Deviance	F value	P	
Date	3	13.72	11.44	<0.001	***
Region	1	0.922	2.305	0.130	
Date*Region	3	0.802	0.668	0.572	
Region(Site)	4	4.554	2.846	0.025	*
Omega-6 Fatty Acids					
	df	Deviance	F value	P	
Date	3	5.838	5.411	0.001	**
Region	1	0.015	0.041	0.840	
Date*Region	3	0.677	0.627	0.598	
Region(Site)	4	1.847	1.284	0.277	
Long Chain Omega-3 Fatty Acids					
	df	Deviance	F value	P	
Date	3	17.33	14.25	<0.001	***
Region	1	1.504	3.711	0.055	
Date*Region	3	1.036	0.852	0.467	
Region(Site)	4	5.221	3.220	0.013	*
Long Chain Omega-6 Fatty Acids					
	df	Deviance	F value	P	

Date	3	4.823	4.148	0.007	**
Region	1	0.167	0.429	0.513	
Date*Region	3	1.094	0.941	0.421	
Region(Site)	4	3.426	2.210	0.069	

Individual Fatty Acids

14:0

	df	Deviance	F value	P	
Date	3	17.34	13.62	<0.001	***
Region	1	8.458	19.93	<0.001	***
Date*Region	3	4.712	3.702	0.012	*
Region(Site)	4	10.62	6.255	<0.001	***

16:0

	df	Deviance	F value	P	
Date	3	10.83	8.218	<0.001	***
Region	1	0.414	0.943	0.332	
Date*Region	3	1.709	1.297	0.276	
Region(Site)	4	8.162	4.645	0.001	**

17:0

	df	Deviance	F value	P	
Date	3	3.738	2.456	0.064	
Region	1	0.688	1.356	0.245	
Date*Region	3	1.973	1.296	0.276	
Region(Site)	4	7.598	3.743	0.006	**

18:0

	df	Deviance	F value	P	
Date	3	1.488	1.612	0.187	
Region	1	0.155	0.505	0.478	
Date*Region	3	0.614	0.665	0.574	
Region(Site)	4	1.312	1.066	0.374	

21:0

	df	Deviance	F value	P	
Date	3	28.31	17.81	<0.001	***
Region	1	15.05	28.39	<0.001	***
Date*Region	3	3.216	2.023	0.111	
Region(Site)	4	8.835	4.168	0.003	**

16:1n-7

	df	Deviance	F value	P	
Date	3	26.88	20.04	<0.001	***
Region	1	0.012	0.027	0.870	
Date*Region	3	0.897	0.678	0.567	
Region(Site)	4	3.063	1.735	0.143	

18:1n-9

	df	Deviance	F value	P	
Date	3	23.87	21.60	<0.001	***
Region	1	0.200	0.544	0.462	
Date*Region	3	3.820	3.457	0.017	*
Region(Site)	4	7.271	4.935	<0.001	***

20:1n-9

	df	Deviance	F value	P	
Date	3	28.73	32.13	<0.001	***

Region	1	0.193	0.647	0.422	
Date*Region	3	0.403	0.451	0.717	
Region(Site)	4	2.068	1.734	0.143	

18:2n-6

	df	Deviance	F value	P	
Date	3	20.91	13.70	<0.001	***
Region	1	1.005	1.974	0.161	
Date*Region	3	1.902	1.246	0.294	
Region(Site)	4	5.533	2.718	0.030	*

18:3n-6

	df	Deviance	F value	P	
Date	3	17.60	13.80	<0.001	***
Region	1	1.455	3.423	0.065	
Date*Region	3	2.514	1.971	0.119	
Region(Site)	4	14.20	8.347	<0.001	***

20:3n-6

	df	Deviance	F value	P	
Date	3	8.002	6.285	<0.001	***
Region	1	0.880	2.073	0.151	
Date*Region	3	1.473	1.157	0.327	
Region(Site)	4	6.173	3.634	0.007	**

20:4n-6 - ARA

	df	Deviance	F value	P	
Date	3	5.179	4.242	0.006	**
Region	1	0.209	0.513	0.475	
Date*Region	3	1.203	0.985	0.400	
Region(Site)	4	4.318	2.653	0.034	*

22:4n-6

	df	Deviance	F value	P	
Date	3	5.840	5.195	0.002	**
Region	1	0.266	0.709	0.400	
Date*Region	3	1.256	1.118	0.343	
Region(Site)	4	3.706	2.473	0.045	*

18:4n-3

	df	Deviance	F value	P	
Date	3	13.72	10.90	<0.001	***
Region	1	0.080	0.190	0.663	
Date*Region	3	3.540	2.811	0.040	*
Region(Site)	4	19.10	11.38	<0.001	***

20:5n-3 - EPA

	df	Deviance	F value	P	
Date	3	14.16	10.58	<0.001	***
Region	1	5.559	12.46	<0.001	***
Date*Region	3	2.308	1.724	0.163	
Region(Site)	4	9.266	5.192	<0.001	***

22:5n-3

	df	Deviance	F value	P	
Date	3	33.17	27.85	<0.001	***
Region	1	0.475	1.196	0.275	
Date*Region	3	1.418	1.191	0.314	
Region(Site)	4	8.446	5.319	<0.001	***

22:6n-3 - DHA					
	df	Deviance	F value	P	
Date	3	16.40	14.23	<0.001	***
Region	1	0.364	0.946	0.332	
Date*Region	3	0.127	0.110	0.954	
Region(Site)	4	3.263	2.122	0.079	

Supplementary Table 3.2a FA composition (mg FA g lipid^{-1}) and health indicator ratios of inshore *Acropora tenuis* from the three sites within the Burdekin region from February 2013 until February 2014. Values are means ± SE. Sites are B1 (Geoffrey Bay; n=12), B2 (Pandora Reef; n=12) and B3 (Pelorus Island; n=8).

	B1				**B2**				**B3**			
	Feb2013	Jun2013	Oct2013	Feb2014	Feb2013	Jun2013	Oct2013	Feb2014	Feb2013	Jun2013	Oct2013	Feb2014
Fatty Acid Classes												
Total Fatty Acids	464.1 ± 76.4	468.0 ± 60.6	661.3 ± 135.9	396.8 ± 59.8	421.6 ± 65.6	596.0 ± 86.7	403.6 ± 43.0	351.1 ± 51.7	292.2 ± 43.1	596.6 ± 68.8	497.4 ± 61.1	285.7 ± 37.9
SFA	232.5 ± 47.3	167.6 ± 22.0	288.9 ± 56.4	179.4 ± 30.5	156.7 ± 29.3	195.8 ± 26.9	129.5 ± 13.5	107.1 ± 14.7	101.6 ± 15.5	209.6 ± 18.6	180.2 ± 20.9	103.6 ± 15.6
MUFA	32.1 ± 6.7	31.7 ± 4.1	43.2 ± 8.2	25.3 ± 4.3	22.7 ± 4.8	36.1 ± 4.7	20.9 ± 2.2	15.8 ± 2.3	11.7 ± 2.0	34.3 ± 3.2	26.0 ± 3.1	12.0 ± 1.9
PUFA	183.5 ± 23.6	241.4 ± 31.6	294.7 ± 65.7	167.7 ± 23.7	214.0 ± 29.8	319.1 ± 47.7	221.0 ± 24.2	188.3 ± 31.5	151.8 ± 22.5	300.0 ± 40.1	245.9 ± 32.1	130.4 ± 15.5
n-3 PUFA	77.9 ± 9.9	119.5 ± 16.2	129.2 ± 29.0	70.6 ± 9.8	87.7 ± 11.6	139.9 ± 21.3	97.0 ± 10.9	78.4 ± 13.2	68.4 ± 10.9	149.2 ± 21.4	118.4 ± 15.7	62.6 ± 6.7
n-6 PUFA	105.5 ± 13.9	121.5 ± 15.6	165.4 ± 36.7	96.8 ± 14.0	126.3 ± 18.3	179.1 ± 26.5	124.1 ± 13.7	109.8 ± 18.5	83.4 ± 12.0	150.8 ± 18.8	127.6 ± 16.7	67.8 ± 8.9
LC n-3 PUFA	54.3 ± 6.8	88.2 ± 11.5	101.8 ± 23.9	48.9 ± 6.9	68.5 ± 9.4	114.0 ± 17.0	84.5 ± 9.5	59.3 ± 9.8	59.1 ± 9.2	134.5 ± 19.7	109.7 ± 14.7	53.9 ± 5.9
LC n-6 PUFA	48.9 ± 5.1	74.3 ± 9.4	94.9 ± 25.2	57.2 ± 8.1	78.5 ± 9.9	111.8 ± 15.6	91.6 ± 9.9	78.9 ± 13.0	71.0 ± 10.2	111.8 ± 14.1	102.3 ± 13.8	52.8 ± 6.6
Individual Fatty Acids												
14:0	28.5 ± 6.5	21.4 ± 2.6	37.1 ± 6.5	21.1 ± 4.1	19.7 ± 4.1	24.8 ± 3.9	15.8 ± 2.0	10.6 ± 1.9	7.2 ± 1.1	19.6 ± 2.9	16.9 ± 2.4	8.2 ± 1.4
16:0	170.1 ± 38.3	105.4 ± 14.4	201.7 ± 39.0	126.2 ± 22.8	99.2 ± 22.0	122.2 ± 17.0	74.4 ± 8.0	60.7 ± 9.6	56.9 ± 9.2	132.6 ± 10.9	112.1 ± 14.0	61.8 ± 10.0
17:0	8.0 ± 1.1	11.8 ± 1.5	13.9 ± 3.9	8.1 ± 1.3	15.4 ± 2.2	19.3 ± 3.5	12.8 ± 1.4	12.4 ± 2.4	15.0 ± 2.0	24.5 ± 4.3	17.4 ± 2.6	10.2 ± 1.3
18:0	30.2 ± 3.2	30.5 ± 3.9	43.0 ± 10.1	28.4 ± 3.8	32.5 ± 3.5	38.5 ± 4.7	33.9 ± 3.3	30.1 ± 2.5	32.6 ± 4.9	44.6 ± 3.9	43.3 ± 5.3	28.9 ± 3.8
21:0	1.9 ± 0.4	7.4 ± 1.2	3.9 ± 0.8	2.5 ± 0.4	2.6 ± 0.5	6.1 ± 1.2	2.3 ± 0.5	3.9 ± 0.8	2.1 ± 0.7	7.4 ± 1.2	3.4 ± 0.5	3.2 ± 0.4
16:1n-7	9.1 ± 2.1	10.3 ± 1.5	13.9 ± 2.3	7.2 ± 1.3	8.1 ± 2.0	12.8 ± 1.8	6.6 ± 0.8	4.9 ± 1.0	4.8 ± 0.7	11.4 ± 1.3	8.8 ± 1.4	4.0 ± 0.7
18:1n-9	17.7 ± 3.6	13.6 ± 1.8	20.9 ± 4.2	12.7 ± 2.3	11.8 ± 2.3	16.2 ± 2.3	9.1 ± 1.0	7.1 ± 0.9	5.5 ± 0.8	14.7 ± 1.4	10.6 ± 1.2	5.0 ± 0.8
20:1n-9	4.1 ± 0.7	7.1 ± 0.9	6.9 ± 1.6	3.9 ± 0.6	2.6 ± 0.6	6.8 ± 0.7	4.8 ± 0.5	3.1 ± 0.3	1.4 ± 0.7	8.1 ± 0.6	6.1 ± 0.7	2.6 ± 0.3
18:4n-3	23.5 ± 3.1	31.4 ± 4.7	27.5 ± 5.2	21.6 ± 3.0	19.2 ± 2.3	25.9 ± 4.4	12.5 ± 1.5	19.1 ± 3.5	9.3 ± 1.7	14.7 ± 2.0	8.6 ± 1.2	8.7 ± 1.0
20:5n-3	23.3 ± 2.9	42.8 ± 6.0	48.3 ± 12.2	23.0 ± 3.2	30.6 ± 3.7	51.3 ± 8.1	39.3 ± 4.6	28.9 ± 5.0	35.8 ± 5.7	74.6 ± 11.7	60.0 ± 8.7	33.5 ± 3.5
22:5n-3	6.9 ± 0.7	17.4 ± 2.2	18.3 ± 4.4	7.9 ± 1.1	12.8 ± 1.6	29.6 ± 4.4	20.6 ± 2.5	12.0 ± 2.0	10.3 ± 1.5	28.9 ± 4.1	23.5 ± 3.1	9.4 ± 1.1
22:6n-3	24.0 ± 4.0	28.0 ± 3.4	35.2 ± 7.4	18.0 ± 2.9	25.1 ± 4.3	33.1 ± 4.6	24.6 ± 2.7	18.4 ± 3.2	13.0 ± 2.1	31.0 ± 4.1	26.3 ± 3.2	11.1 ± 1.4
18:2n-6	8.6 ± 1.7	4.5 ± 0.8	7.9 ± 1.6	5.2 ± 0.9	6.9 ± 1.3	8.0 ± 1.4	3.8 ± 0.4	3.0 ± 0.5	2.2 ± 0.6	5.1 ± 1.0	4.1 ± 0.6	2.4 ± 0.4
18:3n-6	46.9 ± 10.0	41.8 ± 5.6	60.6 ± 10.4	33.0 ± 6.2	39.8 ± 7.6	58.9 ± 9.8	27.5 ± 3.6	26.8 ± 5.0	10.1 ± 1.7	33.7 ± 4.9	20.3 ± 3.0	11.5 ± 1.9
20:3n-6	6.0 ± 1.3	2.7 ± 0.3	5.6 ± 1.0	4.4 ± 0.8	4.1 ± 1.0	4.3 ± 0.7	2.4 ± 0.3	2.9 ± 0.5	1.9 ± 0.4	3.9 ± 0.3	3.1 ± 0.4	1.7 ± 0.3
20:4n-6	30.4 ± 3.6	51.1 ± 6.5	64.5 ± 17.8	38.4 ± 5.4	55.0 ± 7.1	80.0 ± 11.5	65.3 ± 7.3	57.1 ± 10.2	49.4 ± 7.0	77.5 ± 10.4	70.4 ± 9.9	36.0 ± 4.7
Health Indicator Ratios												
n-3:n-6	0.75 ± 0.03	0.98 ± 0.03	0.78 ± 0.02	0.74 ± 0.02	0.71 ± 0.03	0.77 ± 0.02	0.78 ± 0.04	0.72 ± 0.04	0.82 ± 0.04	0.98 ± 0.03	0.94 ± 0.03	0.95 ± 0.04
LC n-3:LC n-6	1.13 ± 0.10	1.19 ± 0.05	1.13 ± 0.08	0.87 ± 0.05	0.88 ± 0.05	1.01 ± 0.03	0.92 ± 0.05	0.76 ± 0.04	0.83 ± 0.05	1.18 ± 0.03	1.09 ± 0.04	1.05 ± 0.05
EPA:DHA	1.15 ± 0.15	1.53 ± 0.05	1.39 ± 0.11	1.40 ± 0.12	1.32 ± 0.07	1.51 ± 0.05	1.61 ± 0.09	1.60 ± 0.17	2.85 ± 0.18	2.38 ± 0.13	2.25 ± 0.11	3.08 ± 0.07
EPA:ARA	0.79 ± 0.06	0.84 ± 0.03	0.78 ± 0.04	0.61 ± 0.03	0.57 ± 0.03	0.63 ± 0.02	0.61 ± 0.05	0.52 ± 0.05	0.73 ± 0.05	0.95 ± 0.04	0.86 ± 0.04	0.96 ± 0.05

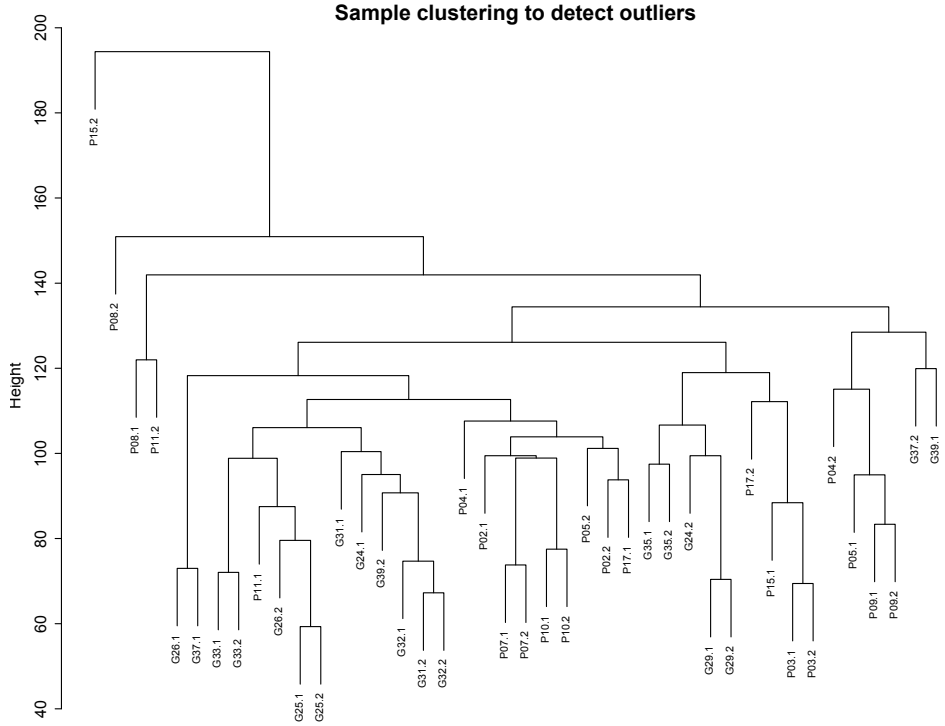
Supplementary Table 3.2b FA composition (mg FA g lipid^{-1}) and health indicator ratios of inshore *Acropora tenuis* from the three sites within the Whitsunday region from February 2013 until February 2014. Values are means ± SE. Sites are W1 (Pine Island; n=12), B2 (Daydream Island; n=12) and B3 (Double Cone Island; n=9).

	W1				**W2**				**W3**			
	Feb2013	Jun2013	Oct2013	Feb2014	Feb2013	Jun2013	Oct 013	Feb2014	Feb2013	Jun2013	Oct2013	Feb2014
Fatty Acid Classes												
Total Fatty Acids	480.1 ± 107.0	650.0 ± 223.1	528.8 ± 75.6	363.4 ± 36.1	448.2 ± 68.9	450.0 ± 64.9	405.3 ± 67.7	354.2 ± 52.1		584.2 ± 191.6	516.4 ± 66.1	342.0 ± 23.2
SFA	177.2 ± 40.2	230.4 ± 68.6	169.6 ± 22.3	104.7 ± 9.4	187.6 ± 29.6	167.5 ± 23.8	154.0 ± 28.3	123.5 ± 21.3		187.5 ± 62.2	187.0 ± 28.1	110.3 ± 7.7
MUFA	22.8 ± 4.7	43.0 ± 11.9	29.5 ± 3.7	14.3 ± 1.2	23.8 ± 3.3	34.9 ± 5.5	25.7 ± 4.5	14.4 ± 2.3		35.4 ± 10.5	34.6 ± 4.9	12.9 ± 0.9
PUFA	245.0 ± 54.7	330.5 ± 124.0	280.0 ± 41.7	202.0 ± 22.0	211.1 ± 33.9	220.7 ± 31.8	194.4 ± 30.9	174.2 ± 25.0		313.6 ± 103.8	248.6 ± 31.6	182.4 ± 15.0
n-3 PUFA	115.34 ± 25.2	164.4 ± 67.9	140.5 ± 21.3	94.5 ± 11.0	92.4 ± 16.6	101.7 ± 14.9	84.9 ± 14.1	67.6 ± 9.3		179.4 ± 63.7	124.9 ± 16.3	94.4 ± 7.8
n-6 PUFA	129.6 ± 29.6	166.0 ± 56.3	139.5 ± 21.0	107.4 ± 11.2	118.8 ± 17.7	119.0 ± 17.3	109.5 ± 17.0	106.7 ± 15.9		134.1 ± 40.2	123.7 ± 15.3	88.0 ± 7.4
LC n-3 PUFA	91.6 ± 20.5	136.1 ± 57.3	126.0 ± 19.3	75.1 ± 8.6	71.3 ± 12.8	83.9 ± 12.5	77.0 ± 12.6	56.1 ± 8.0		146.5 ± 51.0	113.4 ± 15.1	77.0 ± 6.3
LC n-6 PUFA	86.4 ± 19.5	111.7 ± 42.5	106.9 ± 17.3	85.3 ± 9.0	70.1 ± 11.5	72.5 ± 10.7	76.1 ± 10.5	82.4 ± 12.4		92.6 ± 27.4	92.5 ± 11.8	70.2 ± 6.3
Individual Fatty Acids												
14:0	14.9 ± 3.8	20.5 ± 5.3	14.7 ± 2.0	6.5 ± 0.7	17.3 ± 2.8	19.0 ± 3.5	13.5 ± 2.6	7.8 ± 1.3		14.5 ± 4.4	15.0 ± 2.1	7.2 ± 0.9
16:0	111.8 ± 25.3	148.6 ± 42.5	101.4 ± 13.1	56.3 ± 5.2	129.5 ± 21.0	109.4 ± 15.6	105.1 ± 22.1	73.4 ± 14.8		115.3 ± 39.1	122.9 ± 19.8	61.2 ± 4.9
17:0	18.0 ± 4.3	20.2 ± 9.1	17.7 ± 3.2	15.9 ± 1.7	13.2 ± 2.6	11.7 ± 1.9	11.8 ± 1.8	14.4 ± 2.4		18.1 ± 6.1	14.4 ± 2.0	12.9 ± 1.5
18:0	39.4 ± 8.2	44.7 ± 14.4	41.1 ± 5.4	34.1 ± 2.8	33.0 ± 5.4	30.3 ± 4.1	30.4 ± 3.5	36.3 ± 5.3		38.1 ± 11.0	39.2 ± 6.0	32.2 ± 2.6
21:0	7.3 ± 2.0	12.7 ± 6.4	7.2 ± 1.4	5.3 ± 0.7	5.5 ± 1.1	6.4 ± 1.0	2.2 ± 0.5	3.7 ± 0.5		14.5 ± 5.4	5.7 ± 0.7	6.7 ± 0.6
16:1n-7	8.0 ± 1.9	16.4 ± 5.0	9.4 ± 1.3	5.2 ± 0.6	7.5 ± 1.1	12.0 ± 2.1	7.3 ± 1.5	5.4 ± 1.1		12.2 ± 4.0	10.1 ± 1.4	4.1 ± 0.4
18:1n-9	10.1 ± 2.0	18.1 ± 4.3	12.7 ± 1.6	5.5 ± 0.4	12.6 ± 1.8	17.2 ± 2.7	12.2 ± 2.3	6.0 ± 1.1		15.3 ± 4.7	15.2 ± 2.3	5.0 ± 0.4
20:1n-9	4.1 ± 0.7	8.4 ± 2.7	7.2 ± 1.0	3.1 ± 0.2	3.3 ± 0.5	5.8 ± 0.8	6.0 ± 0.8	2.9 ± 0.5		6.5 ± 1.8	8.2 ± 1.4	3.4 ± 0.3
18:4n-3	23.8 ± 4.8	28.3 ± 10.7	14.5 ± 2.1	19.4 ± 2.7	21.0 ± 3.9	17.7 ± 2.6	7.9 ± 1.6	11.4 ± 1.5		32.9 ± 12.7	11.5 ± 1.3	17.4 ± 1.6
20:5n-3	54.8 ± 13.4	71.4 ± 33.1	69.3 ± 11.7	45.6 ± 5.2	41.7 ± 7.6	43.5 ± 6.8	39.5 ± 6.7	32.8 ± 5.3		90.3 ± 31.2	64.1 ± 8.4	47.8 ± 3.9
22:5n-3	14.8 ± 3.0	28.4 ± 11.8	27.1 ± 4.0	13.7 ± 1.6	10.8 ± 2.1	17.0 ± 2.5	16.4 ± 2.6	9.7 ± 1.1		27.0 ± 9.3	23.6 ± 3.2	13.6 ± 1.3
22:6n-3	21.9 ± 4.2	36.3 ± 12.6	29.6 ± 3.9	15.8 ± 1.8	18.9 ± 3.3	23.4 ± 3.5	21.1 ± 3.6	13.6 ± 1.8		29.1 ± 10.4	25.7 ± 3.7	15.5 ± 1.3
18:2n-6	6.2 ± 2.1	6.0 ± 1.6	3.8 ± 0.7	2.2 ± 0.2	6.8 ± 1.0	6.4 ± 1.0	4.2 ± 0.9	3.5 ± 1.1		5.1 ± 1.5	4.2 ± 0.5	2.1 ± 0.2
18:3n-6	35.6 ± 7.8	48.2 ± 13.6	28.2 ± 3.8	18.9 ± 2.0	40.8 ± 5.8	40.1 ± 5.9	28.9 ± 6.4	20.1 ± 2.9		36.2 ± 11.5	26.4 ± 4.0	14.7 ± 1.1
20:3n-6	4.3 ± 1.0	4.2 ± 1.4	2.7 ± 0.3	2.5 ± 0.3	4.8 ± 0.7	3.0 ± 0.4	3.1 ± 0.6	2.8 ± 0.4		2.8 ± 1.0	2.6 ± 0.4	1.8 ± 0.2
20:4n-6	59.1 ± 13.8	77.9 ± 29.6	75.8 ± 12.7	60.6 ± 6.8	47.8 ± 8.1	50.5 ± 7.7	53.9 ± 7.7	58.4 ± 8.8		64.0 ± 18.7	64.4 ± 8.1	49.3 ± 4.5
Health Indicator Ratios												
n-3:n-6	0.90 ± 0.003	0.93 ± 0.05	1.02 ± 0.04	0.87 ± 0.03	0.76 ± 0.03	0.85 ± 0.04	0.77 ± 0.03	0.64 ± 0.03		1.24 ± 0.04	1.00 ± 0.03	1.08 ± 0.03
LC n-3:LC n-6	1.07 ± 0.05	1.20 ± 0.07	1.21 ± 0.05	0.88 ± 0.03	1.05 ± 0.08	1.15 ± 0.07	0.98 ± 0.04	0.69 ± 0.03		1.48 ± 0.05	1.23 ± 0.05	1.11 ± 0.04
EPA:DHA	2.31 ± 0.17	1.83 ± 0.18	2.25 ± 0.13	2.91 ± 0.09	2.19 ± 0.17	1.87 ± 0.12	1.90 ± 0.14	2.35 ± 0.15		3.13 ± 0.12	2.60 ± 0.13	3.10 ± 0.10
EPA:ARA	0.91 ± 0.05	0.86 ± 0.06	0.93 ± 0.05	0.75 ± 0.03	0.90 ± 0.09	0.86 ± 0.06	0.71 ± 0.05	0.56 ± 0.03		1.32 ± 0.06	1.01 ± 0.05	0.98 ± 0.04

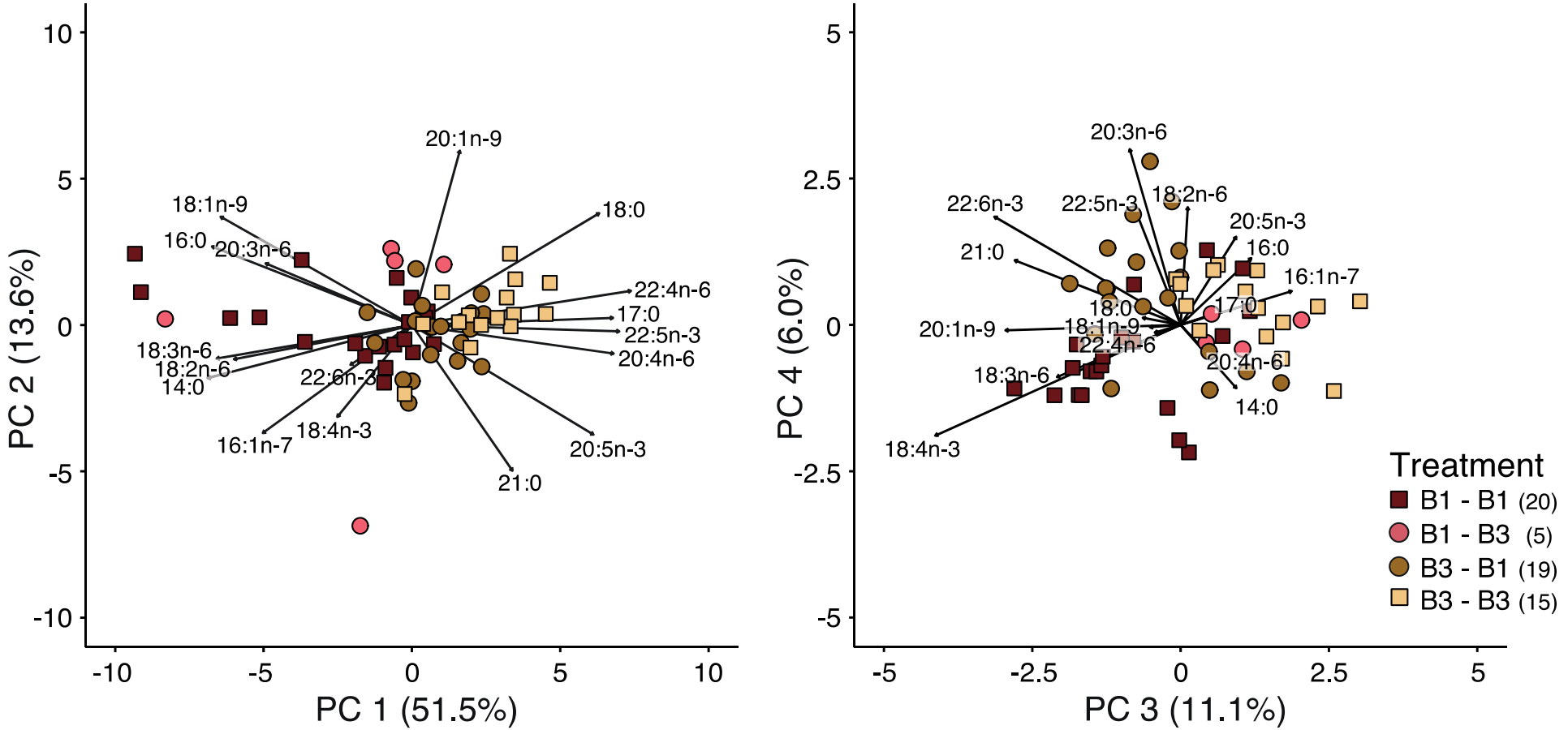
APPENDIX C: Supplementary Material *Chapter 5*

Suppl. Table 5.1 Number of reads remaining following quality trimming, removal of PCR duplicates and mapping by isogroups that were used as input for WGCNA. * Outlier and removed from analyses.

Source Location	Transplant Location	Sample Name	Reads Left
B1	B1	24.1	1938629
B1	B1	25.1	1846568
B1	B1	26.1	704012
B1	B1	29.1	1153647
B1	B1	31.1	134264
B1	B1	32.1	1896101
B1	B1	33.1	1063520
B1	B1	35.1	1113099
B1	B1	37.1	2029927
B1	B1	39.1	115041
B3	B1	02.1	800106
B3	B1	03.1	1590893
B3	B1	04.1	133725
B3	B1	05.1	1098460
B3	B1	07.1	1194817
B3	B1	08.1	1199499
B3	B1	09.1	2359517
B3	B1	10.1	2450805
B3	B1	11.1	1258531
B3	B1	15.1	1137112
B3	B1	17.1	2334508
B1	B3	24.2	1240742
B1	B3	25.2	2072281
B1	B3	26.2	934597
B1	B3	29.2	1158021
B1	B3	31.2	674715
B1	B3	32.2	2129389
B1	B3	33.2	1038656
B1	B3	35.2	1353008
B1	B3	37.2	1448559
B1	B3	39.2	1581242
B3	B3	02.2	1189315
B3	B3	03.2	1259330
B3	B3	04.2	1128288
B3	B3	05.2	1898759
B3	B3	07.2	1181719
B3	B3	08.2	1421536
B3	B3	09.2	1535476
B3	B3	10.2	1900840
B3	B3	11.2	1082512
B3	B3	15.2 *	1283952
B3	B3	17.2	1211691



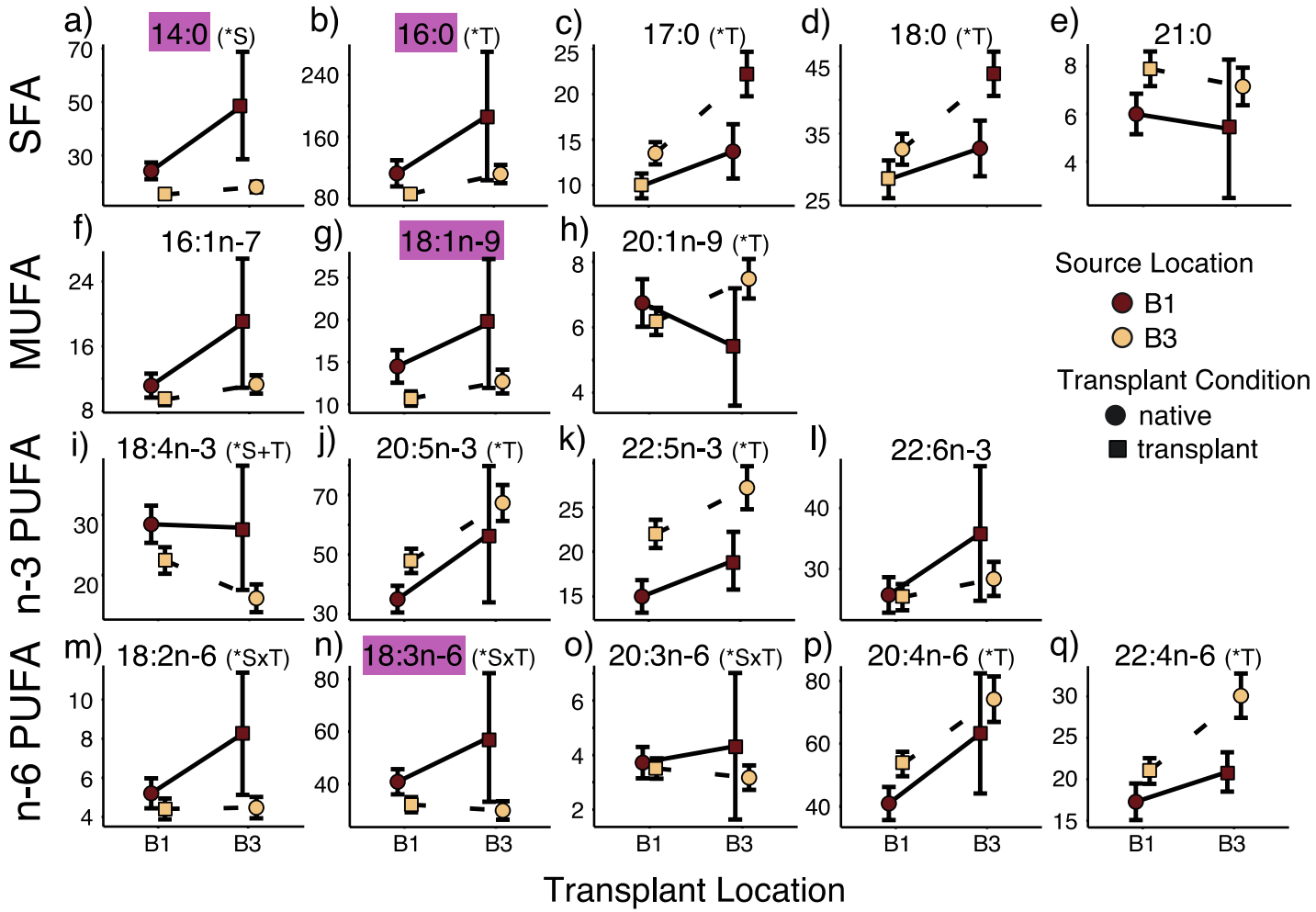
Suppl. Fig. 5.1 Clustering of gene expression samples to detect outliers.



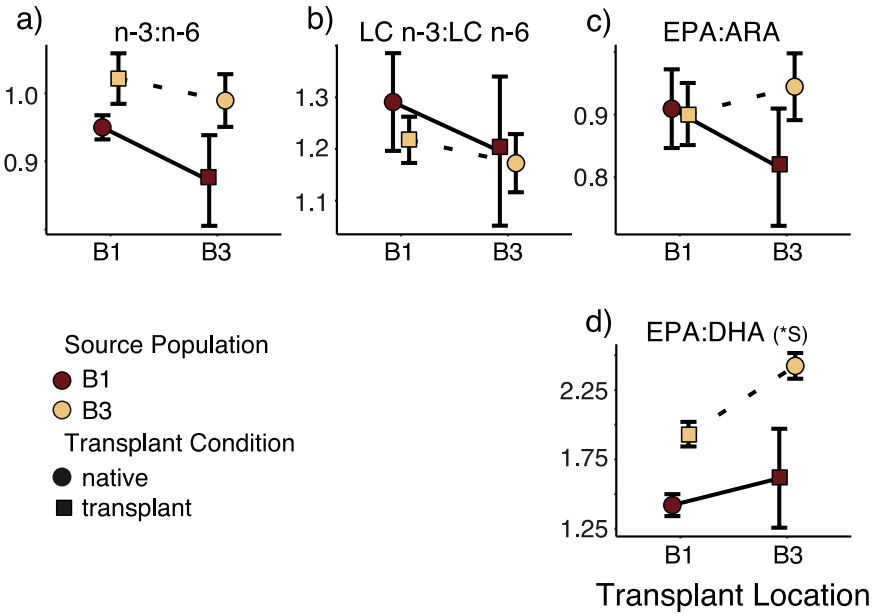
Suppl. Fig. 5.2 Biplots of principal component analysis of FA composition measured in coral colonies reciprocally transplanted between B1 and B3 from the Burdekin region in June 2014, showing relationships between PC1 - PC4. FA are expressed as percentage composition of total FA. Lettering for treatments denote 'source population – transplant location'. Sample sizes for FA analyses are in parentheses.

Suppl. Table 5.2 Standard deviation and proportion of variance explained in principal component analysis (PCA) of individual FA percentage composition of *Acropora tenuis* reciprocally transplanted between B1 and B3 within the Burdekin region. Analysis of variance (ANOVA) of important principal components (> 1.0 standard deviation) was used to determine significant differences between source and transplant locations within this region. *** denotes significance at α = 0.001. ** denotes significance at α = 0.01. * denotes significance at α = 0.05.

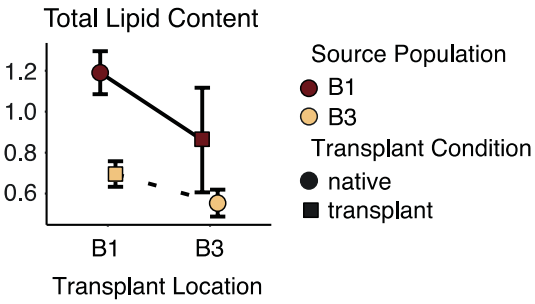
Importance of components						
	PC1	*PC2*	*PC3*	*PC4*	*PC5*	*PC6*
Standard deviation	2.96 *	1.52*	1.38*	1.01*	0.925	0.842
Proportion of variance	0.515	0.136	0.111	0.060	0.050	0.042
Cumulative proportion	0.515	0.651	0.762	0.823	0.873	0.915
Principal component 1						
	df	*SS*	F value	P		
Source	1	198.3	38.16	<0.001	***	
Transplant	1	17.23	3.316	0.074		
Source*Transplant	1	6.790	1.306	0.258		
Residuals	55	285.8				
Principal component 2						
	df	*SS*	F value	P		
Source	1	0.030	0.034	0.905		
Transplant	1	3.070	3.071	0.258		
Source*Transplant	1	1.340	1.342	0.454		
Residuals	55	129.5				
Principal component 3						
	df	*SS*	F value	P		
Source	1	5.970	5.596	0.022	*	
Transplant	1	43.77	41.02	<0.001	***	
Source*Transplant	1	1.270	1.194	0.279		
Residuals	55	58.68				
Principal component 4						
	df	*SS*	F value	P		
Source	1	13.75	16.71	<0.001	***	
Transplant	1	0.180	0.217	0.643		
Source*Transplant	1	0.290	0.354	0.554		
Residuals	55	45.26				



Suppl. Fig. 5.3 Mean concentrations (± SE) of 17 individual FA for corals reciprocally transplanted between B1 (Geoffrey Bay) and B3 (Pelorus Island) in June 2014. Source population is indicated by the colour: B1 (dark red) and B3 (light yellow). Transplant treatment is indicated by the shape of symbols: native (O) and transplant (□). Concentrations of individual FA are standardised to mg FA g lipid^{-1}. Coloured boxes around FA labels represent significant correlations with pink gene modules. Sample sizes for FA analyses: native B1 (n = 20), transplanted B1 (n = 5), native B3 (n = 15) and transplanted B3 (n = 19).



Suppl. Fig. 5.4 Mean concentrations (± SE) of four FA health indicator ratios for corals reciprocally transplanted between B1 (Geoffrey Bay) and B3 (Pelorus Island) in June 2014. Source population is indicated by the colour: B1 (dark red) and B3 (light yellow). Transplant treatment is indicated by the shape of symbols: native (O) and transplant (□). Concentrations of individual FA are standardised to mg FA g lipid^{-1}. Coloured boxes around FA labels represent significant correlations with pink gene modules. Sample sizes for FA analyses: native B1 (n = 20), transplanted B1 (n = 5), native B3 (n = 15) and transplanted B3 (n = 19).



Suppl. Fig. 5.5 Total lipid (mg lipid g dw^{-1}) content of corals reciprocally transplanted between B1 (Geoffrey Bay) and B3 (Pelorus Island) in June 2014. Source population is indicated by the colour: B1 (dark red) and B3 (light yellow). Transplant treatment is indicated by the shape of symbols: native (O) and transplant (□). Concentrations of individual FA are standardised to mg FA g lipid^{-1}. Coloured boxes around FA labels represent significant correlations with pink gene modules. Sample sizes for FA analyses: native B1 (n = 20), transplanted B1 (n = 5), native B3 (n = 15) and transplanted B3 (n = 19).

Suppl. Table 5.3 Generalised linear mixed model (GLMMs) analyses of total lipid content (mg lipid g dry weight^{-1}), individual fatty acids (mg FA g lipid^{-1}) and FA health indicator ratios in June 2014 samples, collected to determine the effects of reciprocally transplanting coral colonies between B1 and B3. Genotype was considered as a random effect. Total lipid content and individual FA use a Gamma error distribution, and FA health indicator ratios use a Gaussian error distribution. The "intercept" parameter corresponds to the predicted response variable for coral colonies back-transplanted at B3. *** denotes significance at α = 0.001. ** denotes significance at α = 0.01. * denotes significance at α = 0.05.

Total Lipid Content					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	1.901	0.187	10.18	<0.001	***
Source (B1)	-0.522	0.276	-1.887	0.059	
Transplant (B1)	-0.354	0.182	-1.944	0.052	
Source*Transplant (B1)	-0.017	0.261	-0.066	0.947	

Individual Fatty Acids

14:0					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.060	0.007	8.009	<0.001	***
Source (B1)	-0.035	0.012	-3.033	0.002	**
Transplant (B1)	0.010	0.007	1.460	0.144	
Source*Transplant (B1)	0.018	0.011	1.692	0.091	
16:0					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.010	0.001	7.190	<0.001	***
Source (B1)	-0.004	0.002	-1.692	0.091	
Transplant (B1)	0.003	0.001	2.762	0.006	**
Source*Transplant (B1)	0.004	0.002	1.941	0.052	
17:0					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.052	0.010	5.507	<0.001	***
Source (B1)	0.020	0.018	1.090	0.276	
Transplant (B1)	0.030	0.007	4.040	<0.001	***
Source*Transplant (B1)	0.009	0.019	0.486	0.627	
18:0					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.024	0.003	8.154	<0.001	***
Source (B1)	0.003	0.005	0.634	0.526	
Transplant (B1)	0.008	0.002	4.178	<0.001	***
Source*Transplant (B1)	0.003	0.005	0.545	0.586	
21:0					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.158	0.023	6.733	<0.001	***
Source (B1)	0.044	0.045	0.982	0.326	
Transplant (B1)	-0.007	0.018	-0.373	0.709	
Source*Transplant (B1)	0.007	0.042	0.169	0.866	
16:1n-7					
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.098	0.014	7.087	<0.001	***
Source (B1)	-0.032	0.023	-1.437	0.151	

Transplant (B1)	0.020	0.011	1.813	0.070	
Source*Transplant (B1)	0.030	0.019	1.572	0.116	

18:1n-9

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.088	0.011	7.705	<0.001	***
Source (B1)	-0.033	0.018	-1.804	0.071	
Transplant (B1)	0.016	0.009	1.817	0.069	
Source*Transplant (B1)	0.023	0.015	1.453	0.146	

20:1n-9

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.145	0.017	8.651	<0.001	***
Source (B1)	-0.004	0.030	-0.150	0.881	
Transplant (B1)	0.031	0.011	2.842	0.005	**
Source*Transplant (B1)	0.006	0.025	0.223	0.823	

18:2n-6

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.263	0.036	7.336	<0.001	***
Source (B1)	-0.116	0.056	-2.064	0.039	*
Transplant (B1)	0.004	0.027	0.138	0.890	
Source*Transplant (B1)	0.113	0.048	2.335	0.020	*

18:3n-6

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.037	0.004	8.258	<0.001	***
Source (B1)	-0.017	0.007	-2.513	0.012	*
Transplant (B1)	-0.002	0.004	-0.509	0.611	
Source*Transplant (B1)	0.013	0.006	2.104	0.354	*

18:4n-3

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.065	0.007	8.699	<0.001	***
Source (B1)	-0.027	0.011	-2.468	0.014	*
Transplant (B1)	-0.016	0.007	-2.152	0.031	*
Source*Transplant (B1)	0.020	0.011	1.858	0.063	

20:5n-3 – EPA

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.017	0.003	5.927	<0.001	***
Source (B1)	0.003	0.005	0.535	0.593	
Transplant (B1)	0.007	0.002	3.466	<0.001	***
Source*Transplant (B1)	0.006	0.005	1.414	0.157	

22:5n-3

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.041	0.006	7.062	<0.001	***
Source (B1)	0.010	0.011	0.879	0.379	
Transplant (B1)	0.009	0.004	2.073	0.038	*
Source*Transplant (B1)	0.013	0.011	1.183	0.237	

22:6n-3 – DHA

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.038	0.005	7.795	<0.001	***
Source (B1)	-0.012	0.008	-1.384	0.166	
Transplant (B1)	0.005	0.004	1.234	0.217	
Source*Transplant (B1)	0.013	0.008	1.677	0.094	

20:3n-6

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.348	0.050	7.009	<0.001	***
Source (B1)	-0.089	0.082	-1.081	0.280	
Transplant (B1)	-0.005	0.033	-0.163	0.871	
Source*Transplant (B1)	0.141	0.065	2.150	0.032	*

20:4n-6 – ARA

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.015	0.002	6.559	<0.001	***
Source (B1)	0.001	0.004	0.327	0.744	
Transplant (B1)	0.005	0.002	2.743	0.006	**
Source*Transplant (B1)	0.005	0.004	1.197	0.231	

22:4n-6

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.037	0.005	6.717	<0.001	***
Source (B1)	0.011	0.010	1.031	0.303	
Transplant (B1)	0.015	0.004	3.637	<0.001	***
Source*Transplant (B1)	0.002	0.010	0.253	0.800	

Coral Health Indicators

Omega-3 to Omega-6 Ratio

	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.950	0.030	31.73	<0.001	***
Source (B3)	0.072	0.043	1.669	0.104	
Transplant (B3)	-0.083	0.058	-1.426	0.171	
Source*Transplant (B3)	0.055	0.069	0.804	0.432	

Long Chain Omega-3 to Long Chain Omega-6 Ratio

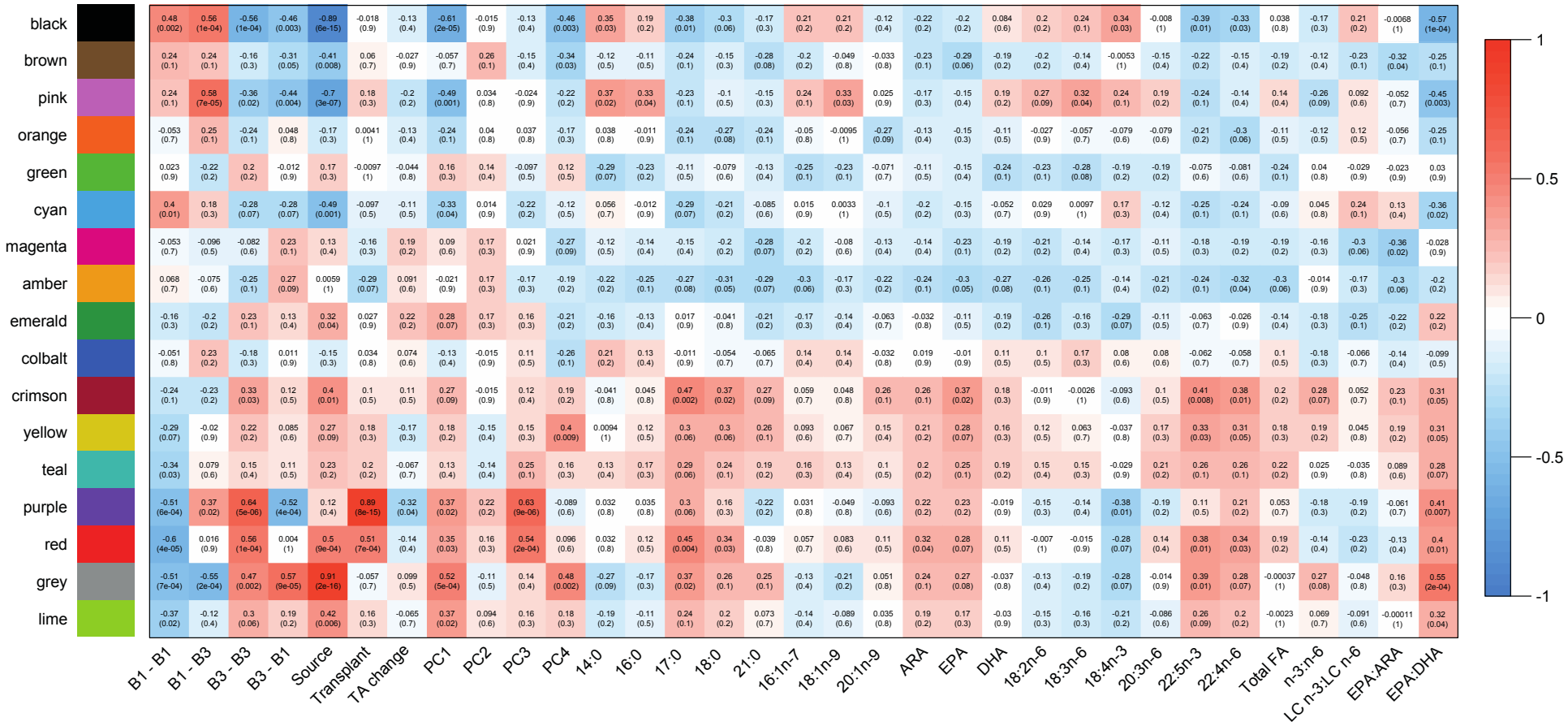
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	1.291	0.073	17.67	<0.001	***
Source (B3)	-0.073	0.105	-0.699	0.489	
Transplant (B3)	-0.003	0.113	-0.023	0.982	
Source*Transplant (B3)	-0.034	0.132	-0.256	0.801	

EPA to ARA Ratio

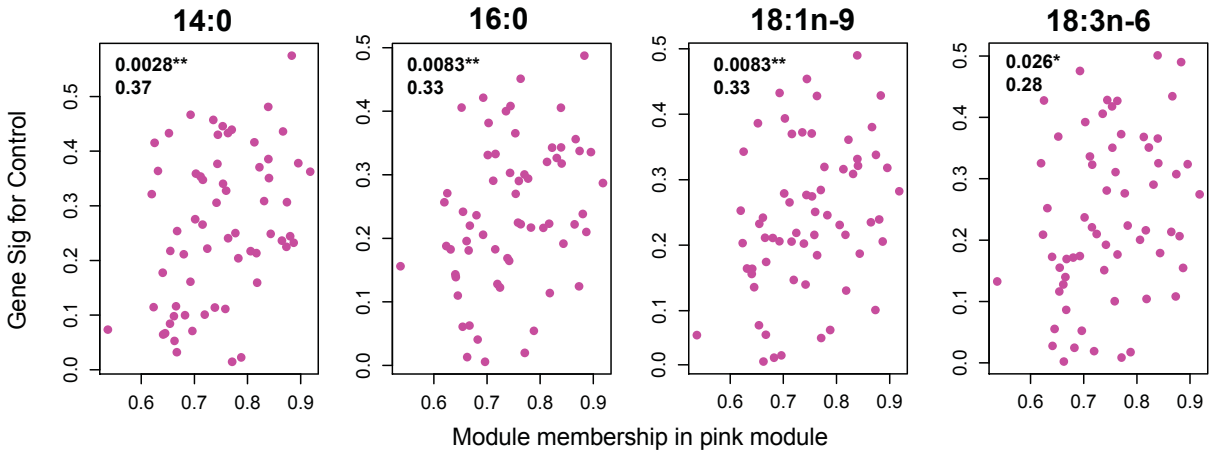
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	0.910	0.056	16.33	<0.001	***
Source (B3)	-0.008	0.080	-0.106	0.916	
Transplant (B3)	-0.039	0.087	-0.450	0.658	
Source*Transplant (B3)	0.095	0.101	0.934	0.363	

EPA to DHA Ratio

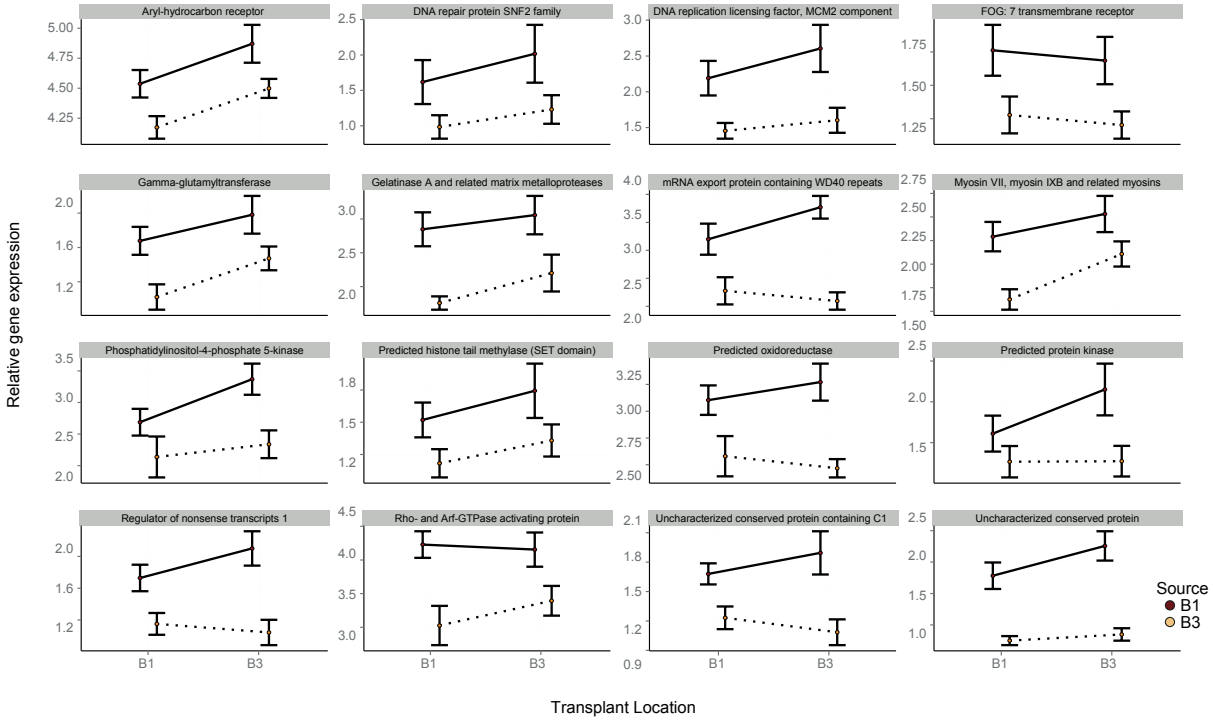
	Estimate	Std Error	*t*-statistic	P	
(Intercept)	1.421	0.092	15.41	<0.001	***
Source (B3)	0.511	0.132	3.871	0.004	**
Transplant (B3)	0.141	0.175	0.805	0.431	
Source*Transplant (B3)	0.353	0.207	1.708	0.105	



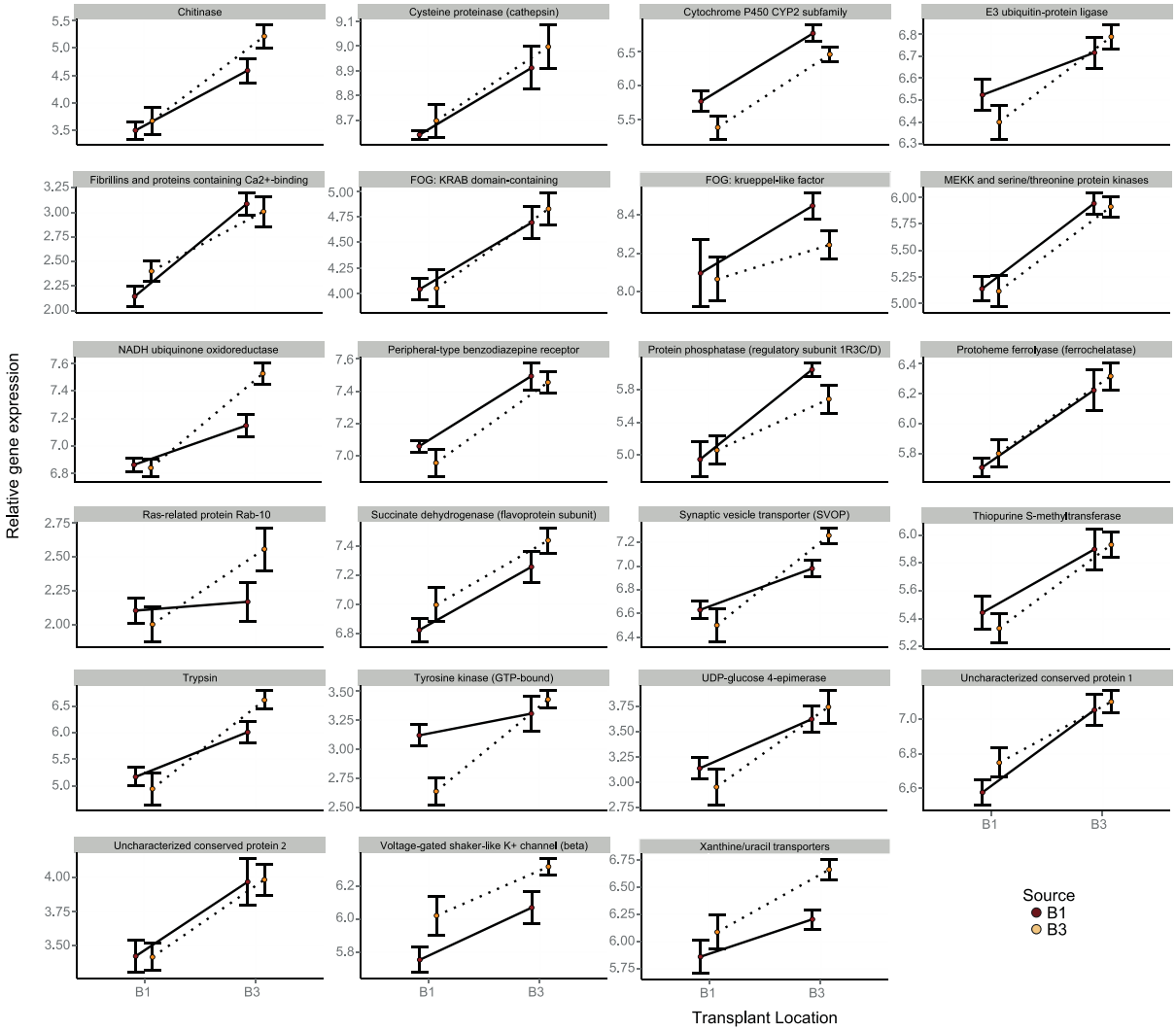
Suppl. Fig. 5.6 Gene module – coral attribute matrix showing correlations between 17 unique gene modules (colours listed vertically) and 33 coral health attributes or experimental parameters (listed horizontally). Unique modules were determined by WGCNA analyses. Coral and experimental attributes include four treatments (lettering denotes 'source population – transplant location'), source and transplant effects, total area change, principal components from PCA of FA percentage composition, individual and total FA concentrations, and coral health indicator ratios. Values in boxes are R^2 and significance of the correlation; heatmap colours indicate the magnitude and direction of the relationship.



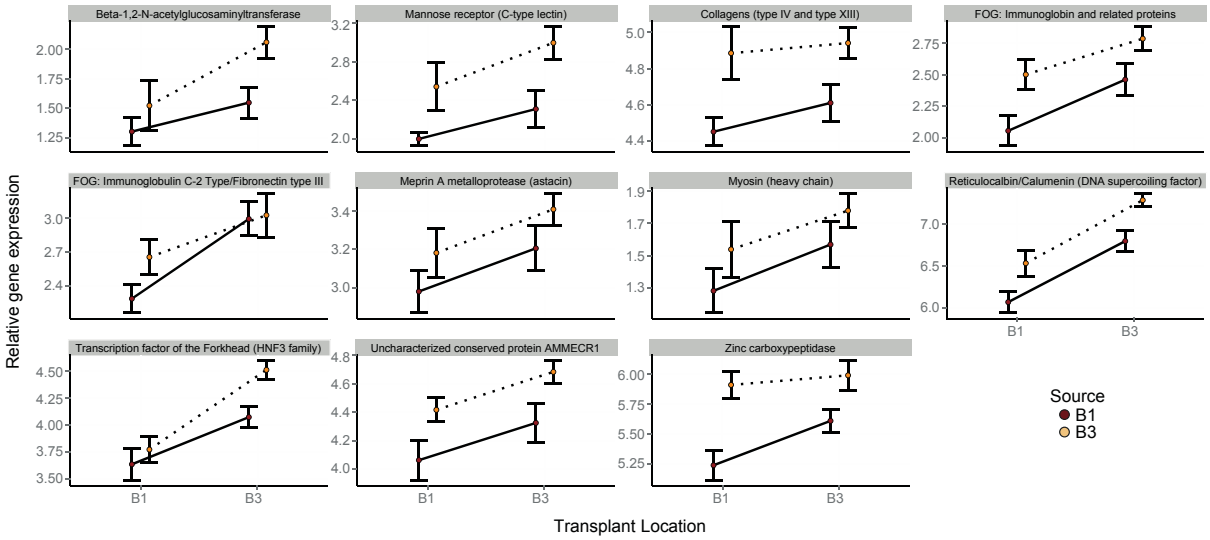
Suppl. Fig. 5.7 Positive correlations with select FA and genes in the pink module.



Suppl. Fig. 5.8 Annotated differentially expressed genes from the pink module.



Suppl. Fig. 5.9 Annotated differentially expressed genes from the purple module.



Suppl. Fig. 5.10 Annotated differentially expressed genes from the red module.

APPENDIX D: Author's publication list

Publications arising from this thesis

Rocker MM, Brandl SJ (2014) Transplantation of corals into a new environment results in substantial skeletal loss in *Acropora* tenuis. Marine Biodiversity 45: 321-326. DOI: 10.1007/s12526-014-0239-y

Rocker MM, Francis DS, Fabricius KE, Willis BL, Bay LK (*in review*) Variation in health and condition of the coral *Acropora tenuis* along two inshore water quality gradients on the Great Barrier Reef. Marine Pollution Bulletin

Additional publications arising during PhD candidature

Rocker MM, Noonan S, Humphrey C, Moya A, Willis BL, Bay LK (2015) Expression of calcification and metabolism-realted genes in response to elevated pCO_2 and temperature in the reef-buildign coral *Acropora millepora*. Marine Genomics 24: 313-318. DOI: 10.1016/j.margen.2015.08.001

Mar Biodiv (2015) 45:321–326
DOI 10.1007/s12526-014-0239-y

SENCKENBERG

SHORT COMMUNICATION

Transplantation of corals into a new environment results in substantial skeletal loss in *Acropora tenuis*

Melissa M. Rocker · Simon J. Brandl

Received: 2 October 2013 / Revised: 1 May 2014 / Accepted: 2 May 2014 / Published online: 20 May 2014

Abstract The degradation of coral reefs, specifically the loss of structural biomass created by coral skeletons, is an important issue in coral reef science. In this study, we give evidence for high skeletal loss in corals transplanted from a high turbidity environment to a low turbidity environment. Specifically, we show that in colonies of *Acropora tenuis*, significantly higher skeletal loss occurred in colonies from Geoffrey Bay (Magnetic Island, Australia, ~8 km offshore) transplanted to Pelorus Island (Palm Islands, Australia, ~16 km offshore), when compared to control colonies and their reciprocally transplanted counterparts. These results may suggest marked intraspecific differences in the physiological condition of coral colonies, possibly causing selective predation by corallivorous organisms, strengthening the need for detailed investigations of the underlying causes as well as the consequences of skeletal loss in an important branching species of coral, *Acropora tenuis*.

Keywords Skeletal loss · Coral physiology · Corallivory

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Expression of calcification and metabolism-related genes in response to elevated pCO_2 and temperature in the reef-building coral *Acropora millepora*

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ABSTRACT

Declining health of scleractinian corals in response to deteriorating environmental conditions is widely acknowledged, however links between physiological and functional genomic responses of corals are less well understood. Here we explore growth and the expression of 20 target genes with putative roles in metabolism and calcification in the branching coral, *Acropora millepora*, in two separate experiments: 1) elevated pCO_2 (464, 822, 1187 and 1638 μatm) and ambient temperature (27 °C), and 2) elevated pCO_2 (490 and 822 μatm) and temperature (28 and 31 °C). After 14 days of exposure to elevated pCO_2 and ambient temperatures, no evidence of differential expression of either calcification or metabolism genes was detected between control and elevated pCO_2 treatments. After 37 days of exposure to control and elevated pCO_2, Ubiquinol-Cytochrome-C Reductase Subunit 2 gene (*QCR2*; a gene involved in complex III of the electron chain transport within the mitochondria and critical for generation of ATP) was significantly down-regulated in the elevated pCO_2 treatment in both ambient and elevated temperature treatments. Overall, the general absence of a strong response to elevated pCO_2 and temperature by the other 19 targeted calcification and metabolism genes suggests that corals may not be affected by these stressors on longer time scales (37 days). These results also highlight the potential for *QCR2* to act as a biomarker of coral genomic responses to changing environments.

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