Changing environmental conditions on the health of two dominant *Acropora* species at Ningaloo Reef

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Abstract

Variations in coral health indices, reflecting autotrophic activity (chlorophyll *a* (chl *a*) and zooxanthellae density), metabolic activity (RNA/DNA ratio and protein) and energy storage (lipid ratios) for two dominant *Acropora* species (*A. digitifera* (Dana, 1846) and *A. spicifera* (Dana, 1846)) were investigated at Ningaloo Reef (North-Western Australia). Health indices were measured before and after spawning in austral autumn and winter 2010 and austral summer 2011, at six stations. Physico-chemical-biological parameters were also measured simultaneously to determine the key environmental factors impacting coral health. In addition, the suitability of these health measures as short-term and long-term indicators of coral health was investigated by comparing diel (throughout the day), daily (between days) and seasonal patterns.

Coral health indices showed clear seasonal and species-specific differences but negligible spatial differences across a reef section (2-5 km). For both species, indices of metabolic activity showed highest and optimal values during autumn (at 26-28°C), while autotrophic indices were highest in winter and summer. The unexpected high autotrophic indices and low metabolic indices and energy stores during summer 2011 are related to the La Niña event. In this case high sea surface temperatures did not result in bleaching since high plankton concentrations in the water led to low light levels in the reef lagoon, counteracting the impact of high seawater temperatures and resulting in a shift towards high coral symbiont densities, but low metabolic activities and energy levels of the coral host. During this time feeding modes shifted for both species, with an increased importance of planktonic food for metabolic activity of *A. digitifera,* but light and plankton-dependence for *A. spicifera*. Under normal conditions metabolic indices for *A. spicifera* correlated with nitrogen supply, temperature and zooplankton concentrations while for *A. digitifera,* light as well as picoplankton concentrations were important. This can possibly be explained by higher energy requirement for lipid

synthesis and storage in *A. digitifera* since lipid ratios were higher for this species and changed throughout the year, while they were stable for *A. spicifera*. The impact of the broadcast spawning event on coral health indices at Ningaloo Reef occurred only as a modest shift against a backdrop of massive seasonal changes in coral physiology. Overall, results show that the two species vary physiologically and react differently to extreme events. This knowledge can be used to make predictions for how changes in environmental factors may impact the health of corals in the future.

Table of Contents

Abstract ... 3

List of figures ... 8

List of tables ... 10

List of major abbreviations ... 11

Acknowledgement ... 12

Statement of candidate contribution ... 14

Publications arising from this thesis ... 15

Chapter 1 Introduction ... 16

1.1 Health indices ... 20

1.2 Temporal variability of health indices ... 23

1.3 Ningaloo Reef ... 25

1.4 Objectives ... 27

Chapter 2 Seasonal variations in energy levels and metabolic processes of two dominant *Acropora* species (*A. spicifera* and *A. digitifera*) at Ningaloo Reef ... 30

2.1 Abstract ... 30

2.2 Introduction ... 31

2.3 Methods ... 33

2.3.1 Study site ... 33

2.3.2 Sampling ... 34

2.3.3 Analysis of health indices ... 35

2.3.4 Statistical analysis ... 39

2.4 Results ... 39

2.4.1 Differences in health indices between species, seasons and stations ... 39

2.4.2 Seasonal variability of health indices ... 43

2.5 Discussion ... 47

2.5.1 Present findings in context with other studies ... 47

2.5.2 Seasonal variability in health indices ... 48

2.5.3 Comparison between metabolic activity and energy levels of *A. spicifera* and *A. digitifera* 51

2.5.4 Implications for Ningaloo Reef 54

Chapter 3 Which environmental factors predict seasonal variation in coral health of *Acorpora digitifera* and *Acropora spicifera* at Ningaloo Reef? 55

3.1 Abstract 55

3.2 Introduction 56

3.3 Methods 59

3.3.1 Study site 59

3.3.2 Sampling 60

3.3.3 Analysis of health indices 60

3.3.4 Analysis of physico-chemical-biological factors 62

3.3.5 Statistical analysis 64

3.4 Results 65

3.4.1 Relationships between coral cover and current speed 65

3.4.2 Seasonal changes in physico-chemical-biological factors 67

3.4.3 Relationships between coral health indices and physico-chemical-biological factors 70

3.4.4 Importance of PAR and nutrients for coral health indices 77

3.5 Discussion 78

3.5.1 Long-term effect of current speed on *Acropora* species coral cover 79

3.5.2 Physico-chemical-biological predictors for coral health during typical seasonal patterns (excluding the La Niña event) 81

3.5.3 La Niña and its impacts on physico-chemical- biological factors and thus coral health 86

3.5.4 Implication for long-term health of Ningaloo Reef 90

Chapter 4 Temporal (diel and daily and seasonal) variations in metabolic and autotrophic indices for *Acropora digitifera* and *Acropora spicifera* – implications for monitoring projects 91

4.1 Abstract 91

4.2 Introduction 92

4.3 Methods 95

4.3.1 Study site 95

4.3.2 Sampling 96

4.3.3 Analysis of health indices 97

4.3.4 Analysis of physico-chemical-biological factors 98

4.3.5 Statistical analysis 100

4.4 Results 102

4.4.1 Temporal variation of health indices 102

4.4.2 Environmental predictors for metabolic indices on a diel basis 105

4.4.3 Time-lag effect of light on health indices 106

4.4.4 Diel patterns of physico-chemical-biological factors 107

4.5 Discussion 109

4.5.1 Temporal changes in health indices and suitability of indicators for monitoring 109

4.5.2 Summary/ Conclusion 117

Chapter 5 General Discussion 119

5.1 Typical seasonal patterns of health indices and main physico-chemical-biological predictors for health indices of *A. digitifera* and *A. spicifera* 119

5.2 Impacts of La Niña on health condition for corals at Ningaloo Reef 122

5.3 Implications for the suitability of coral health indices for monitoring projects 124

5.4 Implications for Ningaloo reef for future scenarios 126

References 129

Appendix A 138

Appendix B 142

List of figures

Figure 2-1: Study location of Sandy Bay and the adjacent Ningaloo Reef section with respect to the Western Australian coastline (right figure). Coral sampling stations are depicted by red diamonds and numbers (left figure). Superimposed blue arrows (left figure) show the characteristic flow pattern across the reef flat and around the lagoon (as described by Lowe et al. 2008). Satellite imagery: Google Earth, 2010. 34

Figure 2-2: Non-metric multidimensional scaling by ordination of health indices (protein concentration (mg µgDNA^{-1}), RNA/DNA ratio, lipid ratio, zooxanthellae density (cells cm^{-2}), chlorophyll *a* (µg cm^{-2})) for (A) two species (*A. digitifera* and *A. spicifera*), (B) four different sampling periods (March and April 2010 (autumn), August 2010 (winter) and February 2011(summer)), (C) six stations at Sandy Bay, Ningaloo Reef. Analyses were performed on Euclidean dissimilarities of log-transformed data. 42

Figure 2-3: Health indices for *A. digitifera* and *A. spicifera* for four different sampling periods at Sandy Bay, Ningaloo Reef. Values are means ± standard error, n = 27 – 37. Same letters indicate no significant difference between seasons for *A. digitifera* (a, b, c, d) and *A. spicifera* (e, f, g, h) separately based on pair-wise analysis (Euclidean distance after log-transformation) of PERMANOVA. 45

Figure 3-1: Percent coral cover of plate *Acropora*, caespito-corymbose *Acropora* and other coral species at Ningaloo reef. Six stations were sampled in autumn (March - April 2010). Numbers in brackets represent long-term average current speed per station and same letters indicate no significant difference in total coral coverage per station. 67

Figure 3-2: Patterns in physico-chemical-biological factors at Ningaloo Reef throughout seasons. (A) Temperature (°C) and light (PAR), (B) current speed, (C) chlorophyll *a* (chl *a*), (D) picoplankton concentration (*Prochloroccocus*, *Synechoccocus* and picoeukaryotes), (E) nutrients (PO_4, NO_x, NH_4, Si and TN) and (F) zooplankton concentrations at six stations at Ningaloo Reef during autumn (March/April) and winter (August 2010) and during the La Niña event in summer (February 2011) (grey column). Data are means ± standard error (n=6). 70

Figure 3-3: Quadratic correlations (R^2) of temperature (˚C) (A-E) and linear correlations (R^2) of PAR (µE m^{-2} s^{-1}) (F-J) with (A) protein concentration (mg µgDNA^{-1}), (B) RNA/DNA ratio, (C) lipid ratio,(D) chlorophyll *a* concentration (chl *a* µg cm^{-2}) and (E) zooxanthellae density (ZD cells x 10^6 cm^{-2}) for *Acropora digitifera* 78

Figure 3-4: Quadratic correlations (R2) of temperature (˚C) (A-E) and linear correlations (R2) of PAR (µE m-2 s-1) (F-J) with (A) protein concentration (mg µgDNA-1), (B) RNA/DNA ratio, (C) lipid ratio,(D) chlorophyll a concentration (chl a µg cm-2) and (E) zooxanthellae density (ZD cells x 106 cm-2) for *Acropora spicifera*. 78

Figure 4-1: Diel pattern for health indices for *Acropora digitifera* and *Acropora spicifera* at Ningaloo. Values represent means ± standard error, n = 9 – 12 for metabolic indices (protein concentration, RNA/DNA ratio) and autotrophic indices (zooxanthellae density (ZD), chlorophyll *a* concentration (per surface area and per cell), yield and rETR)) at four different

times per day in August 2010 (winter) and February 2011 (summer). Stars symbolize significant differences between species and seasons (* P < 0.05; ** P < 0.01; *** P < 0.001). 104

Figure 4-2: Correlation coefficient (R) between health indices and light (PAR) history. Correlation coefficients were determined for (A) protein concentration (mg $mgDNA^{-1}$), (B) RNA/DNA ratio, (C) zooxanthellae density (ZD) (cells cm^{-2}), (D) chlorophyll a per surface area (µg cm^{-2}), (E) chlorophyll *a* per cell (µg $cell^{-1}$) and F) relative electron transport rate (rETR) (Based on DISTLM). 107

Figure 4-3: Diel patterns for physico-chemical-biological parameters at Ningaloo Reef. Patterns in (A) current speed (B) temperature and light intensity (PAR) (C) dissolved inorganic nitrogen (DIN) and organic nitrogen (ON), (D) chlorophyll *a* concentrations and zooplankton and (E) picoplankton carbon and nitrogen content at site 4 during August 2010 and February 2011. Data are means ± standard error (n=4). 109

List of tables

Table 2-1 Results of three-factorial PERMANOVAs for protein concentration, RNA/DNA ratio, lipid ratio, zooxanthellae density (ZD), chlorophyll *a* concentration (chl *a*) per surface area and per cell, comparing two species (*A. digitifera* and *A. spicifera*), four sampling periods (March and April 2010 (autumn), August 2010 (winter) and February 2011 (summer)) at six different stations at Sandy Bay, Ningaloo Reef. Effects tested are species (Sp), season (Se) and Station (St). Significant results printed in bold. 40

Table 2-2: Linear correlations provided as R^2 values, between health indices (protein concentration, RNA/DNA ratio, lipid ratio, zooxanthellae density (ZD) and chlorophyll *a* (chl *a*) for *A. digitifera* and *A. spicifera* at Sandy Bay, Ningaloo Reef. Tests were performed with DISTLM (Primer 6) based on Euclidean dissimilarity matrix after log-transformation (with selection criteria all specified). Grey shading indicates significant negative relationships and bold letters significant positive relationships (* P < 0.05; ** P < 0.01; *** P < 0.001)............. 47

Table 3-1: Main physico-chemical-biological predictors for coral health indices (protein concentration, RNA/DNA ratio, lipid ratio, chlorophll *a* (chl *a*) concentration and zooxanthellae density (ZD) per surface area) for *Acropora digitifera* for (A) typical seasons (autumn and winter 2010) not affected by La Niña event, (B) during the coral spawning event in autumn 2010, when metabolic indices were highest and (C) all sampled seasons including the La Niña event (autumn 2010, winter 2010 and summer 2011) Tests were performed with DISTLM (based on BIC; Primer 6) Grey shading indicates significant negative relationships and no shading significant positive relationships.* P < 0.05; ** P < 0.01; *** P < 0.001. (var = variability, Pro = *Prochlorococcus*, Picoeuk = picoeukaryotes, temp = temperature, ZP = zooplankton)... 75

Table 3-2: Main physico-chemical-biological predictors for coral health indices (protein concentration, RNA/DNA ratio, lipid ratio, chl *a* concentration and zooxanthellae density per surface area) for *Acropora spicifera* for (A) typical seasons (autumn and winter 2010) not affected by La Niña event, (B) during the coral spawning event in autumn 2010, when metabolic indices were highest and (C) all sampled seasons including the La Niña event (autumn 2010, winter 2010 and summer 2011) Tests were performed with DISTLM (based on BIC; Primer 6) Grey shading indicates significant negative relationships, no shading significant positive relationships and italic no significant relationship.* P < 0.05; ** P < 0.01; *** P < 0.001. (var = variability, Syn = *Synechococcus*, Pro = *Prochlorococcus*, Picoeuk = picoeukaryotes, temp = temperature, ZP = zooplankton) 76

Table 4-1: Physico-chemical-biological predictors for variation in protein concentration and RNA/DNA ratio in *A. digitifera* and *A. spicifera* during August 2010 and February 2011.Results are based on BIC tests (Primer). Bold letters symbolize negative correlations while other correlations were positive. * p < 0.05, ** p < 0.01, *** p < 0.001. Non-significant correlations are in italic.var = variability, tot = total. 106

List of major abbreviations

A. spicifera	*Acropora spicifera*
A. digitifera	*Acropora digitifera*
BIC	Bayesian information criterion
Chl *a*	Chlorophyll *a*
DIN	Dissolved inorganic nitrogen
DISTLM	Distance-based linear model
ON	Organic nitrogen
NH_4	Ammonium
NO_x	Nitrate + nitrite
PAR	Light intensity (photosynthetic active radiation)
PERMANOVA	Permutational ANOVA
PO_4	Phosphate
PS	Photosystem
rETR	Relative electron transport rate
Si	Silicate
TN	Total nitrogen
Yield	Effective quantum yield
ZD	Zooxanthellae density
ZP	Zooplankton

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Statement of candidate contribution

I hereby declare that all material presented in this thesis is original except where due acknowledgement is given, and has not been accepted for the award of any other degree or diploma. The body of this thesis (Chapter 2 to 4) is presented as a series of self-contained papers intended for journal publication, and some repetition of the literature review, study site details and methodology has therefore been necessary.

Chapter 2 is in review (Coral Reefs) and is under the joint authorship of myself, Dr. Nicole L. Patten, Prof. Richard J.N. Allcock, Prof. Sandra M. Saunders, Daniel Strickland and Prof. Anya M. Waite. Chapter 3 has the joint authorship of myself, Dr. Nicole Patten, Dr. Ming Feng, Daniel Strickland and Prof. Anya M. Waite and has been submitted for publication (PLoS ONE). Chapter 4 was submitted (PLoS ONE) and has the joint authorship of myself, Dr. Nicole Patten and Prof. Anya M. Waite. These papers were written under joint authorship to acknowledge the input of the different parties involved in parts of the research process. Prof. Anya M. Waite supported the project with funding, gave advice for the sampling design and helped with reviews and discussion. Dr. Nicole L. Patten helped with the sampling design, review and discussion. Prof. Richard J.N. Allcock and Prof. Sandra M. Saunders taught me analysis of RNA/DNA ratio and protein concentration as well as lipid ratio and helped with review and discussion. Daniel Strickland carried out analysis for zooxanthellae density for March and April 2010 and analysed percentage coral coverage at Ningaloo and long-term current speed (Chapter 3) and created map of study site. Dr. Ming Feng helped with review and discussion.

As the author of all material within this thesis, I am completely responsible for all data collection, sample and data analyses (expect as mentioned above), figures and written text contained herein.

Publications arising from this thesis

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Chapter 1 Introduction

Coral reefs are among the most biologically diverse and economically important ecosystems in the world (Sheppard 2009). However, they are under increasing pressure from both climate change and human impacts (Hoegh-Guldberg et al. 2007). For example, episodes of coral mortality, driven by elevated seawater temperature (Hoegh-Guldberg 1999; Buddemeier et al. 2004; Anthony and Marshall 2012) and hydrodynamic stresses (in the form of extreme storm and wave events, wave forcing, sea level rise/fall and shifts in ocean currents) impact coral reefs worldwide (Dollar 1982; Wilkinson 2004; Webster et al. 2005; Kleypas 2008). Normally, coral reefs tend to recover from natural disturbances (e.g., tropical storms, periodic extreme weather events, low tides, predators, disease, variation in temperature) over short ecological timescales (weeks to months). However when the recovery of the system is impaired by, for example, human-induced disturbances, this can result in serious degradation of the coral reef followed by a shift in coral community, or the destruction of reefs as already seen in the loss of 19% of coral reefs worldwide (Wilkinson 2004). As a result, coral species that are more resilient to high temperatures and hydrodynamic stresses may dominate reef communities in future years (Hughes and Connell 1999; Riegl 2003). Moreover, corals are not the only organisms affected by these disturbances; all organisms that depend on the reef are ultimately impacted when the reef system changes as a habitat, nursery area and/or nutritional source. Other major threats to coral reefs include ocean-acidification, that can affect the ability of corals to build their skeleton in the future (Hoegh-Guldberg 2007), outbreaks of *Acanthus*, fishing impacts and nutrient run-off (Sheppard et al. 2009). However, in this thesis we focus on effects of changes in hydrodynamic patterns, temperature, light and food availability on corals,

since for our study site, Ningaloo Reef, human induced threats as fishing or nutrient run-off have not as yet been observed to have had a major impact.

Corals depend on both autotrophic and heterotrophic processes to acquire sufficient energy for metabolic processes (e.g., growth, respiration) and reproduction. Autotrophy is a light-driven process, by which intracellular symbiotic algae (zooxanthellae) transform solar energy through photosynthetic carbon fixation and translocate much of the resulting photosynthates to the coral hosts (Muscatine and Porter 1977; Muscatine 1990; Dubinsky and Jokiel 1994). Heterotrophy is the active uptake and subsequent utilisation of plankton, organic nutrients and organic matter from the water column by the coral to support both coral host and algal symbiont metabolic processes (Sebens et al. 1996; Anthony and Fabricius 2000; Ferrier-Pagès et al. 2003). In the past, it was thought that autotrophy provided corals with the bulk of their energy; however, more recent studies showed heterotrophy to be more important than previously expected, both in supplying essential organic nutrients for growth, and in supplying corals with the energy required for metabolic functions (Grottoli et al. 2006; Ferrier-Pagès et al. 2011). Heterotrophy becomes particularly important in providing corals with nutrients, including nitrogen that is essential for tissue growth, but which is not adequately supplied to the coral through autotrophic processes (D'Elia and Webb 1977; Dubinsky and Jokiel 1994; Mills and Sebens 2004). Previous observations showed that, for example, zooplankton ingestion represents a non-negligible energy input for the growth of two scleractinian corals, *Montastrea annularis* and *Porites furcata* (Witting 1999). This study did not include planktonic food sources smaller than 50µm. In addition, possible feeding rates during the day for corals with continuous polyp extension were not considered, thus it can be expected that the role of heterotrophy in the coral metabolic budget is much higher than current calculations show (Houlbrèque and Ferrier-Pagès 2009).

Whether corals receive energy predominantly through autotrophic or heterotrophic processes is species-specific, depth dependent (Anthony 1999; Anthony and Fabricius 2000, Palardy et al. 2005) and can change with bleaching events (Grottoli et al. 2006). Certain coral species such as *Goniastrea retiformis* (Lamarck, 1816) can shift feeding requirements from autotrophy to heterotrophy depending on the light regime and associated increases or decreases of photosynthetic yields, while other species such as *Porites cylindrica* (Dana, 1846) are not able to use particle feeding to compensate for carbon losses due to shading (Anthony 1999; Anthony and Fabricius 2000). Species-specific differences in feeding mode are complicated by seasonally driven changes in the physico-chemical-biological environment such as light, temperature and nutrient availability. These environmental factors may determine whether corals feed autotrophically or heterotrophically (Ribes et al. 2003; Palardy et al. 2005). In addition, changes in current speed over the reef likely alter the flux of dissolved and particulate nutrients reaching coral reefs, as well as modifying the boundary layer thickness of individual corals (Atkinson et al. 1994; Hearn et al. 2001; Ribes et al. 2003; Ribes and Atkinson 2007) and subsequently affect the feeding mode via heterotrophy (Sebens et al. 1998) and autotrophy (Dennison and Barnes 1988). Depth-defined differences in feeding mode also exist, as shown by Palardy et al. (2005) who documented a shift from photoautotrophy to heterotrophy at different depths for *Pocillopora damicornis* (Linnaeus, 1758)*, Pavona clavus* (Dana, 1846) and *Pavona gigantea* (Verrill, 1869).

Energy gained through both autotrophy and heterotrophy is important for the reproductive success of corals, which is a key component of coral fitness (Beiring and Lasker 2000), and changes in the physico-chemical-biological environment are likely to affect coral reproductive output. Previous work showed that changes in water depth, sea surface temperature, increased sedimentation and run-off (and thereby a decrease in the

light regime), as well as an increase in nutrient concentrations, can all have an effect on the reproductive success of different coral species (Fabricius 2005). Elevated ammonium levels reduced egg sizes in *Montipora capitata* (Dana, 1846) (Cox and Ward 2002) and reduced egg fertilisation rates in *Acropora longicyathus* (Milne Edwards & Haime, 1860) (Harrison and Ward 2001). A shift in energy allocation between growth and reproduction was also found; for example, in *P. damicornis* an increase in one is usually accompanied by a decrease in the other (Ward 1995), resulting in a change of reef growth rates, and eventually in species composition. Such a shift in energy allocation has also been suggested for species that can maintain both skeletal growth and density under reduced carbonate saturation by investing greater energy in calcification. It is likely that this strategy results in a diversion of resources from other essential processes, such as reproduction, as seen in chronic stress (Szmant and Gassman 1990). A result could be an ultimate reduction of the larval output from reefs, impaired with a decreased potential for recolonization following disturbances (Houlbrèque and Ferrier-Pagès 2009).

It is clear that coral health will be affected by human-induced and/or climate-change related environmental changes. Determining a coral baseline of resilience to such changes requires fundamental background knowledge. In particular, an understanding is needed of different coral species' primary energy source under natural conditions (autotrophy vs. heterotrophy), how their metabolic activities vary, and how much energy they can store as lipids. Information on the spatial and temporal variation in these factors is also necessary.

1.1 Health indices

A variety of coral health indices have been developed to describe short-term as well as long-term changes in coral health. To define what is healthy and what is not, is important for monitoring and management plans for coral reefs. For this, it is important to separate natural variability from situations in which coral health has changed due to stress. Corals can be defined as 'healthy' when coral health indices are within the typical range for a species and do not show abnormally high or low values due to e.g., extreme events such as bleaching (e.g., Meesters et al. 2002; Palardy et al. 2008; Cooper et al. 2009). It is important to take temporal and spatial variations into account, since previous studies showed that e.g., RNA/DNA ratios can vary on short time and space scales (Meesters et al.2002; Humphrey 2009).

Total lipid content is widely considered to be a relevant bio-indicator of coral condition, and can represent a measure of resilience to environmental impacts through energy storage (e.g., Anthony and Fabricius 2000). Previous studies showed that species which are largely dependent on photosynthetically fixed carbon, such as e.g., *Porites compressa,* deplete their stored energy reserves (lipids) during extreme events such as bleaching until significant rates of photosynthesis returned (Grottoli et al. 2004). However, species that are able to increase heterotrophy (e.g., *Montipora capitata*) are able to keep up their energy reserves (Grottoli et al. 2006; Rodrigues and Grottoli 2007) and/or regain the original lipid class composition more quickly (Rodrigues et al. 2008). If environmental conditions are favourable and energy requirements are fully met through autotrophy and/or heterotrophy, surplus energy is stored in the form of storage lipids (wax ester or triacylglycerol TG) (Patton et al. 1977; Harland et al. 1991; Harland et al. 1992b), or released as mucus (Crossland et al. 1980). Scleractinian corals are rich in lipids (9 to 47% dry weight) (Stimson 1987; Harland 1993; Yamashiro and Yamashiro 1999), of which 20 to 80% can be in the form of

storage lipids (Patton et al. 1977; Harland 1993; Oku et al. 2003a), while the rest consists of structural lipids utilised for maintaining the structure of cells (phospholipids and cholesterol; Saunders et al. 2005). The concentration of stored lipid varies with environmental condition such as changes in light intensity (Patton et al. 1977; Stimson 1987), water temperature (Oku et al. 2003b) and food availability (Harland et al. 1992a; Harland et al. 1992b). Seasonal increases in the concentration of storage lipids are thought to depend on favourable seasonal conditions, while concentrations of structural lipids are thought to be relatively stable over time (Oku et al. 2003b). Since, under conditions of stress, corals deplete storage lipids before accessing structural lipids (Rodrigues et al. 2008b), the ratio between storage lipids and structural lipids (hereafter "lipid ratio"), has been suggested as a good bio-indicator of coral condition (Saunders et al. 2005; Cooper et al. 2009). Seasonal changes in lipid ratio are expected to be particularly influenced by the annual coral spawning event of broadcasting species, since this massive reproductive output reduces energy storage in form of lipids by more than 85% in post-spawning coral tissue (Ward 1995; Leuzinger et al. 2003; Leuzinger et al. 2012). The reason for this decrease is the release of eggs and sperm, both materials with high lipid content (mainly storage lipids) (Harii et al. 2007). In our study we will use lipid ratio as an indicator of energy storage in corals.

Autotrophic energy supply is especially important for shallow-water corals, with algal photosynthesis essential for maintenance of energy stores (Stimson 1987) and metabolic processes within the corals (Dubinsky and Jokiel 1994). Zooxanthellae density and pigmentation, specifically chlorophyll *a* (chl *a*) content of the coral host, are indicators of the ability of corals to capture light (Harithsa et al. 2005) and therefore to gain energy. However, chlorophyll concentration can also be reduced under high light as a mechanism for photo protection (Titlyanov and Titlyanova 2002). Previous studies have shown seasonal variations in zooxanthellae density and chl *a* concentration

(Fagoonee et al. 1999; Fitt et al. 2000), which were most likely due to changes in light (Fitt et al. 2000), seawater temperature (Coles and Jokiel 1977; Fitt et al. 2000), nutrient availability (Houlbrèque et al. 2003) and flow regime (Nakamura et al. 2005). Corals can acclimatise to new environmental conditions by changing their zooxanthellae density and amount of chlorophyll per unit surface area for example, as a photoacclimatisation process (Cooper et al. 2009). A recent study showed also that both too few and too many zooxanthellae impact corals negatively, suggesting that zooxanthellae can represent a net cost to a host through having parasitic behaviour on the energy budget (Cunning and Baker 2012). Thus, while these indices can be used as autotrophic indices of coral condition, we must approach their use with caution and investigate their environmental correlates to determine how best such indices might be deployed.

Both RNA/DNA ratios and the protein concentration in the tissue of the coral holobiont have been used as indicators of metabolic activity and nutritional condition in corals (Meesters et al. 2002; Ferrier-Pages et al. 2003). Since biochemical components shift with changing environmental factors, resulting in alteration of protein synthesis or metabolism that then alter performance, growth or reproductive output, RNA/DNA ratio and protein concentration can be used as a snapshot of the condition of the organism at the sampling time (Dahlhoff et al. 2004). RNA content is primarily a function of ribosome number and is correlated with new protein synthesis wheras DNA content remains constant in an individual because it is a function of chromosome number (Dahlhoff 2004). Thus, during periods of active protein production such as those associated with growth or differentiation, the RNA/DNA ratio is predicted to be higher than during periods when protein metabolism is low (Buckley and Szmant 2004). RNA/DNA ratio is therefore directly related to both tissue growth and nutritional status and are used in many organisms as an indicator of growth, biomass or metabolic

functioning and thus health (Meesters et al. 2002). Both, autotrophy and heterotrophic feeding in corals can influence RNA/DNA ratios. Irradiance was correlated with RNA/DNA ratio for *Porites lutea* (Milne Edwards & Haime, 1860), *Porites lobata* (Dana, 1846) *and Porites astreoides* (Lamarck, 1816) (Meesters et al. 2002; Buckley and Szmant 2004), and food availability was correlated with RNA/DNA ratios in fish, crustaceans and molluscs (Dahlhoff 2004). Seasonal changes in the surrounding seawater environment including temperature, light and nutrients can also affect RNA/DNA ratios (Meesters et al. 2002; Buckley and Szmant 2004; Harithsa et al. 2005). In this study we will use RNA/DNA ratio to describe variation in metabolic activity inside the coral holobiont.

Protein concentrations can reflect heterotrophic feeding rates (Al-Sofyani and Niaz 2007), since fed colonies in general exhibited significantly higher protein concentrations (Ferrier-Pages et al. 2003; Houlbrèque et al. 2003; Borell et al. 2008). Protein concentrations in corals follow seasonal patterns (Ferrier-Pages et al. 2003; Rossi et al. 2006) and respond to shifts in seawater temperature (Borell et al. 2008; Grottoli and Rodrigues 2011). This, together with the capacity of corals to rapidly modify protein expression as a response to both acute and chronic changes in environmental conditions (Dahlhoff 2004), suggests that protein concentration is likely to be highly indicative of coral condition and will be used in our study to describe metabolic activity inside the coral holobiont.

1.2 Temporal variability of health indices

Previous studies using health indices to describe coral health demonstrated that health indices vary strongly over seasonal scales, following changes in the physico-chemical-biological environment. It is important to understand how autotrophic indices, coral metabolism and energy stores are linked with each other, and further, how changes

in the physico-chemical-biological environment may affect these coral health indices. This information is essential to make future predictions about the impacts that climate-induced changes will have on coral health.

Since corals are strongly dependent on light due to their symbiosis with zooxanthellae, it can be expected that they respond over hourly time scales to intracellular fluctuations in O_2, CO_2 and NH_4 tension and pH, all of which are driven by algal photosynthesis and algal and coral metabolism (respiration rates) (Levy et al. 2006; Yellowlees et al. 2008). At these same time scales, food availability for corals can also vary. For example, highest concentrations of zooplankton often occur at night which suggests that diel cycles (across a 24 hour period) in corals are set by the availability of demersal plankton as food (Alldredge and King 1977; Heidelberg et al. 2004; Yahel et al. 2005). Previous studies identified diel cycles for a variety of indices related to autotrophy and photoacclimatisation. Algal cell division showed a maximum division rate between 0 and 6 am for *P. damicornis* (Hoegh-Guldberg 1994). Effective quantum yield of photosystem II (PSII), a way to assess the photosynthetic performances of PSII (Rodolfo-Metalpa et al. 2008), exhibited highest values at night, a decrease in the morning, low values around noon and an increase towards the evening for e.g., *Stylophora pistillata* (Esper, 1797) and *Goniastrea aspera* (Verrill, 1905) (Brown et al. 1999b; Winters et al. 2003). Relative electron transport rate (rETR), an indicator for photosynthetic activity (Hoogenboom et al. 2006), also developed diel patterns in *Turbinaria mesenterina* (Lamarck, 1816) and *S. pistillata,* with highest values at noon and decreasing towards the evening (Winters et al. 2003; Hoogenboom et al. 2006). Low yield values during noon, when light intensity is normally highest, are related to a photoacclimatisation process to protect the photosystem from too high light intensity even though at the same time photosynthetic rates and thus rETR are highest due to this high light intensity. Since the provision of photosynthetically derived and

translocated products from the zooxanthellae to the coral affect metabolic rates of corals (Falkowski et al. 1993; Dubinsky and Jokiel 1994), we can expect that RNA/DNA ratio and protein concentration will also express diel cycles. In addition, protein concentration has been shown to vary with food availability such as zooplankton and is thus likely influenced by diel fluctuations in plankton availability (Sebens et al. 1996; Ferrier-Pages et al. 2003). Diel variations were also previously observed for RNA/DNA ratios in fish and molluscs (Chicharo et al. 2001; Esteves et al. 2009) and are suggested to differ with metabolic rates, food requirements and digestion times (Buckley et al. 1999).

It is not known how day/night changes in metabolic patterns control metabolic indices (RNA/DNA ratio and protein concentration), or which physico-chemical-biological factors, if any, are responsible. Furthermore, it is unclear whether a relationship exists between diel changes in photosynthetic activity and diel changes in metabolic indices rates such as protein synthesis.

The understanding of diel changes in health indices is essential for implications in monitoring projects, since short-term variations in health indices are larger than long-term ones, and this would mean long-term variations may be masked by short-term ones and may thus be undetectable and unrepresentative.

1.3 Ningaloo Reef

Ningaloo Reef, situated on the northwest coast of Australia, is the world's largest fringing reef (Taylor and Pearce 1999). The reef is highly influenced by two dominant oceanic currents, the Leeuwin Current and the Ningaloo Current (e.g., Rousseaux et al. 2011). The Leeuwin Current is a southward flowing current bringing relatively warm, low salinity ocean water to the region, with strongest flow in autumn

and winter, and relatively high phytoplankton concentrations in autumn (Rousseaux et al. 2011). The wind-induced Ningaloo Current flows in the opposite direction to the Leeuwin Current, and is more commonly documented during summer, when it is associated with sporadic and localized upwelling events (Hanson et al. 2005; Rousseaux et al. 2011). Ningaloo Reef is also subject to local hydrodynamic regimes delivering offshore waters across the reef top (Lowe et al. 2008; Wyatt et al. 2010), and therefore responsible for changes in the biological as well as physical parameters over the reef (i.e. temperature, light, nutrients, phytoplankton and zooplankton abundance). The reef removes up to 90% of the plankton in waters passing over it, suggesting that the ocean delivers nutrients that are heterotrophically important (Wyatt et al. 2010). These uptake rates are both hydronamically and biologically driven (Wyatt et al. 2010).

Local current speeds as well as light intensities are likely to change with sea-level rise (Taebi et al. 2011). In addition, climate-related weather patterns such as El Niño Southern Oscillation (ENSO) and Indian Ocean Dipole can result in changes in the frequency and/or intensity of storm events (Holbrook et al. 2009; Weller et al. 2012). Moreover, water temperature and nutrient availability over the reef can be affected by these weather patterns, since they lead to changes in the broad current patterns, thermocline depth, and marine productivity of Western Australian coastal waters (Feng et al. 2003; Feng et al. 2009). Anthony and Marshall (2012) predicted a gradual temperature rise (2.5°C increase until end of this decade), and in consequence a weakening of the Leeuwin Current. It is unclear what consequences these physico-chemical-biological changes will have for coral health and community compositions at Ningaloo Reef. Since hydrodynamics at Ningaloo Reef have been broadly studied and are potentially predictable (e.g., Lowe et al. 2008; Taebi et al. 2011), this area represented a perfect study site for the present research.

Given that strong seasonal and inter-annual changes in physico-chemical-biological parameters have already been well-described for Ningaloo Reef, e.g., current speeds (Lowe et al. 2008; Taebi et al. 2011), light, temperature, nutrient concentrations and plankton concentrations (Patten et al. 2011; Rousseaux et al. 2011; Wyatt et al. 2012), it is expected that such changes in the physico-chemical-biological environment will also be reflected by shifts in coral health. In particular, Wyatt and co-workers documented the dominance of particulate nutrient uptake during the autumn/winter plankton bloom, while dissolved nutrients were more available in summer (Wyatt et al. 2012). We can thus hypothesize that seasonal changes nutrient supply are likely to drive changes in coral health – specifically, that heterotrophy is likely to be more important in autumn/winter than in summer.

Ningaloo Reef is dominated by corals of the genus *Acropora* (Cassata and Collins 2008), with *A. digitifera* (Dana, 1846) (caespito-corymbose) and *A. spicifera* (Dana, 1846) (plate) as two of the dominant species. An assessment of natural seasonal changes in coral health, and how these are related to physico-chemical-biological environmental factors is critical for understanding how extreme events and climate-driven environmental changes will affect coral health and thus the species composition at Ningaloo Reef.

1.4 Objectives

The overall aim of this project was to determine how coral autotrophic and metabolic indices, and energy stores, vary for *A. digitifera* and *A. spicifera* over a key range of temporal and spatial scales (seasonal, interannual, and diel). To do this, coral health indices were measured during four sampling periods throughout the year; these sampling periods included the before, during and after the major coral spawning event at Ningaloo, which occurred in March/April 2010, and an extreme weather event, i.e. La

Niña in February 2011. In addition, diel (throughout the day) and daily (between days) work was carried out during summer (February 2011) and winter (August 2010) to determine differences in health indices on short-term and long-term scales. Sampling has been carried out on different areas of the reef crest with varying hydrodynamic regimes (current speed and nutrient availability). Three stations were chosen close to the ocean, where nutrient availability was high and three close to the lagoon, where nutrient availability was lower (Wyatt et al. 2012). The two southerly located stations were located in an area with high current speed, while the four northerly located stations are in areas with lower current speed (Taebi et al. 2011). Understanding how coral metabolic processes change over these key temporal and spatial scales is fundamental as background knowledge of coral health at Ningaloo Reef as well as for implications of the suitability of health indices in monitoring projects. In addition, it is important to determine the main physico-chemical-biological predictors for coral health in order to allow predictions about possible impacts of climate-induced changes in coral health and species composition at Ningaloo Reef.

Thus, the overall objectives of this research were to:

(1) determine how energy storage, as well as metabolic and autotrophic indices change seasonally, particularly through the spawning event for *A. digitifera* and *A. spicifera,* and how energy storage and metabolic processes such as metabolic activities and autotrophic indices are linked within each species;

(2) determine which physico-chemical-biological factors are the main drivers of seasonal shifts in coral health indices during typical seasons, as well as during La Niña, for *A. digitifera* and *A. spicifera*;

(3) determine the suitability of metabolic indices (RNA/DNA ratio, protein concentration) and autotrophic health indices (zooxanthellae density, chl *a*

concentration, effective quantum yield and rETR) for depicting diel, daily and seasonal changes in the health condition of *A. digitifera* and *A. spicifera* to implement the use of these indicators for monitoring projects.

Chapter 2 Seasonal variations in energy levels and metabolic processes of two dominant *Acropora* species (*A. spicifera* and *A. digitifera*) at Ningaloo Reef

2.1 Abstract

Seasonal variations in coral health indices reflecting autotrophic activity (chlorophyll *a* (chl *a*) and zooxanthellae density), metabolic activity (RNA/DNA ratio and protein) and energy storage (ratio of storage: structural lipids, or lipid ratios) were examined for two dominant *Acropora* species (*Acropora digitifera* and *Acropora spicifera*) at Ningaloo Reef (north-western Australia). Such detailed investigation of metabolic processes is important in understanding the vulnerability of corals to environmental change. Health indices in *A. digitifera* and *A. spicifera* were measured at six stations, before and after spawning in austral autumn and winter 2010, and austral summer 2011. Health indices showed seasonal and species-specific differences but negligible spatial differences across a reef section. For *A. digitifera*, chlorophyll *a* and zoxanthellae density were negatively correlated with both lipid ratios and metabolic indices. Metabolic indices were significantly higher in *A. spicifera* than *A. digitifera*. No correlation was observed between RNA/DNA ratios or lipid ratios with any autotrophic indices for *A. spicifera*. Lipid ratios were stable throughout the year for *A. spicifera* while they changed significantly for *A. digitifera*. For both species, indices of metabolic activity were highest during autumn, while autotrophic indices were highest in winter and summer. Results suggest that the impact of the broadcast spawning event on coral health indices at Ningaloo Reef occurred only as a backdrop to massive seasonal

changes in coral physiology. The La Niña summer pattern resulted in high autotrophic indices and low metabolic indices and energy stores. Our results imply different metabolic processes are occurring in *A. digitifera* vs *A. spicifera* as well as a strong impact of extreme events on coral physiology. These results suggest that future changing climate will most likely have a strong negative impact on coral health and potentially on coral's reproductive success.

2.2 Introduction

Coral reefs are under increasing pressure from climate change and human impacts (Hoegh-Guldberg 2007). For corals to remain healthy, they require sufficient metabolic energy to support growth and reproduction (Grottoli et al. 2006; Rodrigues and Grottoli 2007). Corals acquire this energy through autotrophic (light-driven) and heterotrophic (nutrient and carbon uptake) processes (Muscatine 1990; Dubinsky and Jokiel 1994; Mills et al. 2004; Houlbrèque and Ferrier-Pagès 2009). In the past, it was thought that autotrophy provided corals with the bulk of their energy; however, more recent studies indicated that heterotrophy plays a bigger role than previously expected, both in supplying essential organic nutrients for growth (e.g., N and P), and in providing corals with the energy required for metabolism (Grottoli et al. 2006; Ferrier-Pagès et al. 2011).

Autotrophy or heterotrophy can both be important in supporting corals' energetic requirements, but the ability of corals to shift from one mode to the other is species-specific and depends on depth (Anthony and Fabricius 2000; Palardy et al. 2005; Houlbrèque and Ferrier-Pagès 2009) as well as on environmental conditions such as light intensity, nutrient availability and flow patterns (Anthony and Fabricius 2000; Ribes et al. 2003; Palardy et al. 2005). Given the fundamental role of seasonal variations in determining local environmental factors, we expect that metabolic

processes and energy levels of different coral species will show strong seasonal variation. Previous studies showed seasonal variations in zooxanthellae density (symbiotic algae which gain energy through photosynthesis) and pigmentation (chl *a* concentration) (Fagoonee et al. 1999; Fitt et al. 2000). These are commonly used as autotrophic indicators since they describe the ability of corals to capture light (Harithsa et al. 2005; Cooper et al. 2009). Seasonal variations have also been reported for protein concentrations (Ferrier-Pagès et al. 2003; Rossi et al. 2006) and RNA/DNA ratios (Buckley and Szmant 2004) for certain species. Protein concentration and RNA/DNA ratio have been applied with some success as indicators of nutritional condition, metabolic activity and potential for tissue growth (Meesters et al. 2002; Buckley and Szmant 2004; Dahlhoff 2004). Protein concentration is also thought to reflect heterotrophic feeding rates for certain coral species, since fed colonies have been shown to exhibit significantly higher protein concentrations and tissue growth (Ferrier-Pagès et al. 2003; Houlbrèque et al. 2003; Houlbrèque et al. 2004b; Al-Sofyani and Niaz 2007).

In corals, energy storage exhibits seasonal shifts, since lipids are stored only when surplus energy is available through autotrophic or heterotrophic feeding. This can be reflected through lipid ratio (storage lipids / structural lipids) (Oku et al. 2003a; Saunders et al. 2005). The annual broadcast coral spawning event can reduce energy storage, documented as decreased lipid content in coral tissue post-spawning (Leuzinger et al. 2003; Leuzinger et al. 2012) and the release of material with high lipid content (mainly storage lipids) during the spawning event (Harii et al. 2007).

We hypothesized that two *Acropora* species, *A. digitifera* (Dana, 1846) and *A. spicifera* (Dana, 1846) would show species-specific differences in feeding modes and energy storage throughout a seasonal cycle. We tested this using a broad range of health indices at one side for the first time. The objectives of this study were to determine (1) how

energy storage, as well as metabolic and autotrophic indices change seasonally, particularly through the spawning event for *A. digitifera* and *A. spicifera,* and (2) how energy storage and metabolic processes such as metabolic activities and autotrophic indices are correlated within each species.

2.3 Methods

2.3.1 Study site

The study site, Sandy Bay Lagoon, is located at Ningaloo Reef, along the North-West Cape of Western Australia (22.23°S, 113.84°E). Sandy Bay Lagoon was chosen, as local hydrodynamic patterns have been well described seasonally (Lowe et al. 2008; Wyatt et al. 2010) and the fringing reef is considered typical for the ~290 km extension of Ningaloo Reef, with shore-parallel reef sections periodically interrupted by channels (Lowe et al. 2008). The steep reef front (~1:50) rises to a shallow reef crest (~1.5 m) where waves break, transporting water into the lagoon across the crest and returning back to the ocean through the channels. At Sandy Bay, the reef crest starts ~50 m from the surf zone, spreads over ~500 m and reaches 1,000 m shore-ward, giving way to a sandy lagoon habitat (depth ~2-3 m) (Fig. 2-1).

Sampling was conducted at six stations, two stations south (1, 2) and four stations north (3, 4, 5, and 6) of the channel (Fig. 2-1). Three of these stations were located closest to the ocean side of the reef (1, 3, and 5) and three stations were closest to the lagoon (2, 4, and 6) (Fig. 2-1). The reef crest at Sandy Bay is dominated by the coral genus *Acropora* (Cassata and Collins 2008).

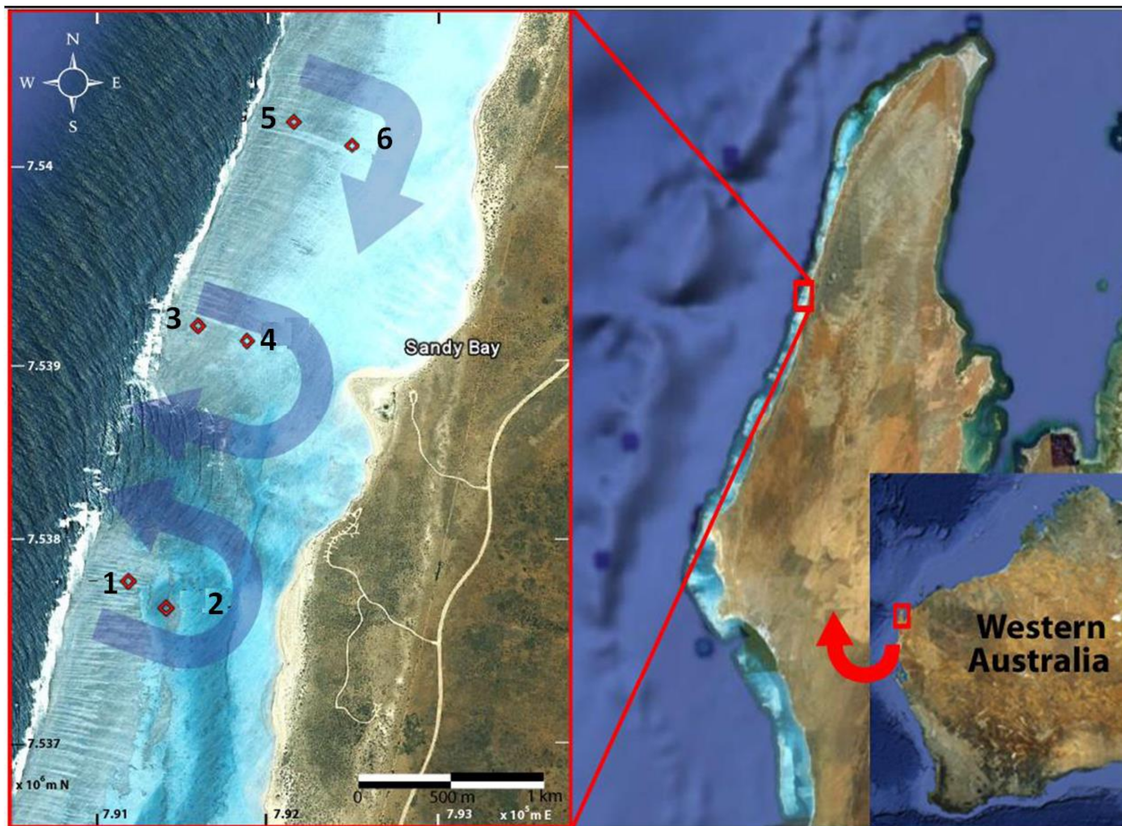


Figure 2-1: Study location of Sandy Bay and the adjacent Ningaloo Reef section with respect to the Western Australian coastline (right figure). Coral sampling stations are depicted by red diamonds and numbers (left figure). Superimposed blue arrows (left figure) show the characteristic flow pattern across the reef flat and around the lagoon (as described by Lowe et al. 2008). Satellite imagery: Google Earth, 2010.

2.3.2 Sampling

Corals of two dominant coral species *A. digitifera* (n = 6 at each station) and *A. spicifera* (n = 6 at each station) were tagged at each of the six stations (Wallace 1999) (depth range of 1 and 2.5 m, depending on tide and location). Only tagged corals were sampled over the study period with the exception of Station 1, where, due to large destruction by *Drupella* spp., no tagged corals survived between August and February; thus, other corals of the same species were sampled. Also, since some tagged colonies could not be re-located on subsequent visits at other stations due to individual tag loss, the number of coral samples differed between seasons. Coral samples were collected between 10:00 and 14:00 in autumn, covering the period before coral spawning (28-30th March 2010) and after the coral spawning event (15-17th April 2010), in winter

(18-27th August 2010), and in summer (12-15th February 2011). Coral spawning was observed directly (by snorkelling) from 4th-8th April 2010, with the majority of spawning (release of eggs during night) occurring on the 7th and 8th April. February 2011 fell within a La Niña year, which resulted in record high seawater temperatures (Pearce et al. 2011), lower light values (Appendix A, Fig. 1), and higher nutrient concentrations and current speeds than during normal summers (Chapter 3). Corals were sampled while snorkelling, with three 1 to 5 cm long coral pieces (tip of the branch) removed (hammer and chisel) from the middle of the coral colony. To be sure that the tagged corals had spawned, tissue and skeleton of the coral samples was inspected before and after the spawning for the presence of eggs. Only corals which had spawned during the April sampling period were included in statistical analyses; thus, seven colonies of *A. spicifera* and one colony of *A. digitifera* were excluded across all stations, leaving a minimum of 4 samples per species per station.

Light (photosynthetically active radiation, PAR) and temperature were measured at all stations using Hobo Pendant Data Loggers deployed at each station at the benthos over the sampling period for 2 weeks.

2.3.3 Analysis of health indices

Zooxanthellae density and chlorophyll a *concentration*

For the analysis of zooxanthellae density (per cm^2) and chl *a* concentration in the tissue ($\mu g\ cm^{-2}$), coral samples were stored at -20°C prior to analysis. Methods for the assessment of zooxanthellae density and chl *a* in coral tissue were adapted from Siebeck et al. (2006). Coral tissue was removed from the skeleton using a jet of pressurized air in filtered seawater (~50 ml) until only the white coral skeleton was visible. The volume was measured and the slurry homogenised using a hand-held blender for 45 s. Four aliquots of 10 ml were taken from each homogenate; one aliquot was fixed with

formaldehyde at a ratio of 2 ml formaldehyde per 10 ml solution, for microscopic analysis of zooxanthellae density, and three aliquots were immediately frozen for chl *a* determination. The number of zooxanthellae per volume slurry was counted using a haemocytometer (Neubauer) with four replicate counts on four haemocytometer plates. The total number of zooxanthellae per area was calculated using the volume of the homogenate and the coral surface area. Coral surface area was measured using the paraffin wax method of Stimson and Kinzie (1991). Chl *a* concentration was determined following the methods of Jeffrey and Humphrey (1975). Briefly, three 10 ml aliquots were centrifuged (3500 x *g* for 15 min), the supernatant was discarded, the pellet re-suspended in 10 ml of 100 % acetone and chl *a* extracted for 24 h in darkness at -20 °C. After centrifugation at 3500 x *g* for 10 min fluorescence was measured in a T700 fluorometer (Turner Designs), and results were standardized to coral surface area.

RNA/DNA and protein analysis

Samples for RNA/DNA and protein assessments were stored at -80 °C immediately (within a couple of minutes) after sampling, prior to analyses. Methods for concentrations of RNA/DNA from the coral holobiont followed Humphrey (2009). RNA content varies with metabolic demand and is correlated with new protein synthesis, while DNA content is largely stable (Buckley and Szmant 2004; Dahlhoff 2004). Coral samples (~1 cm long, tip of the branches) were crushed with pestle and mortar in liquid nitrogen and 10 ml of Tris-EDTA (TE) extraction buffer (10 mmol l^{-1} Tris-HCl, 1 mmol l^{-1} EDTA, pH 7.5) with 1% sarcosyl added. Samples were sonicated (3 min) in an ice bath, centrifuged (1200 x *g* for 3 min) and the supernatant (100 µl) transferred into deep well plates together with TE buffer (900 µl). The same solution was also used for protein analysis. RNA/DNA samples were analysed on three 96 deep-well microplates. To each well, on each plate 75 µl of nucleic acid standards (0–2.5 µg

ml^{-1} for DNA (calf thymus) and RNA (calf liver), duplicates), control homogenates or sample homogenates (triplicates) were added. To plate one, 15 µl of TE buffer was added to each well, while to plate two both, 7.5 µl TE buffer and 7.5 µl RNAse were added. Plate one and two were then incubated for 35 min at room temperature, before adding 75 µl of Ribogreen to each well of each plate. To plate three, 7.5 µl RNAse and 7.5 µl DNAse were added to all wells, and the plate was incubated for 60 min at room temperature, before adding 75 µl Ribogreen to each well. All plates were shaken for 5 min (Microplate Shaker) before examining in a microplate reader (485 nm excitation and 528 nm emission). The total amount of RNA per well was calculated by subtracting the fluorescence readings of plate two from plate one. The total amount of DNA per well was calculated by subtracting the fluorescence readings of plate three from plate two RNA and DNA concentrations based on the standard curves of each plate. Finally the ratio between DNA and RNA concentration was determined.

For protein analysis, a standard detergent-compatible colorimetric assay protein kit was used (procedure following Lowry et al. 1951). From the same sample solutions (described above), 5 µl was placed in a 96 deep-well microplate (triplicates), 25 µl of reagent A and 200 µl of reagent B were added to each well, the plate was shaken for 1 min, and absorbance was read after 15 min at 750 nm. Protein content was calculated using a standard curve by adding protein concentration (0.2-1.5 mg ml^{-1} protein duplicates) to each plate and standardizing all protein concentrations afterwards to DNA values.

Lipid ratio

Coral pieces (~3 cm long) for lipid ratio analysis were stored at -20°C until freeze-dried for 24 h in the laboratory. Lipid analysis followed the methods of Saunders et al. (2005). Lipid analysis describes the ratio between storage lipids (wax esters and

triacylglycerin), those that are stored by corals when environmental conditions are favourable and surplus energy is available (Harland et al. 1992b), and structural lipids (phospholipids and cholesterol), which are used for maintaining cell structure and are therefore known to be more stable than storage lipids (Saunders et al. 2005). Briefly, approximately 5 g of each coral sample was weighed, crushed with mortar and pestle, extracted in 10 ml of chloroform/methanol (2:1 by volume) and sonicated (15 min). The resulting slurry was filtered (Whatman's 540 hardened ashless filter), and the chloroform/methanol solution evaporated with nitrogen. The dry weight of the lipid extract was recorded (using an analytical scale) and then the dried material re-dissolved in chloroform/methanol solution (2:1 by volume) to a constant ratio of 1:50 (weight/volume). Thin layer chromatography (TCL) was used to separate total lipid extract into fractions. Five replicates per coral sample (5 µl each replicate) were transferred to a TLC plate (5x6 cm silica gel; Merck 60,). An additional aliquot of cholesterol (2 µl) was added to the plate in the same way to determine the structural band. Each plate was placed in an elution chamber containing a mixture of hexane/ether/acetic acid (14:6:0.5 ml) to generate the chromatogram. Plates were dried in air (15 min), immersed in a solution of phosphoric acid/33% acetic acid/sulphuric acid/0.5% copper sulphate (5:5:0.5:90 ml) for 30 s, and after drying with warm air for 1 min (to prevent spotting), plates were dried completely at room temperature before baking at 110-115°C for 15 min. The chromatogram was scanned immediately after removal from the oven, and the amounts per different lipid fraction were then estimated from the peak of the greyscale intensity of each lipid band using ImageJ software (RSB, Bethesda, MD). Lipid ratios of each replicate were calculated as the peak greyscale intensity for storage lipids over the structural peak greyscale intensity. Lipid ratios were reported as a means ± standard error (SE) (n = 5).

2.3.4 Statistical analysis

A three-factor PERMANOVA was performed in PRIMER Version 6 (PRIMER-E, Plymouth, UK). This technique was chosen because of its robustness when data do not meet the assumptions of homogeneity of variance and normality required for ANOVA (Anderson 2001; Anderson et al. 2008). PERMANOVA was based on Euclidean distances of protein concentration, RNA/DNA ratio, lipid ratio, chl *a* per cm^2 and per cell, zooxanthellae density (all log-transformed) to test for differences between species, seasons and stations with regard to these health indices. Type I (sequential) sum of squares was used with permutation of residuals under a reduced model (9,999 permutations). Log transformation was done based on the results of a draftsman plot as well as Grubbs' test to reduce outliers and make data more continuous. After log-transformation, outliers (max. n = 2) were excluded from statistical tests. Pair-wise post-hoc tests were performed when significant differences occurred. Furthermore, non-metric multidimensional scaling as ordination of health indices (RNA/DNA ratio, protein concentration, lipid ratio, chl *a* and zooxanthellae density per surface area) was done in relation to species, season and station. Analyses were performed on Euclidian dissimilarities of log-transformed data. To determine correlations between health indices, the coefficient of determination R^2 was tested with DISTLM (Primer 6) based also on Euclidean dissimilarity matrix after log-transformation (with selection criteria all specified).

2.4 Results

2.4.1 Differences in health indices between species, seasons and stations

The two *Acropora* species differed significantly from each other in all measured health indices (Table 2-1) with higher variation in health indices for *A. spicifera* than for *A. digitifera* (Fig. 2-2A). In addition, all coral health indices exhibited strong seasonal

variation (Table 2-1) which is reflected in the clear distinction between autumn (March and April 2010), winter (August 2010), and summer (February 2011) (Fig. 2-2B, Appendix A, Table 1). Despite significant differences in some health indices between stations throughout the year, as well as seasonally (Table 2-1), overall, there were no clear spatial patterns occurring for the measured health indices between stations (Fig. 2-2C, Appendix A, Table 2). Pair-wise tests (species x season x station) revealed that spatial differences were due to seasonal changes of spatial patterns for both *Acropora* species (Appendix A. Table 2). These results indicate that spatial differences between stations within the study area made a negligible contribution towards species-specific and seasonal variability in health indices.

Table 2-1 Results of three-factorial PERMANOVAs for protein concentration, RNA/DNA ratio, lipid ratio, zooxanthellae density (ZD), chlorophyll *a* concentration (chl *a*) per surface area and per cell, comparing two species (*A. digitifera* and *A. spicifera*), four sampling periods (March and April 2010 (autumn), August 2010 (winter) and February 2011 (summer)) at six different stations at Sandy Bay, Ningaloo Reef. Effects tested are species (Sp), season (Se) and Station (St). Significant results printed in bold.

Variable	Effect	Df	SS	MS	Pseudo-F	*P*
Protein ($mg\ \mu gDNA^{-1}$)	Sp	1	4.1308	4.1308	41.70	**0.000**
	Se	3	22.1580	7.3859	74.56	**0.000**
	St	5	0.8257	0.1651	1.67	0.141
	Sp x Se	3	1.3275	0.4425	4.47	**0.005**
	Sp x St	5	0.6547	0.1309	1.32	0.251
	Se x St	14	2.4068	0.1719	1.74	0.052
	Sp x Se x St	13	1.7554	0.1350	1.36	0.185
RNA/DNA ratio	Sp	1	11.5950	11.5950	77.16	**0.000**
	Se	3	9.3512	3.1171	20.74	**0.000**
	St	5	2.2191	0.4438	2.95	**0.015**
	Sp x Se	3	0.2316	0.0772	0.51	0.676
	Sp x St	5	0.3619	0.0724	0.48	0.795
	Se x St	14	5.6721	0.4052	2.70	**0.001**
	Sp x Se x St	13	2.0320	0.1563	1.04	0.413
Lipid ratio	Sp	1	0.9578	0.9578	72.24	**0.000**
	Se	3	0.2328	0.0776	5.85	**0.001**
	St	5	0.1348	0.0270	2.03	0.074
	Sp x Se	3	0.3099	0.1033	7.79	**0.000**

	Sp x St	5	0.2284	0.0457	3.45	**0.006**
	Se x St	15	0.5107	0.0340	2.57	**0.001**
	Sp x Se x St	14	0.1370	0.0098	0.74	0.738
ZD (cells cm^{-2})	Sp	1	7.6864	7.6864	65.69	**0.000**
	Se	3	7.5054	2.5018	21.38	**0.000**
	St	5	3.9202	0.7841	6.70	**0.000**
	Sp x Se	3	1.1063	0.3688	3.15	**0.027**
	Sp x St	5	0.4110	0.0822	0.70	0.627
	Se x St	15	2.4364	0.1624	1.39	0.157
	Sp x Se x St	14	0.6428	0.0459	0.39	0.975
Chl *a* (µg cm^{-2})	Sp	1	0.4060	0.4060	5.49	**0.020**
	Se	3	30.9400	10.3130	139.45	**0.000**
	St	5	2.0623	0.4125	5.58	**0.000**
	Sp x Se	3	0.6598	0.2199	2.97	**0.030**
	Sp x St	5	0.6873	0.1375	1.86	0.101
	Se x St	15	5.5499	0.3700	5.00	**0.000**
	Sp x Se x St	14	1.0046	0.0718	0.97	0.481
Chl *a* (µg $cell^{-1}$)	Sp	1	2.9217×10^{-12}	2.9173×10^{-12}	22.05	**0.000**
	Se	3	28.6400×10^{-12}	9.5468×10^{-12}	72.14	**0.000**
	St	5	2.8680×10^{-12}	0.5736×10^{-12}	4.33	**0.001**
	Sp x Se	3	0.0774×10^{-12}	0.0258×10^{-12}	0.20	0.903
	Sp x St	5	1.1459×10^{-12}	0.2292×10^{-12}	1.73	0.126
	Se x St	15	7.8853×10^{-12}	0.5257×10^{-12}	3.97	**0.000**
	Sp x Se x St	14	1.1257×10^{-12}	0.0804×10^{-12}	0.61	0.852

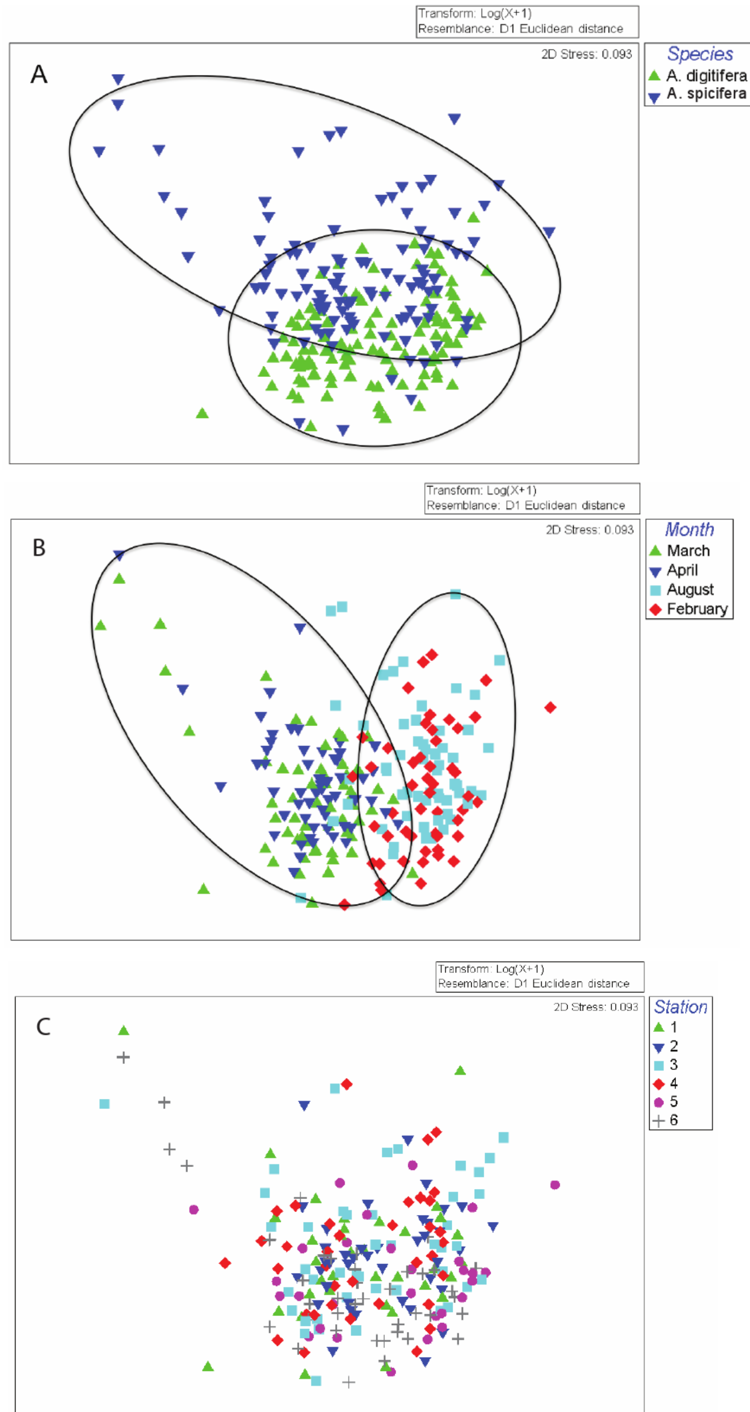


Figure 2-2: Non-metric multidimensional scaling by ordination of health indices (protein concentration (mg µgDNA^{-1}), RNA/DNA ratio, lipid ratio, zooxanthellae density (cells cm^{-2}), chlorophyll *a* (µg cm^{-2})) for (A) two species (*A. digitifera* and *A. spicifera*), (B) four different sampling periods (March and April 2010 (autumn), August 2010 (winter) and February 2011(summer)), (C) six stations at Sandy Bay, Ningaloo Reef. Analyses were performed on Euclidean dissimilarities of log-transformed data.

2.4.2 Seasonal variability of health indices

Due to species-specific variations in health indices, seasonal variability of health indices was investigated separately for *A. digitifera* and *A. spicifera*.

Protein concentrations were up to fivefold higher in *A. spicifera* then in *A. digitifera* throughout all seasons (Fig. 2-3). The protein concentration observed in *A. digitifera* differed significantly between each of the seasons (all $P < 0.001$). Concentrations increased throughout autumn during the spawning event, declined towards winter and then increased slightly over summer but were still relatively low (Fig. 2-3). In contrast, the relatively high protein concentrations for *A. spicifera* did not change significantly before, during or immediately after the autumn coral spawning ($P > 0.05$) but protein concentrations dropped significantly in winter and remained low throughout the early summer (Fig. 2-3, Appendix A Table 1).

Overall, RNA/DNA ratios displayed a decreasing trend from the start of the study (March 2010) to the end of the study (February 2011) for both species, with up to three times the values for *A. spicifera* then for *A. digitifera*. However, for *A. digitifera*, RNA/DNA ratios did not vary throughout the spawning ($P > 0.05$), but decreased towards winter and summer. For *A. spicifera* a more complex pattern was observed with similar RNA/DNA ratios before and after spawning, comparable values between after spawning and winter as well as between winter and summer (all $P > 0.05$ for consecutive pairs of data) (Fig. 2-3).

Parameters indicative of photosynthetic activity (zooxanthellae density, chl *a*) showed an opposite trend to RNA/DNA and protein concentration, with both indices increasing from the start to the end of the study (Fig. 2-3). Zooxanthellae densities were higher for *A. spicifera*, while chl *a* per cell showed higher values for *A. digitifera* (Fig. 2-3). For *A. digitifera,* no significant difference was observed in zooxanthellae density

before and after spawning ($P > 0.05$). Highest zooxanthellae densities occurred in winter and declined significantly to summer ($P < 0.05$). In contrast, zooxanthellae densities for *A. spicifera* were higher in winter and summer than during the spawning period ($P < 0.05$) (Fig. 2-3). Chl *a* concentrations in *A. digitifera* ($cell^{-1}$ and surface $area^{-1}$) exhibited similarly high values in winter and summer ($P > 0.05$), while for *A. spicifera*, this patterns only occurred for chl *a* cm^{-2}. Similar chl *a* concentrations ($cell^{-1}$) for *A. spicifera* occurred before and after the coral spawning event, with highest chl *a* concentrations ($cell^{-1}$) occurring in summer compared with winter ($P < 0.05$) (Fig. 2-3).

Lipid ratios exhibited completely different patterns for the two species with higher values for *A. digitifera* than *A. spicifera*. For *A. digitifera*, similarly high lipid ratios occurred before and after coral spawning ($P > 0.05$), followed by a significant decline between autumn and winter ($P < 0.05$) and similarly lower values between winter and summer (Fig. 2-3). For *A. spicifera*, lipid ratios appeared stable throughout the year ($P > 0.05$) (Fig. 2-3).

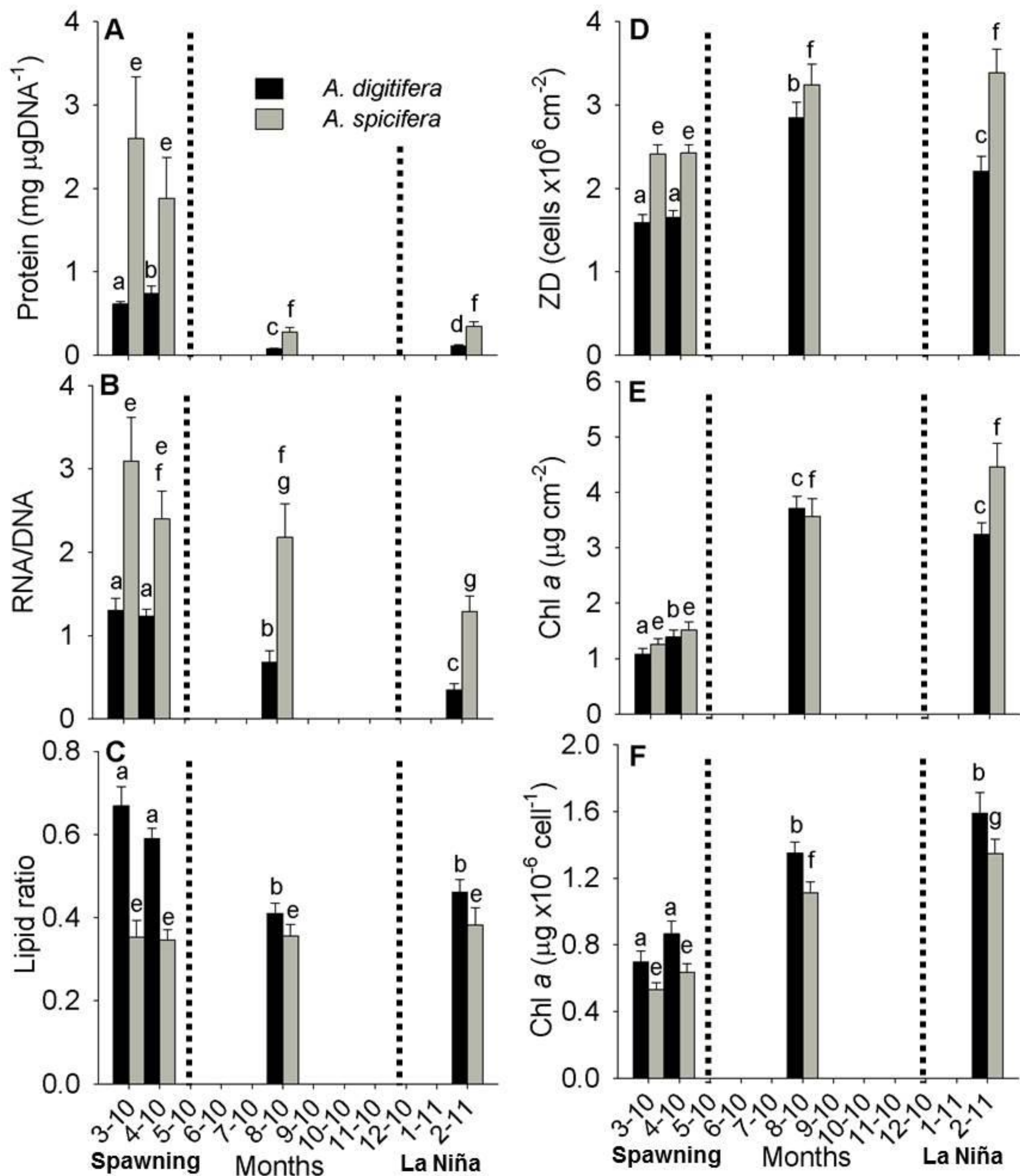


Figure 2-3: Health indices for *A. digitifera* and *A. spicifera* for four different sampling periods at Sandy Bay, Ningaloo Reef. Values are means ± standard error, n = 27 – 37. Same letters indicate no significant difference between seasons for *A. digitifera* (a, b, c, d) and *A. spicifera* (e, f, g, h) separately based on pair-wise analysis (Euclidean distance after log-transformation) of PERMANOVA.

2.4.3 Relationships between health indices for *A. digitifera* and *A. spicifera*

Linear relationships between each of the health indices for *A. digitifera* and *A. spicifera* were determined via linear regression (Table 2-2). Despite some similarities in relationships between health indices for each coral species, overall, more health indices for *A. digitifera* exhibit more significant linear relationships (both positive and negative) than *A. spicifera* (Table 2-2). Protein and RNA/DNA ratios were positively correlated for *A. digitifera* ($R^2 = 0.30$, $F = 48.57$, $P < 0.001$) as well as *A. spicifera* ($R^2 = 0.35$, $F = 60.92$, $P < 0.001$). The negative correlations for both *A. digitifera* and *A. spicifera* occurred for protein and chl *a* (surface area^{-1}) ($R^2 = 0.33$, $F = 57.41$, $P < 0.001$; $R^2 = 0.14$, $F = 17.83$, $P < 0.001$; respectively), as well as for protein and chl *a* cell^{-1} ($R^2 = 0.20$, $F = 29.86$, $P < 0.001$; $R^2 = 0.10$, $F = 12.97$, $P < 0.001$) and protein and zooxanthellae density ($R^2 = 0.11$, $F = 14.12$, $P < 0.001$; $R^2 = 0.04$, $F = 4.96$, $P < 0.05$) and indicate that with increased autotrophic indicators, protein synthesis decreases. Autotrophic indices showed positive relationships, with chl *a* (surface area^{-1}) correlated to zooxanthellae density and chl *a* (µg cell^{-1}), for both *A. digitifera* ($R^2 = 0.40$, $F = 78.43$, $P < 0.001$; $R^2 = 0.51$, $F = 121.54$, $P < 0.001$) and *A. spicifera* ($R^2 = 0.39$, $F = 69.36$, $P < 0.001$; $R^2 = 0.63$, $F = 184.85$, $P < 0.001$).

Lipid ratios showed no significant correlation with any other health indices for *A. spicifera*, while for *A. digitifera* lipid ratios were positively correlated with protein ($R^2 = 0.19$, $F = 25.98$, $P < 0.001$) and negatively with chl *a* surface area^{-1} ($R^2 = 0.17$, $F = 23.84$, $P < 0.001$), chl *a* cell^{-1} ($R^2 = 0.11$, $F = 13.99$, $P < 0.001$) and zooxanthellae density ($R^2 = 0.06$, $F = 7.33$, $P < 0.001$). For *A. digitifera*, RNA/DNA ratio was negatively correlated with chl *a* (cm^{-2}: $R^2 = 0.14$, $F = 18.95$, $P < 0.001$; cell^{-1}: $R^2 = 0.10$, $F = 11.87$, $P < 0.001$) and zooxanthellae density ($R^2 = 0.06$, $F = 7.33$, $P < 0.05$).

Table 2-2: Linear correlations provided as R^2 values, between health indices (protein concentration, RNA/DNA ratio, lipid ratio, zooxanthellae density (ZD) and chlorophyll *a* (chl *a*) for *A. digitifera* and *A. spicifera* at Sandy Bay, Ningaloo Reef. Tests were performed with DISTLM (Primer 6) based on Euclidean dissimilarity matrix after log-transformation (with selection criteria all specified). Grey shading indicates significant negative relationships and bold letters significant positive relationships (* P < 0.05; ** P < 0.01; *** P < 0.001).

A. digitifera	**RNA/DNA**	**Protein (mg/µgDNA)**	**Lipid ratio**	**ZD (cells/cm^2)**	**Chl α (µg/cell)**
Protein (mg µgDNA^{-1})	**0.30*** **				
Lipid ratio	0.03	**0.19*** **			
ZD (cells cm^{-2})	0.06*	0.11***	0.06**		
Chl *a* (µg cell^{-1})	0.10***	0.20***	0.11***	<0.01	
Chl *a* (µg cm^{-2})	0.14***	0.33***	0.17***	**0.40*** **	**0.51*** **
A. spicifera					
Protein (mg µgDNA^{-1})	**0.35*** **				
Lipid ratio	0.03	0.03			
ZD (cells cm^{-2})	<0.01	0.04*	<0.01		
Chl *a* (µg cell^{-1})	<0.01	0.10***	<0.01	0.01	
Chl *a* (µg cm^{-2})	<0.01	0.14***	<0.01	**0.39*** **	**0.63*** **

2.5 Discussion

2.5.1 Present findings in context with other studies

This is the first study to our knowledge that has measured autotrophic indices (zooxanthellae density and chl *a* concentration) in conjunction with metabolic indices (protein concentration, RNA/DNA ratio) and energy storage (lipid ratio) in scleractinian corals throughout a year. Our results clearly show that *A. spicifera* and *A. digitifera* display differences in their metabolic responses and energy levels over a seasonal cycle, while spatial variations within the study area are negligible. Averaged seasonal values of all health indices for both *A. digitifera* and *A. spicifera* were comparable to values reported previously. Studies observed chl *a* concentrations for different coral species from 0.14 – 5.0 x 10^{-6} µg cell^{-1} (Brown et al. 1999a; Chen et al. 2005; Hennige et al. 2009) and for *Acropora* species RNA/DNA ratios from 0.4 to 4 (Harithsa et al. 2005;

Humphrey 2009), zooxanthellae densities from 0.5 to 6.0 x 10^6 cells cm^{-2} (Fitt et al. 2000; Chen et al. 2005) and lipid ratios from 0.05 to 0.6 (Saunders et al. 2005). The slightly higher lipid ratio of 0.7 for *A. digitifera* just prior to spawning may be linked with increased energy being allocated to reproductive processes (Leuzinger et al. 2003). Protein/DNA ratios were difficult to compare to previous studies, since most other studies normalised protein concentration to surface area or organic matter which was not possible due to a different method used in our study. These results indicate that the metabolic activity and energy levels and thus coral health of corals in Ningaloo Reef are comparable to those of other coral reefs in the world and suggest that our data can be used for interpretation and management of other reef ecosystems.

2.5.2 Seasonal variability in health indices

Seasonal responses of autotrophic and metabolic indices

Seasonal variability in health indices for *A. digitifera* and *A. spicifera* observed in our study reflects a shift in both metabolic activity and energy storage throughout the year. Seasonal patterns of higher chl *a* concentration and zooxanthellae densities in winter than in autumn have been observed for a variety of coral species, including *Acropora* spp., as a consequence of photoacclimation (Fagoonee et al. 1999; Fitt et al. 2000; Ulstrup et al. 2008). Symbiont densities are usually inversely correlated with increases in temperatures and light (Stimson 1997; Fitt 2000); therefore, the higher values in our study during summer were unusual (see Fagoonee et al. 1999; Fitt et al. 2000; Ulstrup et al. 2008), which we interpret as being due to anomalous conditions during a well-documented La Niña event with record high temperatures during summer (Pearce et al. 2011). While we might have expected coral bleaching at our study site, as was observed at this time at other reefs of Western Australia (Pearce et al. 2011), zooxanthellae densities were all well above threshold values of 0.5 x 10^6 units for

bleached corals (Fagoonee et al. 1999). However, previous studies have shown that there is a high variability for what is visibly recognised as bleaching since e.g., in some cases of coral bleaching, chl *a* did not change, while other accessory pigments showed noticeable differences (Iglesias-Prieto and Trench 1997). Interestingly, low light values during this period in our study area (Appendix A, Fig. 1, Fig. 3-2) may have buffered the impact of temperature on coral bleaching (Mumby et al. 2001). High light has been shown to exacerbate the bleaching response as a secondary response when photoprotective mechanisms are overwhelmed (Jones et al. 1998). In addition, high availability of dissolved and particulate food for corals can sometimes be preferentially sequestered by the zooxanthellae, essentially a retention of photosynthate for the symbionts' own requirements (Clayton Jr. and Lasker 1984; Ferrier-Pages et al. 2003; Houlbrèque et al. 2004b; Chapter 3). Such a decrease in flux of translocated photosynthate to the coral host can leave the animal more dependent on heterotrophic feeding; in some cases corals will lower protein and lipid stores to sustain their metabolism (Rodrigues and Grottoli 2007; Borell et al. 2008; Rodrigues et al. 2008a). Thus, the low values for RNA/DNA ratios, protein concentrations and lipid ratios during summer could possibly be explained by a shift in energy allocation from supporting metabolic activities and building up energy stores towards increased symbiont growth.

Highest RNA/DNA ratios occurred during autumn and were lowest in winter, in accordance with previous studies (Buckley and Szmant 2004). Protein concentrations also followed previous study trends (Rossi et al. 2006). Seasonal changes in coral metabolism have been attributed to food availability, changes in light levels and temperature (Ferrier-Pages et al. 2003; Buckley and Szmant 2004; Rossi et al. 2006). Thus low metabolic indices in winter could be related to decreased respiration rates with low temperatures (Yamaguchi 1974; Howe and Marshall 2001), less available

photosynthate due to lower light levels (Dubinsky and Jokiel 1994) and/or utilization of energy and protein stores to keep up metabolic activities (Davies 1991). It seems that even though particulate nutrient supply to Ningaloo Reef is in general highest in winter, due to the autumn plankton bloom (Rousseaux et al. 2011), these food sources seem to be insufficient to build up energy storage based on the low lipids and protein values. Further investigations of correlations between physico-chemical-biological factors and health indices to explain also seasonal variations and are discussed in Chapter 3.

Spawning and energy allocation

The release of eggs and sperm into the water is a major event for corals each year; however, our results suggest that spawning represented only a minor perturbation in comparison to the backdrop of large seasonal changes. No significant differences for lipid ratios were observed for either *A. digitifera* or *A. spicifera* throughout the spawning event. This is in contrast to other studies showing significant decreases in lipids one day before and a week after a spawning event (Leuzinger et al. 2003). Others found neither seasonal changes of lipid levels, nor a correlation with gonadal output (Rossi and Tsounis 2007). Clearly, at the time scale over which our samples were collected (five days previous and seven days after the spawning) lipid ratios did not vary in a manner that suggests any major perturbation of corals' energy stores.

In fact, while there was a decline in lipid ratios from autumn to winter for *A. digitifera* in accordance with previous studies (Oku et al. 2003b; Leuzinger et al. 2012), lipid ratios were largely constant for *A. spicifera* throughout the year. Low lipid values during summer in *A. digitifera* are somewhat unexpected, since previous studies showed an increase in lipids (total and storage in % per tissue) throughout spring and constant values during summer (Oku et al. 2003b; Leuzinger et al. 2012), most likely linked with increased lipid biosynthesis due to oocyte development and/or temperature and light

differences (Ward 1995; Oku et al. 2003b; Leuzinger et al. 2012). We hypothesised that abnormal environmental conditions occurring during the La Niña summer affected lipid synthesis either through low light levels (Oku et al. 2003a), and/or energy allocation into growth and survival (Ward 1995) or an increased production of symbionts as a photoacclimatisation process (Clayton Jr. and Lasker 1984; Ferrier-Pages et al. 2003) instead of reproductive material. This suggests that climatic factors might have serious consequences for coral reproduction in some species; if energy is used for metabolic processes and thus energy supply to the coral host is reduced, reproduction halts (Leuzinger et al. 2012). Therefore subsequent spawning after future La Niña events is likely to affect spawning in these species. However, since we did not measure lipid ratios immediately before and after spawning during La Niña further investigation is needed.

2.5.3 Comparison between metabolic activity and energy levels of *A. spicifera* and *A. digitifera*

Acropora digitifera

In general, photosynthetic rates increase under high light, resulting in increased translocation of photosynthetic products to the coral host and thus increased coral metabolism (respiration) (Dubinsky et al. 1990; Davies 1991; Titlyanov and Titlyanova 2002). This is supported by studies showing high RNA/DNA ratios (Meesters et al. 2002; Buckley and Szmant 2004), protein concentration (Ferrier-Pages et al. 2003) and lipid levels with increased irradiance (Stimson 1987; Oku et al. 2003a). Over the seasonal study period for *A. digitifera*, symbiont densities and chl *a* concentration were both negatively correlated with RNA/DNA ratio, protein concentration and lipid ratio. We conclude that these negative correlations are a likely consequence of an increase of zooxanthellae density as well as size of photosynthetic units, and hence chl *a*

zooxanthellae^{-1} due to photoacclimatisation to lower light levels from March to August 2010 (Sheppard 2009). This is an important factor to consider when using so-called "autotrophic indices" of coral health: In fact, these indices are indicative of photoacclimation, not photosynthesis, and can in fact be negatively correlated with the latter. This might be superficially confusing if the index is used broadly as an indicator of autotrophy.

In addition, R^2 values of up to 0.33 between autotrophic indices, metabolic indices and energy levels suggest that autotrophy alone is not controlling coral metabolism, and thus most likely heterotrophic feeding is also important for both protein accumulation and lipid synthesis (Al-Moghrabi et al. 1995; Anthony and Fabricius 2000; Treignier et al. 2008). This is most likely due to the provision of essential nutrients such as nitrogen and phosphorus not commonly provided through photosynthesis (Davies 1991; Dubinsky and Jokiel 1994). Understanding the links between photosynthesis, metabolic activity and energy storage are complicated further by the fact that changes in zooxanthellae density and chl *a* concentrations depend not only on light but on symbiont clades (e.g., Rowan 1997), photoprotection status of the tissue (Titlyanov and Titlyanova 2002) and tissue thickness (e.g., Hoegh-Guldberg 1999). Our data suggest heterotrophic food supply is likely to be important in supporting metabolic activities and energy stores in *A. digitifera*.

Acropora spicifera

For *A. spicifera,* no correlation was observed between autotrophic indices and RNA/DNA or lipid ratios, suggesting that metabolic activities as well as energy stores are largely independent of autotrophic processes. Seasonal patterns of RNA/DNA revealed a superficial trend of increasing RNA/DNA ratios and protein concentrations as autotrophic indicators decreased similar to those seen for *A. digitifera*, suggesting

similar processes such as photoacclimatisation are occurring at a lower magnitude. Protein concentration and RNA/DNA ratios are known to increase with feeding rates, and reflect tissue growth (Ferrier-Pages et al. 2003; Dahlhoff 2004; Al-Sofyani and Niaz 2007). Fivefold higher RNA/DNA and protein concentrations overall in *A. spicifera* compared to *A. digitifera* may therefore reflect higher heterotrophic feeding rates and tissue growth in this species. The lack of seasonal change in lipid ratios, as well as the lack of correlation with any metabolic indices, indicates no observable significant seasonal change in the conservation of excess energy in this species, and suggests minimal seasonal shift in lipid investment for reproduction. It is possible that *A. spicifera* invests energy primarily into metabolic processes such as cell and tissue growth (Ward 1995; Yamashiro et al. 2001).

Acropora digitifera compared to *A. spicifera*

The observed species-specific differences might in fact represent morphologically-driven differences in coral growth rates and their overall energy balance (Chappell 1980; Muko et al. 2000; Todd 2008). Plate-shaped corals such as *A. spicifera* have the highest light capture rates over the corals' entire depth range, but in very shallow water, they perform worse with regards to energy acquisition than vertically oriented coral forms, due to light stress resulting in high energetic costs associated with photo-protection (Hoogenboom et al. 2008). Muko et al. (2000) showed that with decreasing light, the morphology of corals tends to flatten while with increasing light, more branches grow outward. These findings are consistent with our observation that metabolic activity and energy storage in *A. digitifera*, a caespito-corymbose shaped coral, are correlated to a larger degree with autotrophic indices than for *A. spicifera*, likely due to different abilities of these two species to utilize available light and to photoacclimatise.

2.5.4 Implications for Ningaloo Reef

Higher protein concentration and RNA/DNA ratios and thus possibly higher feeding rates in *A. spicifera* than *A. digitifera* might represent a longer-term advantage, as shown by faster recovery rates after bleaching events with high feeding rates and protein concentration (Grottoli et al. 2006). The importance of heterotrophic feeding for Ningaloo Reef has previously been suggested, since the Ningaloo Reef community relies to a large degree on particulate food including live phytoplankton (Wyatt et al. 2010; Patten et al. 2011). Predicted reduction in light intensity and current speed over the reef from climate-driven sea level rise (Taebi et al. 2011) therefore may impact heterotrophic feeding rates as well as autotrophy at Ningaloo Reef. Based on our La Niña data (showing similar scenarios as predictions for sea-level rise such as lower light levels, Chapter 3) we would expect higher autotrophic indices due to photoacclimatisation, lower metabolic activity, and lower energy storage. This critically remains to be resolved. Additionally, ENSO-related events such as the one we observed will likely affect local seawater temperature as well as nutrients through changes in the broad current patterns, thermocline depth, and marine productivity of Western Australian coasts (Feng et al. 2003; Feng et al. 2009). These changes can affect coral heterotrophic metabolism, autotrophy and, for *A. digitifera*, energy storage, impacting both health and reproductive success of Ningaloo Reef corals in the future.

Chapter 3 Which environmental factors predict seasonal variation in coral health of *Acorpora digitifera* and *Acropora spicifera* at Ningaloo Reef?

3.1 Abstract

The impact of physico-chemical-biological factors on coral cover and coral health was examined on a spatial basis for two dominant *Acropora* species, *A. digitifera* and *A. spicifera,* at Ningaloo Reef (north-western Australia) in the southeast Indian Ocean. Coral health was investigated by measuring metabolic indices (RNA/DNA ratio and protein concentration), energy levels (lipid ratio) and autotrophic indices (chlorophyll *a* (chl *a*) and zooxanthellae density) at six stations during typical seasons (austral autumn 2010 (March and April), austral winter 2010 (August)) and during an extreme La Niña event in summer 2011 (February)). These indices were correlated with 15 physico-chemical-biological factors (measured immediately following coral sampling) to identify predictors for health indices. Variations in metabolic indices (protein concentration and RNA/DNA ratio) for *A. spicifera* were mainly explained by nitrogen, temperature and zooplankton concentrations under typical conditions, while for *A. digitifera*, light as well as phytoplankton, in particular picoeukaryotes, were important, possibly due to higher energy requirement for lipid synthesis and storage in *A. digitifera*. Highest metabolic values occurred for both *Acropora* species at the intermediate temperatures 26-28°C when autotrophic indices (chl *a* and zooxanthellae density) were lowest due to photoacclimatisation. The extreme temperature during the

La Niña event resulted in a shift of feeding modes with increased importance of water column plankton concentrations for metabolic activity of *A. digitifera* and light and plankton for *A. spicifera*. Our results suggest that impacts of high sea surface temperatures during extreme events such as the La Niña may be mitigated via reduction of metabolic activity in the coral host. The high water column plankton concentrations and resulting low light levels resulted in a shift towards high symbiont densities, with lower metabolic activity and energy levels than the seasonal norm for the coral host. Overall, data suggests that physico-chemical-biological factors driving coral health indices are species-specific and change with environmental conditions. Therefore, we expect climate change to impact coral health at Ningaloo Reef and likely coral community composition.

3.2 Introduction

Coral reefs are under increasing pressure from climate change and human impacts (Hoegh-Guldberg 2007) such as e.g., hydrodynamic stresses in the form of extreme storm and wave events, sea-level rise/fall and changes in ocean currents (Dollar 1982; Wilkinson 2004; Webster et al. 2005; Kleypas 2008). In addition, episodes of coral bleaching, driven by elevated sea water temperature are predicted to increase in frequency and severity to cause up to 60% coral mortality globally within the next few decades (Hoegh-Guldberg 1999; Buddemeier et al. 2004; Anthony and Marshall 2012). Coral species that are more resilient to high temperatures and hydrodynamic stresses might therefore dominate reef communities in future years (Hughes and Connell 1999; Riegl 2003).

Ningaloo Reef, the world's largest fringing reef (Taylor and Pearce 1999), is situated on the northwest coast of Australia in the southeast Indian Ocean. The physico-chemical-biological marine environment of Ningaloo is strongly influenced by climate

variability in the Indo-Pacific Ocean (Feng et al. 2003; Holbrook et al. 2009; Weller et al. 2012). It has been predicted that climate-driven sea-level rise will affect the hydrodynamic regime of the reef, including changes in local current speeds and light intensity reaching benthic communities (Taebi et al. 2011). The hydrodynamics of Ningaloo Reef are also influenced by changes in the frequency and/or intensity of storm events due to climate-related variability, leading to changes in the broad current patterns, thermocline depth and subsequent marine productivity off the Western Australia coast (Rousseaux et al. 2011). Such large scale events would likely result in changes in seawater temperatures over the reef, as well as changes in the concentration of nutrients reaching the reef (Feng et al. 2009).

Given that seasonal and inter-annual changes in physico-chemical-biological factors have already been observed at Ningaloo Reef, including current speeds (Lowe et al. 2008; Taebi et al. 2011), light, temperature, nutrient concentrations and plankton concentrations (Patten et al. 2011; Rousseaux et al. 2011; Wyatt et al. 2012), we expect that such physico-chemical-biological changes will drive shifts in coral health indices.

Our results from Chapter 2 already indicated the overriding importance of seasonal variations in driving changes in coral health indices including metabolic activity (RNA/DNA ratio, protein concentration), autotrophic indices (zooxanthellae density, chl *a* concentration, used here to identify photoacclimation) and energy levels (lipid ratio). The two studied *Acropora* species (*A. digitifera* (Dana, 1846) and *A. spicifera* (Dana, 1846)), which are dominant species at Ningaloo Reef, showed very different patterns of seasonal variation (Chapter 2) as well as strong species-specific differences, while small-scale spatial variations in metabolism have been shown to be negligible. However, it is still unknown which physico-chemical-biological factors were the most important factors driving changes in these coral health indices over seasonal scales and how they vary due to species-specific differences. However, this knowledge is critical

for understanding how extreme events and climate-driven environmental changes will affect coral communities.

For corals to remain healthy in unfavourable conditions, they require sufficient metabolic energy to support growth and reproduction (Grottoli et al. 2006; Rodrigues and Grottoli 2007) through autotrophic (light-driven) and/or heterotrophic (active uptake of nutrients) processes (Muscatine 1990; Dubinsky and Jokiel 1994; Mills and Sebens 2004, Houlbrèque and Ferrier-Pagès 2009). Autotrophic processes depend mainly on the absorption of light and the uptake of dissolved nutrients and result in translocation of photosynthetically fixed carbon by intracellular symbionts (zooxanthellae) to their coral host (Muscatine and Porter 1977; Dubinsky and Jokiel 1994). In contrast, heterotrophic processes rely on the uptake and subsequent utilisation of plankton, particulate nutrients and dissolved organic matter by corals to support coral host and symbiont metabolic processes (Sebens et al. 1996; Anthony and Fabricius 2000; Ferrier-Pages et al. 2003, Houlbrèque and Ferrier-Pagès 2009). Whether corals predominantly receive energy through autotrophic or heterotrophic processes has been shown to be species-specific and dependent on seasonally driven changes in the physico-chemical-biological seawater environment as well as depths (Anthony and Fabricius 2000; Ribes et al. 2003, Palardy et al. 2005; Houlbreque and Ferrier-Pagès 2009). For example, a decrease in protein concentration, an indicator of metabolic activity, has been observed with fast increasing temperatures (3-4˚C per day) (Borell et al. 2008). Also total and storage lipids have been shown to decrease with extreme temperatures (Grottoli and Rodrigues 2011) but increase during normal summer seasons (Oku et al. 2003b). Increasing values with an increase in light intensity have been observed for RNA/DNA ratios (Buckley and Szmant 2004) and lipid content (Stimson 1987), and an increase in protein concentration, RNA/DNA ratio and lipid content has been observed with an increasing supply of particulate nutrients (Houlbrèque et al.

2003; Dahlhoff 2004; Borell et al. 2008). In addition, shifts in the seawater hydrodynamics can impact both autotrophy and heterotrophy (Dennison and Barnes 1988; Sebens et al. 1998; Ribes and Atkinson 2007, Houlbrèque and Ferrier-Pagès 2009). For example, changing current speeds can alter the concentration of dissolved and particulate nutrients reaching coral reefs, the boundary layer thickness of individual corals and subsequent coral uptake rates (Atkinson et al. 1994; Ribes and Atkinson 2007). However, it is not known which physico-chemical-biological factors are the main driving forces for coral metabolism and energy stores, or how they are related to autotrophic indices. To date, no study has determined how changes in the physico-chemical-biological environment may affect a variety of coral health indices simultaneously under normal as well as extreme conditions in the natural environment.

We hypothesize, that seasonal variations in physico-chemical-biological factors drive coral health, and hypothesize further that these parameters can be used to predict changes in these coral health indices under normal conditions as well as under extreme weather conditions (La Niña) at Ningaloo reef. Having identified earlier that species-specific and seasonal differences were the main drivers of coral health (Chapter 2, this study), we aimed to determine the specific physico-chemical-biological factors in the environment that were most important in driving the observed changes.

3.3 Methods

3.3.1 Study site

The study site, Sandy Bay Lagoon (Sandy Bay), is located at Ningaloo Reef, along the North-West Cape of Western Australia (22.23°S, 113.84°E). Detailed description of the study area is given in Chapter 2.

Sampling was conducted at six stations, two stations south (1, 2) and four stations north (3, 4, 5, and 6) of the channel (Fig. 2-1). Higher average long-term current speeds occur at stations to the south of the channel compared to stations to the north of the channel (Taebi et al. 2011). Three of these stations were located close to the ocean side of the reef (1, 3, and 5) and three stations were close to the lagoon (2, 4, and 6) (Fig. 2-1). The dominant coral genus at the reef crest at Sandy Bay is *Acropora* (Cassata and Collins 2008).

3.3.2 Sampling

Two different coral species; the caespito-corymbose form, *A. digitifera* and the plate form *A. spicifera* (Wallace 1999) (n=6 at each station) were tagged at each of the six stations. Coral samples were taken between 10:00 and 14:00 in autumn covering the period before coral spawning (28-30th March 2010) and after the coral spawning event (15-17th April 2010), in winter (18 - 27th August 2010) and summer (12-15th February 2011). February 2011 was the peak of a strong La Niña event and oceanic conditions were warmer and more nutrient rich than in typical summers in this region (Pearce and Feng 2012). Corals were sampled while snorkelling. Three 1–5 cm long coral pieces were removed from the middle of the coral colony (tip of the branch) for each of the health indices (see below).

3.3.3 Analysis of health indices

<u>*Coral cover*</u>

Estimates of percent hard coral cover (% coral cover) at each station were determined from photographs taken along two parallel transects, of 100 m length, which were spaced 10 m apart. Photographs were taken at 5 m intervals along each 100 m

transect. A metal disc of known diameter was placed in each photograph and the time recorded to allow photo area and the corresponding tide adjusted depth to be calculated from each photograph. Percent hard coral cover was estimated for each coral category (plate corals such as *A. spicifera*, caespito-corymbose corals such as *A. digitifera* as well as all other live hard coral) in each photograph. A pixel counting technique was facilitated in *MATLAB* after visually inspecting and colouring the corals with solid, distinct colours (e.g., red, green, blue) in *Microsoft Paint*. The time each transect was conducted was combined with local tidal records to standardize all estimated depths to a 2.2 m tide.

Zooxanthellae density and chlorophyll a

Coral samples (~3–5 cm long) for the analysis of zooxanthellae density (per cm^2) and chlorophyll *a* (chl *a*) concentration in the tissue (µg cm^{-2}) were stored at -20°C prior to analysis. Methods for zooxanthellae density and chl *a* in coral tissue followed those in Chapter 2, which were adapted from Siebeck et al. (2006). Coral surface area was measured using the paraffin wax method of Stimson and Kinzie (1991).

Chl *a* concentration was determined following the methods of Jeffrey and Humphrey (1975) as described in Chapter 2. In general, these indices were found to be good markers of photacclimation, which is often inversely correlated with photosynthetic rates (Davies 1991).

RNA/DNA and protein analysis

Samples (1 cm long) for RNA/DNA and protein analysis were stored at -80°C prior to analysis. Methods for analysis of RNA/DNA followed Humphrey (2009) and are detailed in Chapter 2.

For protein analysis, a standard detergent-compatible colorimetric assay protein kit was used (Lowry et al. 1951).

Lipid ratio

Coral pieces (~3 cm long) for lipid ratio analysis were stored at -20°C until freeze-dried for 24 hours in the laboratory. Methods for lipid analysis followed Saunders et al. (2005) and are described in detail in Chapter 2.

3.3.4 Analysis of physico-chemical-biological factors

Physico-chemical-biological factors known to be important for coral health were sampled immediately following coral sampling (ca. 0.5 m below water surface). These included light, temperature, current speed, nutrients (nitrogen, phosphate, and silicate), and phytoplankton and zooplankton concentrations.

Physical factors

Light, measured as photosynthetically active radiation (PAR), and temperature were measured using Hobo Pendant Data Logger deployed at each station at the benthos over the sampling period for 2 weeks.

Short-term current speed was estimated using drifters. Two crucifix design drifters (Wyatt et al. 2010) containing a GPS were simultaneously deployed for approximately 10 minutes and the GPS location and time of their deployment and collection recorded. The distance and time of each deployment allowed a simple estimate of surface flow speed. Long-term estimates (≈ months) of current speed were based on data from previous investigation at the site. Data collected by Lowe et al. (2008) and Taebi et al. (2011) provided average current speed near the reef crest for stations 1, 3 and 5 and were based on hourly current measurements of NORTEK Vector ADVs deployed 0.5 m

above the sea floor. Estimates of mean current speed at stations 2, 4 and 6 were derived by applying conservation of mass for flow near the reef crest (station 1, 3, 5), based on the ratio of average depths at the sites (estimated by analysis of photo-quadrate size). The technique provides very conservative estimates of mean current speed, assuming flow is direct between the two sites and that vertical flow profiles at each are comparable.

Chemical and biological factors in the water column

Water samples were taken for the analysis of dissolved nutrients (Nitrate + Nitrite (NO_x), ammonium (NH_4), phosphate (PO_4) and silicate (Si)), total nitrogen (TN) chlorophyll a (chl *a*) (chl *a* < 2 µm, chl *a* < 5 µm and Total chl *a*), and picoplankton (*Synechoccocus*, *Prochloroccocus* and picoeukaryotes). Chl *a* < 5 µm will be defined as large phytoplankton.

For the analysis of dissolved NO_x, NH_4, PO_4 and Si, water samples (40 ml) were filtered through 0.45 µm filters and stored at -20 °C, before flow injection analysis (FIA) with detection by absorbance at specific wavelengths for Si (QuikChem Method 31-114-27-1-D), NO_x (Quickchem Method 31-107-04-1-A) and PO_4 (QuikChem Method 31-115-01-1-G) (Instruments 2000). NH_4 was measured by fluorescence (GlobalFIA high sensitivity gas diffusion unit- HPMSD, Shimadzu RF-10Axl Fluorescence detector) (Watson et al. 2004). For TN, unfiltered water samples (50 ml) were stored at -20 °C until analysis. TN was determined from autoclave digests with potassium persulphate (Lachat Quick-Chem 8500 Automated Flow Injection Analyser) (Valderrama 1981).

Seawater samples (1 l) for the Total chl *a* size fraction were filtered onto Whatman GF/F filters (norm size 0.7 µm); seawater samples (0.5 l), for chl *a* < 2 µm fraction were filtered onto 2 µm nuclepore polycarbonate membrane filters and seawater

samples (2 l) for chl *a* < 5 µm fraction were filtered onto 5 µm nitex mesh. The filters were stored at -20 °C in the dark until fluorometric analysis of duplicate 90% acetone extracts was conducted (Parsons et al. 1984). Chl *a* < 2 µm fraction was calculated by subtracting chl *a* > 2 µm from Total chl *a* and chl *a* 2-5 µm by subtracting chl *a* > 5 µm from chl *a* > 2 µm.

To determine concentrations of autotrophic picoplankton groups (*Synechoccocus*, *Prochlorococcus* and picoeukaryotes), duplicate 1.5 ml seawater samples were fixed with gluteraldehyde (0.5% final concentration) for 10 minutes and then snap frozen in liquid nitrogen. Samples were analysed using flow cytometry (Patten et al. 2011); using a FACSCANTO II (Becton-Dickinson) flow cytometer fitted with a 488 nm laser on high throughput mode at a flow rate of 60 µl min^{-1} for 100 s.

Zooplankton were sampled with a 90 µm plankton net towed for 10 min at 2.5 kn h^{-1} for 50 m at each station and preserved with formaldehyde (ca. 5%). Dry weight analyses were carried out for zooplankton size fractions of 100 to 500 µm and 500 to 1000 µm. Containers were weighted, wet samples added after cleaning samples of salt with deionised water (30 s) and dried in an oven (60°C) for 24 hours. Afterward samples were cooled in a desiccator and weighted for subsequent calculation of dry weight (DW) (Harris 2000).

3.3.5 Statistical analysis

Correlations between five coral health indices (RNA/DNA ratio, protein concentration, lipid ratio, chl *a* concentration and zooxanthellae density) and 15 physico-chemical-biological factors (light, temperature, current speed (drifter), water column Chl *a* concentration (chl *a* < 2µm, chl *a* 2-5 µm, chl *a* >5µm), picoplankton (*Synechoccocus*, *Prochlorochoccus*, picoeukaryotes), zooplankton dry weight (100-500µm and 500-1000µm) and dissolved nutrients (NO_x, NH_4, Si, PO_4)) were tested with

DISTLM (Primer 6) based on Bayesian information criterion (BIC) (forward procedure, 9999 permutations) (Anderson et al. 2008). Data for health indices were log-transformed based on results of draftmans plot and Grubb's test to reduce outliers and make data more continuous. Physico-chemical-biological data were square root transformed and DISTLM was performed based on Euclidean dissimilarity matrix. This test was done 1) for all data excluding the La Niña event as well as 2) only including the spawning event (autumn) and 3) all data including the La Niña event (in this case data were square root transformed except chl *a* concentration (log-transformed) and zooplankton concentrations (forth root transformed)) to determine which physico-chemical-biological factors drive coral health in the presence and absence of extreme events. Seasonal differences in physico-chemical-biological factors were tested with PERMANOVA after transformation based on Euclidean distance using 9999 permutations with Type III (partial) sum of square and permutation of unrestricted permutation of raw data (Anderson et al. 2008). In addition, univariate relationships were determined for PAR and temperature with each health indices since temperature exhibited quadratic function with all health indices (except lipid ratios of *A. spicifera*) (Sigma Plot). To determine correlations between % coral cover and long-term current speed the coefficient of determination R^2 was tested with DISTLM (Primer 6) based on Euclidian dissimilarity matrix (9999 permutations, with selection criteria all specified) (Anderson et al. 2008).

3.4 Results

3.4.1 Relationships between coral cover and current speed

The mean % coral cover of *A. spicifera*, *A. digitifera* and other live hard corals at the six stations at Ningaloo Reef are displayed in Figure 3-1. Total % coral cover

ranged between 4.9% and 39.6% across the six stations, with lowest and highest % total coral cover occurring at Station 1 and Station 2 respectively and with similar coral cover occurring at Station 3, 4 and 5. *A. spicifera* dominated coral cover at each of the stations, averaging 56.9% of the total % coral cover (ranging between 21.1 and 78.3%). At station 1, *A. spicifera* and *A. digitifera* displayed similar cover (ca. 2%), disproportionate to other near-reef crest sites (Station 3 and 5) where *A. spicifera* displayed ca. 3-fold greater cover then *A. digitifera* (Fig. 3-1).

Long-term averaged current speeds ranged between 8.4 and 20.3 cm s^{-1}, with highest and lowest current speeds occurring at Station 2 and 4 respectively (Fig. 3-1). Overall, % coral cover was not correlated with long-term current speed ($p > 0.1$). However, the relationships were confounded by comparatively low coral cover occurring at Station 1, corresponding with high average (long-term) flow speed but also due to a high density of *Drupella* spp. found at this station. The removal of this site from the data set improved the correlations considerably with high R^2 values for relationship between long-term current speed and plate *Acropora* ($R^2 = 0.76$) and total % coral cover ($R^2 = 0.69$), however correlations were still not significant probably due to small sample size (n=5).

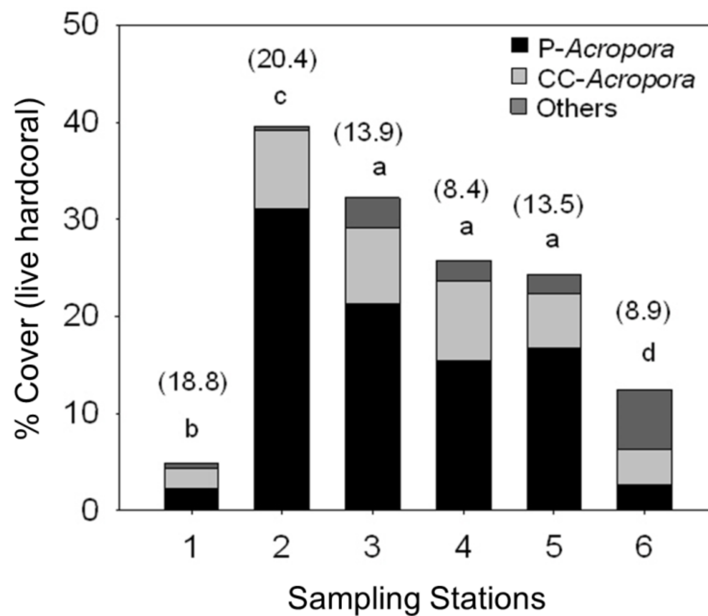


Figure 3-1: Percent coral cover of plate *Acropora*, caespito-corymbose *Acropora* and other coral species at Ningaloo reef. Six stations were sampled in autumn (March - April 2010). Numbers in brackets represent long-term average current speed per station and same letters indicate no significant difference in total coral coverage per station.

3.4.2 Seasonal changes in physico-chemical-biological factors

PAR and temperature both decreased from March/April 2010 (autumn) to August 2010 (winter) from 70 to 30 µmol m^{-2} d^{-1} and 27 to 23 °C, while current speed increased from 14 to 18 cm s^{-1}. NO_x as well as NH_4 showed a decrease from March/April 2010 to August 2010, while TN, water column chl *a*, *Synechoccocus* and picoeukaryotes showed increasing concentrations (Fig. 3-2). *Prochlorococcus* dropped from 30 to 20 x 10^6 cells ml^{-1} from autumn to winter, while zooplankton concentration did not significantly vary between seasons.

During the study, a near-record La Niña event occurred during the austral summer of 2010-2011 (sampling period in February 2011). At this time, seawater

temperature reached a maximum of 30.8°C, while current speed and PAR were variable, ranging between 5.8 and 31.5 cm s^{-1} and between 14.8 and 70.2 µmol photons m^{-2} d^{-1} respectively, with these values most comparable to the winter situation in August 2010 (Fig. 3-2). Significant differences for temperature occurred between February 2011 and March/April 2010 and February 2011 and August 2010 ($F_{3, 23} = 134.3$, $P < 0.001$, pair-wise comparison $P < 0.05$) and for PAR between February 2011 and March/April 2010 ($F_{3, 23} = 4.61$, $P < 0.05$, pair-wise comparison $P < 0.05$).

Water column chl *a* (all size fractions) peaked during the La Niña event, with concentrations two times higher than during any other sampling period, reaching a maximum of 0.10 µg l^{-1} for both chl *a* 2-5 µm and chl *a* > 5 µm, and 0.15 µg l^{-1} for chl *a* < 2 µm. However, only chl *a* in the 2-5 µm size fraction differed significantly to March/April 2010 and August 2010 ($F_{3, 23} = 13.9$, $P < 0.001$, pair-wise comparison $p < 0.05$). The trend for picoplankton was more complex and varied for each group. *Synechococcus* concentrations were five times higher in February 2011 than during any other month ($F_{3, 23} = 19.03$, $P < 0.001$, pair-wise comparison $P < 0.05$). Picoeukaryote concentrations peaked in August 2010 at 1.13×10^6 cells ml^{-1}, however were comparably high in February 2011 ($F_{3, 23} = 7.01$, pair-wise comparison $P > 0.05$), while *Prochloroccocus* concentrations were lowest during the summer La Niña event but did not show significant seasonal differences ($P > 0.05$). The same trend as observed for chl *a* concentration and *Synechococcus* occurred for zooplankton concentrations three times higher during the La Niña summer. Highest zooplankton concentrations occurred in the 500-1000 µm fraction in February 2011 and differed significantly to those in March/April and August 2010 ($F_{3, 22} = 3.56$, $P < 0.05$, pair-wise comparison $p < 0.05$). Zooplankton in the 100-500 µm size fraction also showed highest values in summer however significant differences only occurred between February 2011 and August 2010 ($F_{3,23} = 3.49$, $P < 0.05$, pair-wise comparison $P > 0.05$) (Fig. 3-2).

Nutrient concentrations during the summer La Niña event were similar to March/April 2010, with relatively high NO_x as well as NH_4 concentrations that differed significantly from concentrations in August 2010 (NO_x: $F_{3, 23} = 5.16$, $P < 0.01$; NH_4: $F_{3, 23} = 5.25$, $P < 0.01$; pair-wise comparison $P < 0.05$). Lower concentrations of PO_4 were observed in February 2011 compared to March/April and August 2010 ($F_{3, 23} = 4.12$, $P < 0.05$; pair-wise comparison $P < 0.05$) (Fig. 3-2). TN showed relatively low values in February 2011 compared to those in March/April 2010 with significantly different values from March 2010 ($F_{3, 22} = 7.17$, $P < 0.001$; pair-wise comparison $P < 0.05$) (Fig. 3-2).

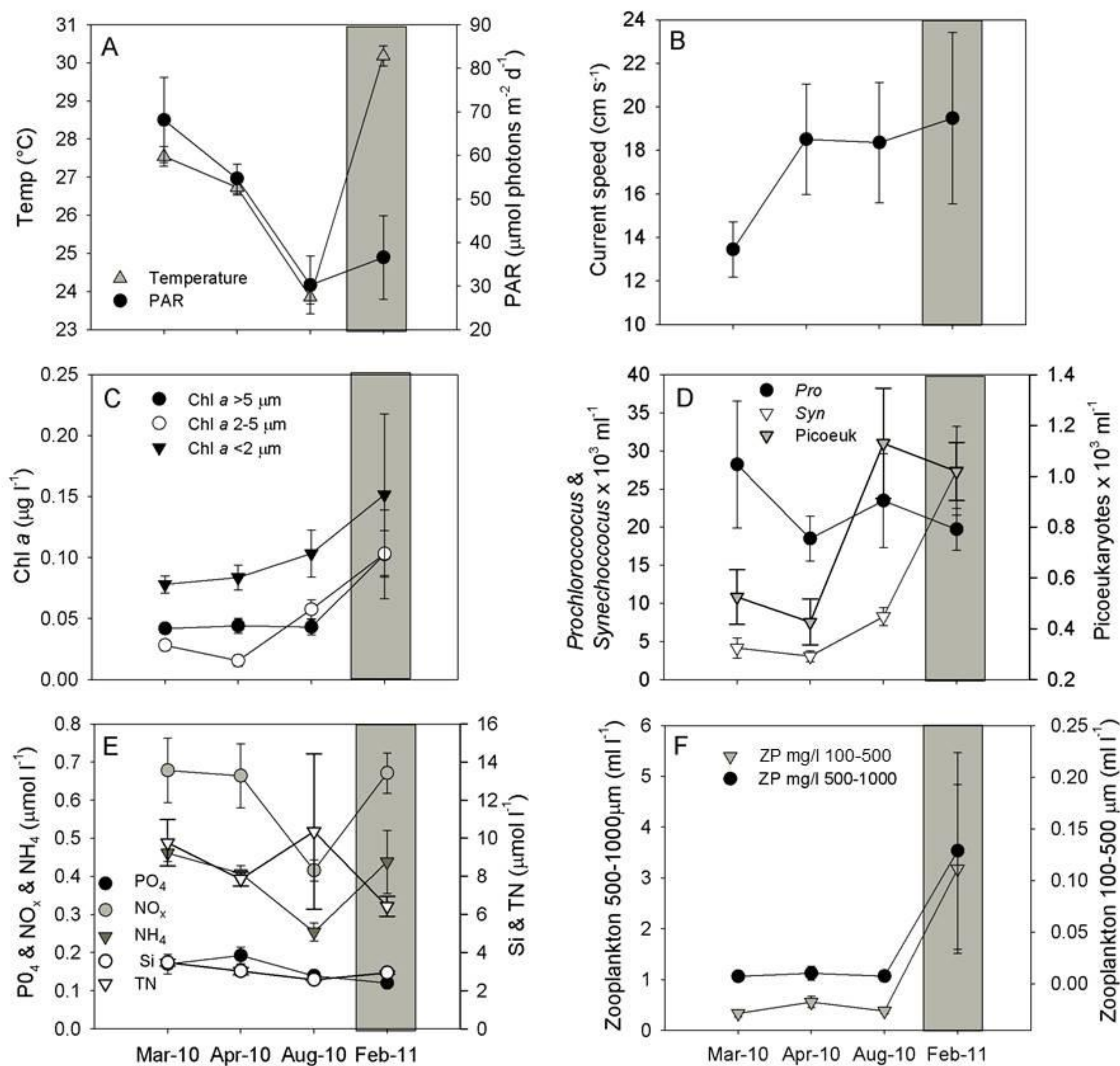


Figure 3-2: Patterns in physico-chemical-biological factors at Ningaloo Reef throughout seasons. (A) Temperature (°C) and light (PAR), (B) current speed, (C) chlorophyll *a* (chl *a*), (D) picoplankton concentration (*Prochloroccocus*, *Synechoccocus* and picoeukaryotes), (E) nutrients (PO_4, NO_x, NH_4, Si and TN) and (F) zooplankton concentrations at six stations at Ningaloo Reef during autumn (March/April) and winter (August 2010) and during the La Niña event in summer (February 2011) (grey column). Data are means ± standard error (n=6).

3.4.3 Relationships between coral health indices and physico-chemical-biological factors

To determine which physico-chemical-biological factors were most important for metabolic activity, autotrophic indices and energy stores for *A. digitifera* and *A. spicifera*, multivariate analysis was performed across seasons that showed 1) typical seasonal patterns in physico-chemical-biological factors (March/April 2010 and August

2010) (Table 3-1A, 3-2A); 2) during March/April 2010 alone (during the spawning event), since metabolic activity was highest during this time (Chapter 2) (Table 3-1B, 3-2B) and 3) during all sampled seasons including the La Niña event (autumn 2010, winter 2010 and summer 2011 (La Niña)) (Table 3-1C, 3-2C). Relationships between physico-chemical-biological factors with each measured coral health indices are described below.

In general, picoplankton and chl *a* concentration in the water column were negatively correlated with protein concentration and lipid ratio, while temperature, PAR, dissolved nutrients (with exception of TN) and zooplankton were positively correlated with protein concentration and lipid ratio for both *Acropora* species. In contrast, chl *a* in coral tissue and zooxanthellae density exhibited negative correlations with temperature, PAR and dissolved nutrients but positive correlations with plankton (picoplankton, chl *a* and zooplankton with the exception of water column chl *a* > 5 µm) for both *Acropora* species. Correlations between RNA/DNA ratio and PAR, temperature chl *a* > 5 µm and zooplankton were positive (Table 3-1A/B and Table 3-2A/B).

<u>*During typical seasonal patterns (autumn and winter 2010)*</u>

For *A. digitifera*, water column chl *a* in the 2-5 µm size fraction explained 54.9% of the protein concentration variability. Temperature, PO_4 and *Prochlorococcus* explained a further 16.8% of the variability in protein concentration (Table 3-1A). In contrast for *A. spicifera*, 44.7% of the variability in protein concentration was best explained by temperature (35.7%), NH_4 (4.5%) concentration and zooplankton in the 500-1000 µm size fraction (4.6%) (Table 3-2A).

For *A. digitifera*, RNA/DNA ratios exhibited strongest correlations with PAR (31.5% of the variability) and to a lesser extent, with chl *a* > 5 µm (5.1% of the

variability). For *A. spicifera*, temperature best explained RNA/DNA ratio variability (14.3%) (Table 3-1A).

For both *A. digitifera* and *A. spicifera*, lipid ratios were correlated with Si concentrations in the water column, explaining 9.5% and 10.9% of the variability respectively. For *A. digitifera*, 24.7% of the variability in lipid ratios was best explained by picoeukaryote (Table 3-1A) while for *A. spicifera* 6.5% of protein concentration variability was explained by TN (Table 3-2A).

For *A. digitifera*, picoeukaryote concentration in the water column was responsible for 48% of the variability of tissue chl *a*, with temperature and PAR then explaining a further 18.1% of this variability (Table 3-1A). For *A. spicifera*, temperature showed the highest correlation with tissue chl *a*, explaining 39.6% of the variance, with picoeukaryotes and zooplankton in the 500-1000 µm fraction and TN explaining a further 12.1% of the variability (Table 3-2A).

For *A.digitifera*, temperature explained 30.1% of the variation in zooxanthellae density followed by picoeukaryotes (4.0%) (Table 3-1A), while zooxanthellae density in *A. spicifera* was most strongly correlated with NH_4 (7.7%) (Table 3-2A).

During the coral spawning event (autumn 2010)

During autumn 2010 over the spawning period when metabolic activity was at its maximum, water column chl *a* in the 2-5 µm size fraction was still the best predictor of protein concentration for *A. digitifera* (23.1% of the variability), with temperature and NO_x explaining a further 11.6% (Table 3-1B). At this same time for *A. spicifera* NH_4 was the main predictor of protein concentration (8.8%) followed by current speed (6.6%) (Table 3-2B).

PAR remained the best predictor of RNA/DNA ratio for *A. digitifera* (10.9%) during autumn (Table 3-1B), while for *A. spicifera* no significant correlation was found with any of the measured physico-chemical-biological factors (Table 3-2B).

For lipid ratios, the main predictors remained the same as during typical seasons. However, for *A. digitifera Prochloroccocus* instead of picoeukaryotes showed the strongest correlation with lipid ratio (Table 3-1B).

Water column chl *a* < 2 µm was the main predictor of tissue chl *a* for *A. digitifera*, explaining 36.8% of the variability, while water column chl *a* > 5 µm explained a further 6.3 % of the variability (Table 3-1B). For *A. spicifera*, water column chl *a* in the size fractions 2-5 µm exhibited the strongest correlation with tissue chl *a* (18.5%) followed by NH_4 (13.3%) (Table 3-2B).

During autumn 2010, temperatures remained the best predictor of zooxanthellae density in *A. digitifera* (Table 3-1B), while NO_x were responsible for 9.1% in the variation in zooxanthellae density in *A. spicifera* (Table 3-2B).

During all sampled seasons including the summer La Niña event (autumn and winter 2010 and summer 2011)

During the La Niña event, the physico-chemical-biological water column environment was very different to that during typical summers (Pearce and Feng 2012). These differences in the physico-chemical-biological environment had a significant impact on the measured coral health indices, with overall lower metabolic indicators and lipid stores and higher zooxanthellae densities and chl *a* concentrations in the tissue during the La Niña event then expected during typical summer (Chapter 2).

For *A. digitifera*, chl *a* in the 2-5 µm size fraction was the best predictor of protein concentration, explaining 49.5% of variability. Zooplankton in the 100-500 size fraction and Si explained a further 17.5% of the variability (Table 3-1C). For *A.*

spicifera variability in protein concentration was best predicted by picoeukaryote concentration (24.4%) followed by PAR (11.5%) and PO_4 concentration (4.8%) (Table 3-2C).

RNA/DNA ratio for *A. digitifera* showed the strongest correlations with water column chl *a* in the 2-5 µm size fraction, explaining 33.7%, with *Prochloroccocus* and picoeukaryotes the next best predictors of RNA/DNA ratio (Table 3-1C). RNA/DNA ratio for *A. spicifera* were correlated with water column chl *a* in the 2-5 µm size fraction and PAR explaining 12.6 and 5.1% of the variability, respectively (Table 3-2C).

Variation in lipid ratio for both *Acropora* species was still correlated with those same physico-chemical-biological factors whether statistical analyses were executed with or without inclusion of the La Niña event (Table 3-1C/2C).

Picoeukaryotes and PAR were still predictors for chl *a* concentration in the tissue of *A. digitifera* with 46.5% and 4.1%, respectively. Another 10.2% of the variability of chl *a* was explained by TN (Table 3-1C). Variability of chl *a* in the tissue of *A. spicifera* was most strongly correlated with *Synechoccocus* (36.5%). PAR, large phytoplankton and zooplankton in the 500-1000 µm size fraction explained another 21.2% (Table 3-2C).

For variability in zooxanthellae density, PAR and NH_4 explained 23.1 and 6.7% respectively for *A. digitifera* and picoeukaryotes explain further 3.8% (Table 3-1C). Variability of zooxanthellae density for *A. spicifera* showed strongest correlation with PAR, explaining 10.0% of the variability (Table 3-2C).

Table 3-1: Main physico-chemical-biological predictors for coral health indices (protein concentration, RNA/DNA ratio, lipid ratio, chlorophll *a* (chl *a*) concentration and zooxanthellae density (ZD) per surface area) for *Acropora digitifera* for (A) typical seasons (autumn and winter 2010) not affected by La Niña event, (B) during the coral spawning event in autumn 2010, when metabolic indices were highest and (C) all sampled seasons including the La Niña event (autumn 2010, winter 2010 and summer 2011) Tests were performed with DISTLM (based on BIC; Primer 6) Grey shading indicates significant negative relationships and no shading significant positive relationships.* P < 0.05; ** P < 0.01; *** P < 0.001. (Var = variability, Pro = *Prochlorococcus*, Picoeuk = picoeukaryotes, temp = temperature, ZP = zooplankton)

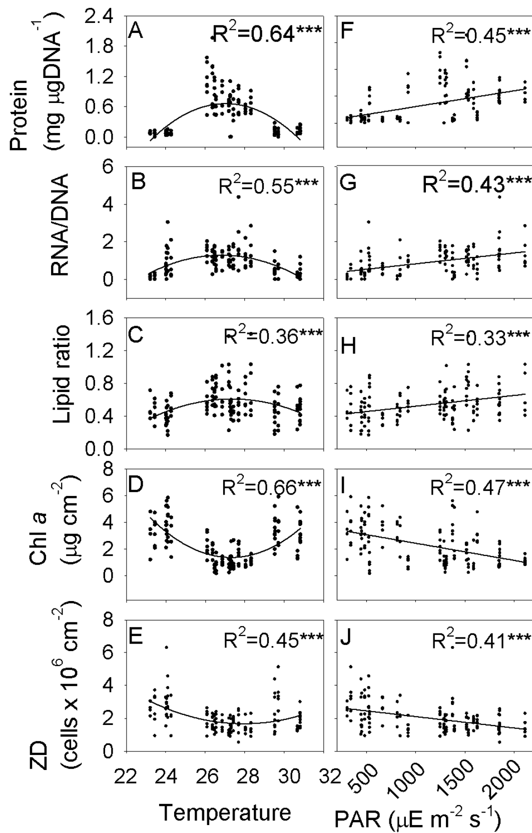
A	**Typical seasons (Autumn and Winter 2010)**				**B Spawning event (Autumn 2010)**				**C All seasons (Autumn & Winter 2010 and Summer 2011)**			
Health indices	**Predictor**	**BIC**	**Pseudo-F**	**% var**	**Predictor**	**BIC**	**Pseudo-F**	**% var**	**Predictor**	**BIC**	**Pseudo-F**	**% var**
Protein	Chl *a* 2-5 µm (µg l^{-1}) ***	-292.9	103.48	54.9	Chl *a* 2-5 µm (µg l^{-1}) ***	-229.7	18.35	23.1	Chl *a* 2-5 µm (µg l^{-1}) ***	-357.6	106.67	49.5
(mg µg^{-1}DNA)	Temp (°C) ***	-312.8	27.15	11.0	Temp (°C) *	-231.7	6.09	7.1	ZP 100-500 µm (mg l^{-1}) ***	-382.9	33.53	12.0
	PO_4 (µmol l^{-1}) ***	-319.7	11.58	4.2	$NO_3 + NO_2$ (µmol l^{-1})*	-231.8	4.11	4.5	Si (µmoll^{-1}) ***	-395.4	17.87	5.5
	Pro (cells µl^{-1}) *	-320.1	4.72	1.6								
RNA/DNA	PAR (µE m^{-2} s^{-1}) ***	-213.8	39.06	31.5	PAR (µE m^{-2} s^{-1}) **	-171.7	7.43	10.9	Chl *a* 2-5 µm (µg l^{-1}) ***	-257.9	55.48	33.7
	Chl *a* >5 µm (µg l^{-1}) *	-216.0	6.68	5.1					Pro (cells µl^{-1}) ***	-277.3	26.23	13.0
									Picoeuk (cells µl^{-1}) **	-280.6	8.00	3.7
Lipid ratio	Picoeuk (cells µl^{-1}) ***	-362.9	27.82	24.7	Pro (cells µl^{-1}) *	-255.2	6.61	9.8	Picoeuk (cells µl^{-1}) ***	-465.9	28.19	20.5
	Si (µmol l^{-1}) **	-370.1	12.05	9.5	Si (µmol l^{-1}) **	-258.5	7.50	10.0	Si (µmol l^{-1}) ***	-475.4	14.74	9.5
Chl *a* (µg cm^{-2})	Picoeuk (cells µl^{-1}) ***	-191.3	78.60	48.0	Chl *a* < 2 µm (µg l^{-1}) ***	-164.9	35.57	36.8	Picoeuk (cells µl^{-1}) ***	-241.2	94.9	46.5
in coral tissue	Temp (°C) ***	-213.8	30.56	13.9	Chl *a* >5 µm (µg l^{-1}) *	-167.3	6.64	6.3	TN (µmol l^{-1}) ***	-260.0	25.48	10.2
	PAR (µE m^{-2} s^{-1}) **	-219.5	10.30	4.2					PAR (µE m^{-2} s^{-1}) **	-266.3	11.21	4.1
ZD (cells cm^{-2})	Temp (°C) ***	-182.2	36.53	30.1	Temp (°C) *	-138.4	4.52	6.9	PAR (µE m^{-2} s^{-1}) ***	-230.9	32.8	23.1
	Picoeuk (cells µl^{-1}) *	-182.8	5.07	4.0					NH_4 (µmol l^{-1}) **	-236.3	10.29	6.7
									Picoeuk (cells µl^{-1}) *	-237.7	6.04	3.8

Table 3-2: Main physico-chemical-biological predictors for coral health indices (protein concentration, RNA/DNA ratio, lipid ratio, chl *a* concentration and zooxanthellae density per surface area) for *Acropora spicifera* for (A) typical seasons (autumn and winter 2010) not affected by La Niña event, (B) during the coral spawning event in autumn 2010, when metabolic indices were highest and (C) all sampled seasons including the La Niña event (autumn 2010, winter 2010 and summer 2011) Tests were performed with DISTLM (based on BIC; Primer 6) Grey shading indicates significant negative relationships, no shading significant positive relationships and italic no significant relationship.* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (Var = variability, Syn = *Synechococcus*, Pro = *Prochlorococcus*, Picoeuk = picoeukaryotes, temp = temperature, ZP = zooplankton)

A	**Typical seasons (Autumn and Winter 2010)**				**B**	**Spawning event (Autumn 2010)**			**C All seasons (Autumn & Winter 2010 and Summer 2011)**			
Health indices	**Predictor**	**BIC**	**Pseudo-F**	**% var**	**Predictor**	**BIC**	**Pseudo-F**	**% var**	**Predictor**	**BIC**	**Pseudo-F**	**% var**
Protein	Temp (°C) ***	-100.8	44.32	35.7	NH_4 (µmol l^{-1}) *	-50.0	18.35	8.8	Picoeuk (cells µl^{-1}) ***	-128.0	33.51	24.4
(mg µg^{-1}DNA)	NH_4 (µmol l^{-1}) *	-102.3	5.89	4.5	Current speed (cm s^{-1})*	-50.1	6.09	6.6	PAR (µE m^{-2} s^{-1}) ***	-140.8	18.49	11.5
	ZP 500-1000 µm (mg l^{-1}) *	-104.5	6.54	4.6					PO_4 (µmol l^{-1}) **	-144.4	8.26	4.8
RNA/DNA ratio	Temp (°C) ***	-108.5	13.39	14.3	ZP 500-1000 µm (mg l^{-1})	-75.5	4.11	4.7	Chl *a* 2-5 µm (µg l^{-1}) ***	-142.5	14.94	12.6
									PAR (µE m^{-2} s^{-1}) *	-144.3	6.42	5.1
Lipid ratio	TN (µmol l^{-1}) *	-332.7	5.52	6.5	TN (µmol l^{-1}) *	-223.1	7.43	11.7	TN (µmol l^{-1}) *	-421.4	4.31	4.0
	Si (µmol l^{-1}) **	-338.4	10.39	10.9	Si (µmol l^{-1}) **	-228.1	6.61	13.3	Si (µmol l^{-1}) **	-424.6	7.91	6.8
Chl *a* (µg cm^{-2})	Temp (°C) **	-182.7	52.51	39.6	Chl *a* 2-5 µm (µg l^{-1}) **	-139.9	7.50	18.5	Syn (cells µl^{-1}) ***	-193.9	59.73	36.5
in coral tissue	Picoeuk (cells µl^{-1}) *	-185.5	7.31	5.1	NH_4 (µmol l^{-1}) **	-145.7	35.57	13.3	PAR (µE m^{-2} s^{-1}) ***	-214.1	27.13	13.2
	ZP 500-1000 µm (mg l^{-1}) *	-187.0	5.83	3.8					Chl *a* >5 µm (µg l^{-1}) ***	-222.8	13.73	6.0
	TN (µmol l^{-1}) *	-187.8	5.04	3.2					ZP 500-1000 µm (mg l^{-1}) *	-222.9	4.67	2.0
ZD (cells cm^{-2})	NH_4 (µmol l^{-1}) *	-178.8	6.68	7.7	$NO_3 + NO_2$ (µmol l^{-1})*	-156.7	6.64	9.1	PAR (µE m^{-2} s^{-1}) **	-213.1	11.56	10.0

3.4.4 Importance of PAR and nutrients for coral health indices

Since our results suggested that PAR and temperature were important drivers of the variability in the measured coral health indices (Table 3-1), univariate relationships between these factors were further tested (Fig. 3-3, 3-4). Temperature displayed a non-linear relationship with all health indices (except lipids in the case of *A. spicifera*), resulting in highest metabolic activity and lowest autotrophic indices occurring between 26 and 28°C for both *Acropora* species(Fig. 3-3, 3-4). Temperatures above or below 26 and 28°C led to decreased metabolic activity but increased autotrophic indices. Metabolic values increased linearly with increasing PAR intensity while autotrophic values declined for both *A. spicifera* and *A. digitifera* (Fig. 3-3, 3-4). Linear relationships between lipid ratios and temperature and PAR showed the same trend as metabolic indices for *A. digitifera* with a linear increase with increasing PAR intensity (Fig. 3-3), however there was no significant relationship between lipid ratios and PAR or temperature for *A. spicifera* (Fig.3-4).



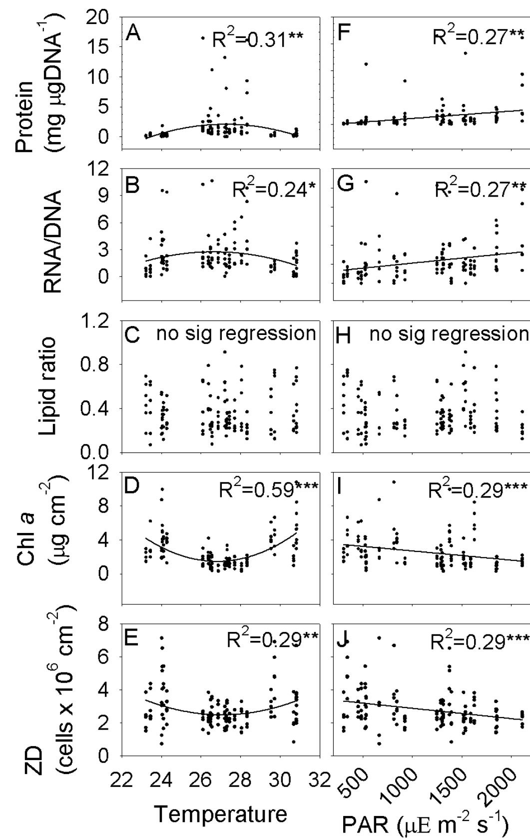


Figure 3-3: Quadratic correlations (R^2) of temperature (˚C) (A-E) and linear correlations (R^2) of PAR (µE m^{-2} s^{-1}) (F-J) with (A) protein concentration (mg µgDNA^{-1}), (B) RNA/DNA ratio, (C) lipid ratio,(D) chlorophyll *a* concentration (chl *a* µg cm^{-2}) and (E) zooxanthellae density (ZD cells x 10^6 cm^{-2}) for *Acropora digitifera*

Figure 3-4: Quadratic correlations (R^2) of temperature (˚C) (A-E) and linear correlations (R^2) of PAR (µE m^{-2} s^{-1}) (F-J) with (A) protein concentration (mg µgDNA^{-1}), (B) RNA/DNA ratio, (C) lipid ratio,(D) chlorophyll *a* concentration (chl *a* µg cm^{-2}) and (E) zooxanthellae density (ZD cells x 10^6 cm^{-2}) for *Acropora spicifera*.

3.5 Discussion

Here we show that under natural environmental conditions, there are clear species differences between the two dominant *Acropora* species at Ningaloo Reef, Western Australia, in their physiological response to physical, chemical and biological variables in the marine environment. We argue that *A. digitifera* is more dependent than *A. spicifera*

on autotrophy, and uses autotrophy to develop long-term energy stores as lipids. Previous studies showed that *Pocillopora domicornis* can store enough lipids to sustain normal caloric demand for at least for 28 days (Davies 1991) and that these reserves can be used (e.g., for *Pocillopora compressa*) to support recovery from extreme events (e.g., post-bleaching) until rates of photosynthesis return to normal (Grottoli *et al.* 2006).

In contrast, *A spicifera* has a higher metabolic activity overall, depends more on heterotrophic food sources such as e.g., picoplankton and zooplankton and does not show seasonal changes in stored energy, not even during the La Niña event (Chapter 2). This is in accordance with previous studies showing that certain species such as *M. capitata* increased feeding rates and acquired a large quantity of food in excess of daily metabolic requirements (Grottoli et al. 2006). Thus the species-specific differences shown in our study support the statement that species, which are able to increase their heterotrophic intake of carbon during bleaching and recovery have a better capacity to maintain and restore energy reserves (Grottoli et al. 2006; Rodrigues and Grottoli 2007), compared to species, which are largely dependent on photosyntetically fixed carbon (Rodrigues and Grottoli 2007). It follows that changes in environmental conditions, including climatic shifts, are likely to impact the community quickly, as demonstrated through our documentation of significant changes in coral health through a natural La Niña event.

3.5.1 Long-term effect of current speed on Acropora species coral cover

Results of % coral cover indicated that averaged long-term current speed plays a role in determining reef community composition spatially at Ningaloo Reef. The high % coral cover of plate shaped corals including the dominant plate form *A. spicifera* occurred at sites with high averaged long-term current speed while for caespito-corymbose shaped corals such as *A. digitifera*, no difference in % coral cover was found. Current speeds shape boundary layer dynamics around corals and subsequently affect the rate at which

corals can take up dissolved and particulate nutrients from the water column, especially at Ningaloo (Wyatt et al 2012), with increased current speed uptake of dissolved and particulate nutrients increased for e.g., *Porites compressa, Montipora capitata* and *Pocillopora damicornis* (Atkinson et al. 1994; Ribes and Atkinson 2007). During autumn, when temperatures were optimal for coral health across all indices, we found that protein concentrations in *A. spicifera* were correlated with both current speed and NH_4 concentrations. We infer that current speed may play an important long-term role in the long-term growth of corals by supporting nutrient delivery to the coral surface. Uptake of dissolved inorganic nitrogen (DIN= ammonium and nitrate) depends not only on current speed but also on DIN concentration in the seawater (Wyatt et al., 2012). In addition, feeding history of corals is important since fed corals show lower uptake rates (Grover et al. 2002). Since concentration of NH_4 was lower in autumn than during the other months, current speed may play a bigger role during this time to increase the supply of NH_4 to the reef.

Plate-shaped corals appear more impacted by acute physical stress events such as cyclones and storm surges (Hughes and Connell 1999) than caespito-corymbose corals. This might explain the disproportionate coverage of plate *Acropora* at Station 1 which was/is more likely to be exposed to these events (Lough 1998) than other stations. Alternatively, shifts in coral-algal dynamics can also result in decreased coral cover. For example algal overgrowth of corals can lead to hypoxia, increased pathogens and viruses along the coral-algal interface (Dinsdale et al. 2008; Bruce et al. 2012) and thickening of the boundary layer around corals (Barott and Rohwer 2012). Shifts from coral to algal dominated systems are linked with anthropogenic influences including increased nutrients in the water column e.g., from land run off (Dinsdale et al. 2008) and overfishing (Bruce et al. 2012). However, at Ningaloo, we do not expect algae

coverage to be the main driver of changes in coral health since this area is not particularly exposed to anthropogenic impacts.

3.5.2 Physico-chemical-biological predictors for coral health during typical seasonal patterns (excluding the La Niña event)

A. digitifera

For *A. digitifera*, light (PAR) and temperature were the main predictors for both autotrophic and metabolic indices, suggesting that this species relies to a large degree on photosynthetically derived products. The statistical decline of chl *a* and zooxanthellae density with high PAR and temperatures is in itself likely to be solely the result of photoacclimatisation (Titlyanov and Titlyanova 2002). Zooxanthellae densities can vary between 0.5 x 10^6 to 5 x 10^6 cells per cm^2 over time (Hoegh-Guldberg and Smith 1989a; Sheppard 2009) in response to seasonal variability such as PAR and temperature (Fagoonee et al. 1999; Fitt et al. 2000). Despite decreased zooxanthellae densities under high PAR conditions due to photoacclimatisation, previous studies have shown that photosynthetic rates are higher than under low PAR conditions resulting in an increase of photosynthetically derived products to the coral host (Davies 1991). The positive correlation of PAR with RNA/DNA ratio could thus be related to increased translocation of photosynthates to the coral host under high PAR conditions resulting in increased metabolic activity (Falkowski et al. 1993; Dubinsky and Jokiel 1994). Previous studies have indeed shown an increase of RNA/DNA ratios with PAR intensity (Meesters et al. 2002; Buckley and Szmant 2004; Humphrey 2009) and negative correlations between so-called "autotrophic indices" and RNA/DNA ratio (Chapter 2). In fact, given all this, chl *a* and zoxanthellae density would be better described as "photoacclimation indices" than "autotrophic indices", since they are, in general, inversely proportional to photosynthetic rates.

Results of this study also reveal that picoplankton, particularly the flow cytometrically identified picoeukaryote group, are possibly important predictors for coral metabolic activity, autotrophic indices as well as energy stores in *A. digitifera*. This is in accordance with previous studies, suggesting a selective grazing of picoplankton by the benthic community and the importance of picoplankton for the trophic dynamics on coral reefs (Houlbreque et al. 2006). In general, this implies that *A. digitifera* relies on heterotrophy in addition to autotrophy, possibly as an additional nitrogen source. Photosynthates translocated to the coral host do not, in general, provide corals with sufficient nitrogen for growth (D'Elia and Webb 1977; Mills and Sebens 2004) and previous studies have shown that picoplankton can provide corals with an important source of nitrogen (Ribes et al. 2003; Houlbrèque et al. 2004a; Houlbrèque and Ferrier-Pagès 2009). At Ningaloo Reef, picoplankton dominates the phytoplankton community virtually year-round and picoplankton has been suggested to be an important nitrogen source for the coral community (Patten et al. 2011; Wyatt et al. 2012). Picoplankton is also possibly important for the maintenance of storage lipids since feeding has been shown to increase lipid content in particular under low light when transfer of photosynthates is low (Treignier et al. 2008). Our data suggests that heterotrophy is, in addition to autotrophy, important in supporting lipid synthesis, as also previously shown for other coral species (Al-Moghrabi et al. 1995; Treignier et al. 2008). Our results further suggest that heterotrophic feeding on picoplankton, in particularly picoeukaryotes, is also important for the maintenance of healthy zooxanthellae density and chl *a* concentrations in coral tissues. This has previously been observed and was explained by an increased supply of nitrogen from the coral host to the symbiont resulting in increased symbiont growth (Dubinsky et al. 1990; Titlyanov et al. 2001a).

Protein concentration and lipid ratio are negatively correlated with both water column chl *a* in the size fraction 2-5 µm and picoplankton. This could be the result of a time-lag effect, since an increase in protein and lipid concentrations due to increased food availability can only be detected after weeks to months (Houlbrèque et al. 2003; Rodrigues and Grottoli 2007; Cooper et al. 2009).

<u>*A. spicifera*</u>

Results of this study revealed a different pattern for *A. spicifera* with nitrogen, temperature and zooplankton as the dominant predictors of the measured health indices. A previous study showed that inorganic (75%) and organic (24%) nitrogen sources can provide up to 100% of the nitrogen required for tissue growth (Grover et al. 2008). Thus results imply that both NH_4 and other components of TN could be an important nitrogen source for *A. spicifera* at Ningaloo Reef.

Light, even if important (Fig. 3-3), was not the main predictor for any of the measured health indices in *A. spicifera,* suggesting that this species is less dependent on photoautotrophy than *A.digitifera* and possibly gains less carbon-rich products through photosynthesis. However, corals' uptake of NH_4 can be light dependent (Grover et al. 2008) since syntheses into amino acid is only possible if enough carbon, mainly supplied through photosynthesis, is available (Dubinsky and Jokiel 1994). The positive correlation between coral protein concentration and zooplankton carbon confirms that zooplankton is an important source for daily carbon requirements in protein synthesis, demonstrated by twice as high protein concentrations in fed than starved corals. (Houlbrèque et al. 2003; Houlbrèque et al. 2004b; Grottoli et al. 2006; Palardy et al. 2008). Thus heterotrophic carbon sources seem to be a good substitute for carbon supply through photosynthesis for this species. When metabolic activity showed highest values, we note that protein concentrations were inversely correlated with current speed,

contradictory with the hypothesis that protein synthesis increases with an increase in current speed due to increased boundary layer thickness around corals and thus increased uptake rates of nutrients (Atkinson et al. 1994; Reidenbach et al. 2006). This suggests a more complex pattern of dependence between current speed and particle uptake, possibly involving dependence on the physical mechanics of settling particles, for example. While of interest, this effect is beyond the scope of the current study.

Given that it takes weeks to months to detect a change in protein concentrations with changes in food availability (Houlbrèque et al. 2003; Rodrigues and Grottoli 2007; Cooper et al. 2009), it was surprising that NH_4 concentration as well as zooplankton showed a positive correlation with protein concentration for *A. spicifera*. Since this result is contradictory with previous studies further investigation is needed. The positive correlation between temperature and protein concentration and RNA/DNA ratio suggests that metabolic activities increase with increasing temperatures as shown previously (Howe and Marshall 2001). Previous studies have shown changes in zooxanthellae density and coral tissue chl *a* to be correlated with NH_4 in the surrounding water (Hoegh-Guldberg and Smith 1989a; Dubinsky et al. 1990; Muscatine et al. 1998). In our study, however, the negative correlations between NH_4 and zooxanthellae density, and between TN and chl *a* concentration in the coral tissue is contradictory with these results and may be due to a time lag effect, since changes in zooxanthellae density are detectable only after weeks with increased NH_4 supply (Hoegh-Guldberg and Smith 1989a; Cooper et al. 2009). The positive correlation of picoeukaryotes and zooplankton with chl *a* concentration in the coral tissue may be due to the increase in zooplankton ingestion since a previous study by Houlbrèque et al. (2003) showed that chl *a* concentrations were 4 to 7 times greater in zooplankton fed corals than in control corals. It might also be partly explained by an additional

shading effect, since high plankton concentrations result in low PAR levels in the water column (Anthony and Connolly 2004).

Comparison between species

We hypothesise that the dependence of health indices of *A. digitifera* and *A. spicifera* on different physico-chemical-biological factors might be at least partly related to differences in coral morphology. Coral shape has been shown to directly affect flow patterns, turbulence and thickness of the boundary layer around corals, and thus impacts uptake rates of particulate and dissolved nutrients (Atkinson et al. 1994; Reidenbach et al. 2006). Branching species rely at least to some extent on particulate matter (Anthony 1999) and have been shown to be better adapted to high light intensity (Muko et al. 2000; Hoogenboom et al. 2008) while plate-shaped corals encounter higher rates of finer suspended particles than branch-shaped corals (Abelson 1993). These differences might partly explain why variations in RNA/DNA ratio and chl *a* concentration in the tissue of the caespito-corymbose shaped *A. digitifera* are better explained by PAR and particulate feeding while variations in protein concentration, lipid ratio and autotrophic indices in plate-shaped *A. spicifera* are better explained by TN and NH_4 in the water as well as current speed. In short, *A.spicifera* seems more dependent on smaller particles and dissolved nutrients than the branch-like *A.digitifera.*

A. digitifera builds up and stores higher concentrations of lipids than *A. spicifera* (Chapter 2), which is provided most likely through photosynthesis but also heterotrophic food sources. This is in agreement with previous studies that showed the importance of heterotrophic feeding to support lipid synthesis, while under high light energy provided by feeding is directed towards an increase in calcification, chlorophyll and protein

content instead (Al-Moghrabi et al. 1995; Grottoli et al. 2006; Treignier et al. 2008, Houlbrèque and Ferrier-Pagès 2009).

Since the results presented here are based only on data collected during daylight hours, further data is needed about the importance of nocturnal food sources for coral metabolism. It is assumed that corals feed primarily during night, when zooplankton densities are highest (Heidelberg et al. 2004; Yahel et al. 2005). We address this in Chapter 4. Since the physico-chemical-biological factors we measured did not explain all of the variability in health indices, others factors such as bacterial uptake (Sorokin 1973; Patten et al. 2011) or nitrogen fixation through coral-associated bacteria (Rohwer et al. 2001) could be also important.

3.5.3 La Niña and its impacts on physico-chemical- biological factors and thus coral health

Differences in physico-chemical-biological factors between La Niña and normal years

Light, current speed, temperature, nutrients and plankton concentrations off Ningaloo Reef in summer 2011 (February) differed statistically from average values of previous years (1982-2008) (Wyatt et al. 2010; Patten et al. 2011; Rousseaux et al. 2011) with temperatures more than 2 degrees higher (Pearce and Feng 2012), lower PAR levels (around ~30 instead of 55 mol photons m^{-2} d^{-1}) (Rousseaux et al. 2011) and higher current speed for summer than winter, (winter is normally the period of highest wave height and current speed at Ningaloo Reef; Taebi et al. 2012). NO_x and NH_4 concentrations in February 2011 were also more typical of higher concentrations normally observed in autumn, and chl *a* concentration in the water in fact exceeded typical winter values (Rousseaux et al. 2011). These differences were a result of the strongest La Niña event on record, an anomalously high air-sea heat flux into the ocean and a record strength Leeuwin Current (Pearce and Feng 2012), which delivered high

nutrient concentrations, and as a consequence, a phytoplankton bloom, high chl *a* and picoplankton concentrations as well as high zooplankton concentrations. The shift from *Prochlorochoccus* to *Synechoccocus* and picoeukaryotes reflects a clear nutrient input to the system (Staehr et al. 2009).

Physico-chemical-biological predictors for coral health during La Niña event

During the La Niña event, increases in plankton gained importance as a predictor for health indices of *A. digitifera* and *A. spicifera*, while temperature lost importance. This was unexpected, since temperature has been shown to strongly affect autotrophic indicators as well as metabolic rates in corals (Brown et al. 1999a; Howe and Marshall 2001) and abnormally high temperatures during La Niña resulted in bleaching in some areas of Ningaloo Reef (Pearce and Feng 2012). The significance of temperature may have been underestimated since multivariate analysis was based on linear regression while temperature followed a quadratic function. In general, metabolic activity (measured as respiration rates) increase with temperature until maximum is reached and decrease there after (Yamaguchi 1974; Howe and Marshall 2001). In our study this trend was observed for protein concentrations and RNA/DNA ratio with temperature. Our results show that coral metabolism for both morphologies, and in the case of *A. digitifera* also energy storage (as lipid ratio), was highest at 26 to 28°C (autumn), pointing towards optimal metabolic activity and thus coral health at this temperature. This temperature range has been identified as optimal for corals elsewhere (Achituv and Dubinsky 1990). Zooxanthellae densities at these temperatures were lowest, despite being within the range typical during normal conditions (ca. 1 to 2.5 x 10^6 cm^{-2}) (Drew 1972; Jones and Yellowlees 1997). This suggests that higher algae densities and chl *a* concentrations in the tissue together with low metabolic activity may

in fact reflect a decline in coral health. This is an important point when considering appropriate indices for coral health generally.

An increase in "autotrophic" indices at abnormally high temperatures suggests corals were stressed physiologically at these temperatures. Other studies have shown a decline of these indices with increasing temperatures (Brown et al. 1999a; Fitt et al. 2000), eventually resulting in coral bleaching (Hoegh-Guldberg 1999). Low light levels at our sample site at Ningaloo during the La Niña, together with high supply of particulate nitrogen as phytoplankton, might have counteracted the negative effect of high temperatures, since light has been shown to be an important co-factor mitigating the coral bleaching response (Jones et al. 1998; Mumby et al. 2001). High nitrogen concentrations can result in retention of photosynthate for the symbiont's own requirement, and increased symbiont growth rates (Clayton Jr and Lasker 1984; Davy and Cook 2001; Ferrier-Pages et al. 2003; Houlbrèque et al. 2004b). This might explain the relatively high zooxanthellae densities and chl *a* concentration found in *A. digitifera* in February 2011 as well as picoeukaryotes and nitrogen (NH_4 and TN) as the driving physico-chemical-biological factors for autotrophic indices. Given that low light conditions have the biggest impact on symbiont growth and lead to increased photoacclimatisation potential (Titlyanov et al. 2000; Titlyanov et al. 2001a; Houlbrèque et al. 2003) this could also explain the significant correlation between PAR and autotrophic indices during La Niña for *A. digitifera*.

Under the abnormal conditions during La Niña, our data support the hypothesis that less photosynthate is transferred to the coral host, and instead heterotrophically derived carbon and nitrogen is required to keep metabolic rates up (Grottoli et al. 2006; Borell et al. 2008, Houlbrèque and Ferrier-Pagès 2009). This was also reflected in correlations between plankton concentrations (picoplankton concentrations, chl *a* in the 2-5 µm size fraction as well as small zooplankton) and metabolic indices. Hughes et al.

(2010) also showed that heterotrophically acquired carbon is retained in both coral host and endosymbiotic algae while photoautotrophically acquired carbon is used mainly to meet short-term metabolic demands and calcification, in accordance to our explanation that acquired carbon was mainly used for zooxanthellae growth. Overall, our measured health indices showed that *A. digitifera* under stress conditions expresses lower metabolic indices and has lower energy stores possibly due to a depletion of protein and lipid stores to sustain their metabolism (Rodrigues and Grottoli 2007; Borell et al. 2008) since heterotrophic feeding seems not to provide sufficient carbon or nitrogen to the coral.

A. spicifera showed a different pattern then *A. digitifera*. Metabolic activities during the La Niña period were low (Chapter 2) and PAR as well as phytoplankton (picoplankton and water column chl *a* in 2-5 µm fraction) concentrations the main predictors for variations in metabolic indices. Since metabolic activities in *A. spicifera* depend strongly on temperatures overall, the abnormally high temperatures during La Niña have a deleterious effect on the physiology of this species. Some species have shown a decline in protein levels with abnormal temperatures (Rodrigues and Grottoli 2007; Borell et al. 2008). Increased zooxanthellae density in *A. spicifera* during La Niña was only correlated with PAR, suggesting that in this case it is a pure photoacclimatisation response (Fitt and Cook 2001; Titlyanov and Titlyanova 2002) and not significantly related to nutrient supply through heterotrophic feeding.

Overall our indices suggest that autotrophy combined with heterotrophy during stress events is not sufficient to supply enough energy to sustain high metabolic activities in *A. spicifera.*

3.5.4 Implication for long-term health of Ningaloo Reef

Our analysis demonstrates a strong impact of extreme events on coral health. At the highest current speed, for example, coral cover was extremely low. Furthermore, the shift in main physico-chemical-biological predictors for coral health during the La Niña event indicates that during such an event, corals depend more heavily on resource availability. However, species' food preference also seems to play a role: though zooplankton and nutrient concentrations in the water were high during La Niña, the general preference was towards picoplankton, possibly due to their importance as a nitrogen source and their presence in relatively high concentrations year-round (Ribes et al. 2003; Houlbrèque et al. 2004a; Patten et al. 2011). While high sea surface temperatures did not result in bleaching of corals, high seawater temperature reduced coral metabolism and lipid stores and hence, coral health. In analysing this process we show that high chl *a* and zooxanthellae densities are not an appropriate indicator for good coral health, thus we recommend the use of metabolic indices and energy stores instead of autotrophic indices when assessing the health of natural coral populations.

Overall our results imply that changes in hydrodynamic regimes, temperature, light and availability of particulate and dissolved nutrients all impact coral health. However, it is the very specific combination of factors and sensitivities that determines the actual outcome for a given coral community, such that no single index or environmental factor can be seen to dominate. It should be noted that there are differences in biogeochemical responses to short term extreme temperature and long term temperature rise. Climate change impacts will impact coral health at Ningaloo reef, and are likely to impact coral community composition. However, when predicting the outcome of climate change, it is important to take all changes in environmental factors into account since effects on coral health indices are complex.

Chapter 4 Temporal (diel and daily and seasonal) variations in metabolic and autotrophic indices for *Acropora digitifera* and *Acropora spicifera* – implications for monitoring projects

4.1 Abstract

Coral health indices are important components of the management assessments of coral reefs, providing insight into local variation in reef condition, as well as tools for comparisons between reefs and across various time scales. Understanding how such health indices vary in space and time is critical to their successful application as management tools. Here we compare autotrophic and heterotrophic coral health indices, examining specifically the temporal variation driven by the local environmental variation, at three scales (diel (throughout day), daily (between days) and seasonal). We compared metabolic indices (RNA/DNA ratio, protein concentration) and autotrophic indices (chlorophyll *a* (chl *a*), zooxanthellae density, effective quantum yield (yield) and relative electron transport rate (rETR)) for two dominant *Acropora* species, *A. digitifera* (caespito-corymbose form) and *A. spicifera* (plate form) at Ningaloo Reef (north-western Australia) in August 2010 (austral winter) and February 2011 (austral summer). Clear seasonal patterns were documented for metabolic indices, zooxanthellae density and rETR, while cyclic diel patterns only occurred for yield and rETR, and RNA/DNA ratio. Significant daily variation was observed for RNA/DNA ratio, chl *a* concentration, yield and rETR. Results suggest that zooxanthellae density and protein concentrations are good long-term indicators of coral health whose variance is largely

seasonal, while RNA/DNA ratio and rETR can be used for both long-term (seasonal) and short-term (diel) coral monitoring. Chl *a* can be used to describe changes between days and yield for both diel and daily variations. Correlations between health indices and light history showed that short-term changes in irradiance had the strongest impact on all health indices except zooxanthellae density for *A. digitifera*; for *A. spicifera* no correlation was observed at all. However, cumulative irradiance over the several days before sampling showed significant correlations with most health indices suggesting that a time-lag effect has to be taken into account when interpreting diel variations in coral condition. These results suggest that diel and daily variations have to be taken into account for the use of chl *a* concentration and yield in long-term monitoring projects. We recommend the use of protein concentrations, RNA/DNA ratios, zooxanthellae density and rETR as long-term bioindicators.

4.2 Introduction

Reef-building corals live in symbiosis with zooxanthellae (endosymbiotic algae), which enables the coral to obtain energy through autotrophy (light-derived) as well as through heterotrophy (active uptake of particles). Photosynthesis in the symbionts of corals provide a carbon source for the coral host that is energy-rich but nitrogen-poor, while the zooxanthellae benefit from high nitrogen and phosphorus metabolic waste products of the corals (Muscatine et al. 1984; Dubinsky and Jokiel 1994; Muller-Parker and D'Elia 1997). The presence of these photoautotrophic symbionts within the coral tissue suggests that corals should experience large daily fluctuations in O_2, CO_2 and NH_4 tension and pH driven by algal photosynthesis and coral metabolism (respiration rates) over a normal light/dark cycle (Levy et al. 2006; Yellowlees et al. 2008). Indeed, oxygen concentrations in the boundary layer of corals vary over diel cycles, with an anoxic state occurring at night and supersaturation

occurring in day light (Shashar et al. 1993). Lipid body formation, which is dependent upon the symbiotic status between the coral host and its symbionts, also exhibits diel rhythmicity with increased lipid density and size occurring during high light periods (Chen et al. 2012). Previous studies showed that the light/dark cycle (Levy et al. 2006) imposed a diel cycle on algal cell division (Hoegh-Guldberg 1994; Fitt 2000). Diel variations in the effective quantum yield of photosystem II (PSII) have therefore been used to assess the photosynthetic performance of PSII, which varies on a diel basis for some coral species (Brown et al. 1999b; Rodolfo-Metalpa et al. 2008). There are also diel fluctuations in relative electron transport rate, an indicator for photosynthetic activity (Hoogenboom et al. 2006).

Since metabolic rates in the coral host depend on photosynthetically derived and translocated products (Falkowski et al. 1993; Dubinsky and Jokiel 1994), the RNA/DNA ratio is correlated with light (Buckley and Szmant 2004) and can be expected to show diel changes. The RNA/DNA ratio is related to protein synthesis (Buckley and Szmant 2004) thus protein concentration might also express diel variations. Plankton and nitrogen concentrations in the water have also been shown to drive changes in the RNA/DNA ratio and protein concentration (Ferrier-Pages et al. 2003; Dahlhoff 2004) and might result in diel variations, even though previous studies showed that significant changes in protein concentrations were only detected in coral tissue after weeks (Ferrier-Pages et al. 2003). Previous work suggests that diel cycles are set by the availability of demersal planktonic food (Johannes and Tepley 1974; Alldredge and King 1977) and alternate sources of nitrogen (Fitt et al. 1995) with high concentration of zooplankton and possible highest feeding rates during night (Heidelberg et al. 2004; Yahel et al. 2005). Diel variations in RNA/DNA ratios have been observed for fish and molluscs (Chicharo et al. 2001; Esteves et al. 2009) due to diel fluctuations in metabolic rates, food requirements and digestion times (Buckley et

al. 1999). However, to our knowledge no study so far has investigated diel patterns in RNA/DNA ratio and protein concentration, or investigated which physico-chemical-biological factors might be responsible for these changes. Mechanisms driving links between diel changes in photosynthetic activity and diel changes in metabolic indices remain largely unknown. This knowledge is important for a better understanding for how autotrophic and metabolic processes are linked with each other and the environment.

Seasonal cycles have been observed for a variety of indices describing coral metabolism such as RNA/DNA ratio (Buckley and Szmant 2004) as well as autotrophic indices such as effective quantum yield (yield), relative electron transport rate (rETR) (Piniak and Storlazzi 2008; Piniak and Brown 2009) zooxanthellae density and pigments (Fagoonee et al. 1999; Fitt et al. 2000). Here we investigate the importance of diel or daily variations, specifically whether these variations occur and if so, whether they occur at the scale of those observed seasonally. Previous studies investigated temporal scales over which metabolic and autotrophic indices change due to changes in water quality (minutes to weeks) (Cooper et al. 2009) and thus the suitability for these indices as monitors of coral health. However, no study so far has investigated simultaneously a variety of metabolic (protein concentration, RNA/DNA ratio) and autotrophic indices (zooxanthellae density and chlorophyll a concentration (chl *a*), yield and rETR) to determine the relative importance of diel, daily and seasonal changes. This understanding is essential for future interpretation of changes in health indices. In addition, the response time of health indices to light has to be taken into account when interpreting diel variation since previous studies showed that translocation of photosynthetically derived carbon can take up to days until it is integrated in the coral tissue (Tremblay 2012).

Our earlier work at Ningaloo Reef determined seasonal changes in health indices (chl *a* concentration, zooxanthellae density, RNA/DNA ratio and protein concentration) for two dominant *Acropora* species as well as driving physico-chemical-biological factors for those seasonal changes (Chapter 2 and 3) and showed unexpected values for autotrophic and metabolic indices during the La Niña event. Here we determine diel and daily changes for these same two *Acropora* species for a better understanding for how useful metabolic and autotrophic indicators are for short-term as well as long-term monitoring projects since we hypothesise that these health indices are likely also presenting diel and daily cycles. In this study we define coral health as relative physiological activities – thus our autotrophic and metabolic measurements are used as indicators for physiological changes inside the coral occurring under a range of both normal and extreme conditions (La Niña). Measurements were taken during a normal winter season and during an extreme summer season (La Niña), and so include a wide range of normal as well as abnormal variations of physiological indices. Thus the aim of this study is to determine 1) how changes in metabolic indices and autotrophic indices occur on a diel, daily and seasonal basis for *A. digitifera* (Dana, 1846) and *A. spicifera* (Dana, 1846), 2) how indices which express diel changes are correlated and 3) which physico-chemical-biological factors are most likely to drive metabolic indices (RNA/DNA ratio and protein concentration) on a diel basis and 4) the time-lag between changes in light and the response of health indices.

4.3 Methods

4.3.1 Study site

The study site, Sandy Bay Lagoon (Sandy Bay), is located at Ningaloo Reef, along the North-West Cape of Western Australia (22.23°S, 113.84°E) and a detailed

description of the study site given in Chapter 2. The dominant coral genus on the reef crest at Sandy Bay is *Acropora* (Cassata and Collins 2008). Day and night sampling was conducted at one station, Station 4, which was easy accessible during most tidal levels (Fig. 2-1). Additionally, seasonal sampling was done at six stations (Fig. 2-1) on a diurnal basis to determine time-lag effects with light.

4.3.2 Sampling

Two different coral species, *A. digitifera* (Dana, 1846) and *A. spicifera* (Dana, 1846) (Wallace 1999) (n=3) were tagged at Station 4. These two corals are distinct in growth form, with *A. digitifera* a caespito-corymbose coral, and *A. spicifera* a plate coral. Coral samples were collected four times daily (morning, noon, evening, midnight) at four days (when weather conditions were convenient) during winter (August 2010) and summer (February 2011) at Station 4 (Fig.2-1). February 2011 was the peak of a strong La Niña event and therefore physico-chemical-biological conditions were different to other summers in this region (Pearce and Feng 2012) with higher sea surface temperatures, comparably low light levels and high plankton and nutrient concentrations (Chapter 3). To determine time-lag effects, six coral colonies of each species (*A. digitifera* and *A. spicifera*) were tagged at six stations in the reef lagoon (Station 1-6, Fig.2-1) and coral samples taken for symbiont density and chl *a* concentration, RNA/DNA ratio, protein concentration during winter 2010, summer 2011 and additionally in autumn 2010 (March and April 2010 - before and after coral spawning). Corals were sampled while snorkelling; with direct measurement on the colony for yield and rETR and removal of 1–5 cm length coral pieces from the middle of the coral colony (tip of the branch) for each of the other health indices (see below).

4.3.3 Analysis of health indices

Zooxanthellae density and chlorophyll a

Coral samples (~3–5 cm long) for the analysis of zooxanthellae density (per cm^2) and chl *a* concentration in the tissue (µg cm^{-2}) were immediately stored at -20°C after sampling and prior to analysis. Methods for zooxanthellae density and chl *a* in coral tissue were adapted from Siebeck et al. (2006) and are described in detail in Chapter 2. Coral surface area was measured using the paraffin wax method of Stimson and Kinzie (1991).

Chl *a* concentration was determined following the method**s** of Jeffrey and Humphrey (1975), described in detail in Chapter 2.

RNA/DNA and protein analysis

Samples for RNA/DNA and protein analysis were stored immediately after sampling (within minutes) at -80°C prior to analysis. Methods for analysis of RNA/DNA followed Humphrey (2009) and can be found in detail in Chapter 2.

For protein analysis, a standard detergent-compatible colorimetric assay protein kit was used (Lowry et al. 1951) as described in Chapter 2 in detail.

Effective quantum yield and relative electron transport rate

Variations in the effective quantum yield (yield) of photosystem II (PSII), a way to assess the photosynthetic performance of PSII, were measured using an underwater pulse-amplitude modulated (PAM) fluorometer (Diving-PAM, Walz) on 10 sections of the tagged coral colony. The fiber was placed at a fixed distance (1cm) in front of the coral tissue. The yield (ΔF/Fm') was measured by exposing 10 sections of the colony separately to a 0.8 s period of saturating light (ca. 8000 µmol m^{-2} s^{-1}) (Rodolfo-Metalpa et al. 2008). Yield measurements were converted to relative eletron transport rate (rETR) with the formula rETR = ΔF/Fm' x PAR x 0.5 with PAR as the immediate

radiance and 0.5 as the factor that accounts for the distribution of electrons between photosystem I and photosystem II (Hoegh-Guldberg and Jones 1999). Relative measure was used for the rate of electron transport since light absorption characteristics of tissue are unknown for these species.

4.3.4 Analysis of physico-chemical-biological factors

Physico-chemical-biological factors known to be essential for coral health were sampled at each station during the sampling period: light, temperature, current speed, nutrients (dissolved inorganic nitrogen), phytoplankton and zooplankton.

Abiotic factors

Light and temperature were measured using temperature loggers (Hobo Pendant Data Logger) that were deployed between 3 to 7 days prior to sampling.

Short-term current speed was estimated using drifters. Two crucifix design drifters (Wyatt et al. 2010) containing a GPS were simultaneously deployed for approximately 10 minutes and the GPS location and time of their deployment and collection recorded. The distance and time of each deployment allowed a simple estimate of surface flow speed.

Biotic factors

Water quality samples were taken for the analysis of dissolved nutrients, total nitrogen (TN), chl *a*, and picoplankton (*Synechoccocus*, *Prochloroccocus* and picoeukaryotes).

Water samples (40 ml) were filtered through 0.45 μm filters and stored at -20 °C, prior to analysis of dissolved inorganic nitrogen (DIN) (NO_x (NO_3 + NO_2)) and NH_4). Flow injection analysis (FIA) with detection by absorbance at specific wavelengths was done to determine concentrations of nitrate (NO_3) /nitrite (NO_2)

(QuikChem FIA + Lachat 8000 series). Briefly, nitrate was reduced to nitrite through a copperized cadmium column that than reacted with sulphanilamide under acid conditions to form a diazonium ion. The diazonium ion is coupled with N- (1-naphthyl) ehthylenediamine dihydrogenchloride that has an absorbance maximum at 520 nm (Quikchem Method 31-107-04-1-A (Instruments 2000). Ammonium was measured by fluorescence (GlobalFIA high sensitivity gas diffusion unit- HPMSD, Shimadzu RF-10Axl Fluorescence detector) following the method of Watson et al. (2004). Briefly, ammonium was liberated as ammonia by sodium hydroxide and passed subsequently through a porous PTFE membrane (HPMSD), reacted with ortho-phthaldiadehyde and sulphite and formed a fluorescent derivative. The derivative was measured with an excitation wavelength of 310 nm and an emission wavelength of 390nm. Dissolved inorganic nitrogen (DIN) is the sum of ammonium and nitrate and nitrite.

For total nitrogen (TN), unfiltered water samples (50 ml) were stored at -20 °C prior to analysis. TN was determined from autoclave digests with potassium persulphate (Valderrama 1981). All analyses were carried out on a Lachat Quick-Chem 8500 Automated Flow Injection Analyser in accordance with NATA accreditation. Organic nitrogen (ON) was calculated by subtracting DIN from TN.

For chl *a* analysis, seawater samples (1 l) were filtered onto 0.7 µm filters (Whatman GF/F) and the filters stored at -20 °C in the dark until fluorometric analysis of duplicated 90% acetone extracts was carried out (Parsons et al. 1984).

Concentrations of autotrophic picoplankton groups (*Synechoccocus*, *Prochlorococcus* and picoeukaryotes) were determined following the technique of Patten et al. (Patten et al. 2011). 1.5 ml seawater was fixed with gluteraldehyde (0.5% final concentration) for 10 minutes and then snap frozen in liquid nitrogen. Samples were analysed using flow cytometry; samples were thawed at 37°C, 1µm fluorescent beads (Molecular Probes) added as an internal standard and samples were analysed

using a FACSCANTO II (Becton-Dickinson) flow cytometer fitted with a 488 nm laser on high throughput mode at a flow rate of 60 µl min^{-1} for 100 s. Nitrogen (N) and carbon (C) content of picoplankton was calculated based on fixed factors for picoeukaryotes (39.2 fg N and 836 fg C) (Verity et al. 1992; Caron et al. 1995), *Prochloroccocus* (4 fg N and 46 fg C) and *Synechoccocus* (30 fg N and 213 fg C) (Bertilsson et al. 2003; Heldal et al. 2003). Total nitrogen and carbon content for picoplankton was then calculated by the sum of nitrogen and carbon concentrations respectivley for *Prochloroccocus*, *Synechoccocus* and picoeukaryotes.

Zooplankton was sampled with a plankton net (90 cm diameter, 10 min in the water with a speed of 2.5 km h^{-1} for 50 m around the station) and preserved with formaldehyde (ca. 5%). Dry weight analyses were carried out for zooplankton between 100 and 1000 µm. Wet zooplankton samples were added to a pre-weighed container after cleaning samples of salt with DI water (30 s) and dried in an oven (60°C for 24 hours). Samples were then cooled down in a desiccator and weighted for subsequent calculation of dry weight (DW) (Harris 2000).

4.3.5 Statistical analysis

A four-factor PERMANOVA was performed in PRIMER Version 6 (PRIMER-E, Plymouth, UK) with species, seasons and times per day as fixed terms and days nested in season (crossed factors) (Anderson 2001; Anderson et al. 2008). PERMANOVA was based on Euclidean distances of protein concentration, RNA/DNA ratio, chl *a* per cm^2 and per cell, zooxanthellae density, yield and relative ETR (all log-transformed) and analysed with Type I (sequential) sum of squares with permutation of residuals under a reduced model (9999 permutations). Health indices, which showed diel patterns, were in addition tested for day/night differences (morning and noon combined and evening and midnight combined) with the same design as described

above but night/day instead of times per day. Log-transformation was done based on results of a draftman plot as well as Grubbs' test to reduce outliers and make data more continuous. Pair-wise tests were performed when significant differences occurred. To determine correlations between health indices, that displayed diel patterns the coefficient of determination R^2 was tested with DISTLM (Primer 6) based also on Euclidean dissimilarity matrix after log-transformation (with selection criteria all specified).

To determine which physico-chemical-biological factors best explain changes in protein concentration and RNA/DNA ratio on a diel basis, health indices and physico-chemical-biological factors (light, temperature, current speed, chl *a* concentration in the water, nitrogen and carbon content of picoplankton, zooplankton (dry weight) and DIN and ON were tested with DISTLM (Primer 6) based on Bayesian information criterion (BIC) (forward procedure, 9999 permutations) (Anderson et al. 2008). Protein concentration and RNA/DNA ratio were log-transformed while physico-chemical-biological factors were square root transformed. Correlations were done for species and seasons separately.

Correlation coefficients (R) between health indices and light history, were determined using DISTLM (Primer 6) based on Euclidean dissimilarity matrix after log-transformation of health indices for integrated light during 0, 1, 3, 6, 12, 24, 36, 48 and 72 hours prior to sampling (with selection criteria all specified). Diel as well as seasonal data was included to get a broader light spectrum.

4.4 Results

4.4.1 Temporal variation of health indices

Differences between species, seasons, days and diel patterns (morning, noon, evening, midnight) were tested for *A. digitifera* and *A. spicifera* between summer 2011 (February) and winter 2010 (August). Diel patterns (means ± standard error) are displayed in Fig. 4-1.

Significant differences between species (sp) and seasons (se) were found for protein concentration (sp: $F_{1, 179} = 58.22$, $p < 0.001$; se: $F_{1, 179} = 27.44$, $p < 0.01$; sp x se: $F_{1, 179} = 2.41$, $p < 0.01$), RNA/DNA ratio (sp: $F_{1, 178} = 81.83$, $p < 0.001$, se: $F_{1, 178} = 4.87$, $p < 0.001$) and zooxanthellae density (sp: $F_{1, 179} = 58.22$, $p < 0.001$; se: $F_{1, 183} = 4.94$, $p < 0.05$). However, pair-wise tests for protein concentration revealed that only *A. spicifera* showed seasonal differences ($p < 0.01$) with higher protein concentrations in summer than in winter, while for zooxanthellae density significant differences were only found for *A. digitifera* ($p < 0.001$) based on higher values during winter (Fig. 4-1) compared with summer. RNA/DNA ratios showed seasonal differences for both *A. digitifera* and *A. spicifera* (sp x se: $F_{1, 178} = 6.34$, $p < 0.05$; pair-wise comparison $p < 0.05$ for both) with highest values occurring during summer compared to winter for both species (Fig. 4-1).

Chl *a* concentration per surface area also varied significantly between species (sp: $F_{1, 183} = 91.98$, $p < 0.001$) due to higher concentrations in summer for *A. spicifera* than for *A. digitifera* (sp x se: $F_{7, 183} = 51.33$, $p < 0.001$, pair-wise comparison $p < 0.01$) while no differences occurred in winter between species for chl *a* concentration per surface area ($p > 0.05$) (Fig. 4-1). No seasonal patterns were observed when analysing species individually. No significant differences between the two *Acropora* species were found for chl *a* concentration per cell, yield and rETR ($p > 0.05$), with only rETR

expressing seasonal differences with higher rates during summer ($F_{1, 164} = 28.83$, $p < 0.001$) (Fig. 4-1).

Protein concentration and zooxanthellae density did not significantly vary over diel or daily time scales for any of the two species. In contrast, RNA/DNA ratio ($F_{7, 178} = 10.53$, $p < 0.001$), chl *a* (chl *a* per cm^2: $F_{7, 183} = 6.89$, $p < 0.001$; chl *a* per cell: $F_{7, 183} = 3.21$, $p < 0.01$), yield ($F_{7,167} = 11.40$, $p < 0.001$) and rETR ($F_{7, 164} = 17.23$, $p < 0.001$) exhibited significant differences between days within each season. Significant changes throughout the day were observed for yield as well as rETR ($F_{3, 167} = 3.10$, $p < 0.05$; $F_{3, 167} = 413.57$, $p < 0.001$) even though this pattern was not consistent throughout all days (da (se) x di: $F_{13, 167} = 7.55$, $p <0.001$; $F_{13, 164} =34.37$, $p <0.001$) (Appendix B, Table 1). In general for yield, evening and midnight values were higher than morning and midday values (Fig. 4-1) and pair-wise tests revealed that morning yield values differed significantly from those at when species were pooled ($p < 0.05$). rETR displayed the opposite pattern with highest rates occurring during midday and zero values occurring during evening and night due to a lack of light (PAR = 0) (Fig. 4-1). Significant differences in rETR occurred when data was pooled between all times with the exception of midnight and evening (since both zero values)) ($p < 0.05$). When morning and noon values were combined as well as evening and midnight values day/night patterns still occurred for yield values ($F_{4, 167} = 5.04$, $p < 0.001$) even though day/light variations were only apparent for some and not all days. For rETR, daily variation lost importance when testing day/night differences with day/night differences occurring for summer and winter ($F_{1, 167} = 13.19$, $p < 0.05$; pair-wise comparison $p < 0.001$). For RNA/DNA, diel patterns were observed within a day but without a consistent pattern (i.e. changing between days) (Appendix B, Table 2). However, when species were investigated separately, no diel difference could be resolved (sp x da(se) x di $p > 0.05$).

In addition, when diurnal and nocturnal values were pooled, there was no significant day/night variation (p > 0.05).

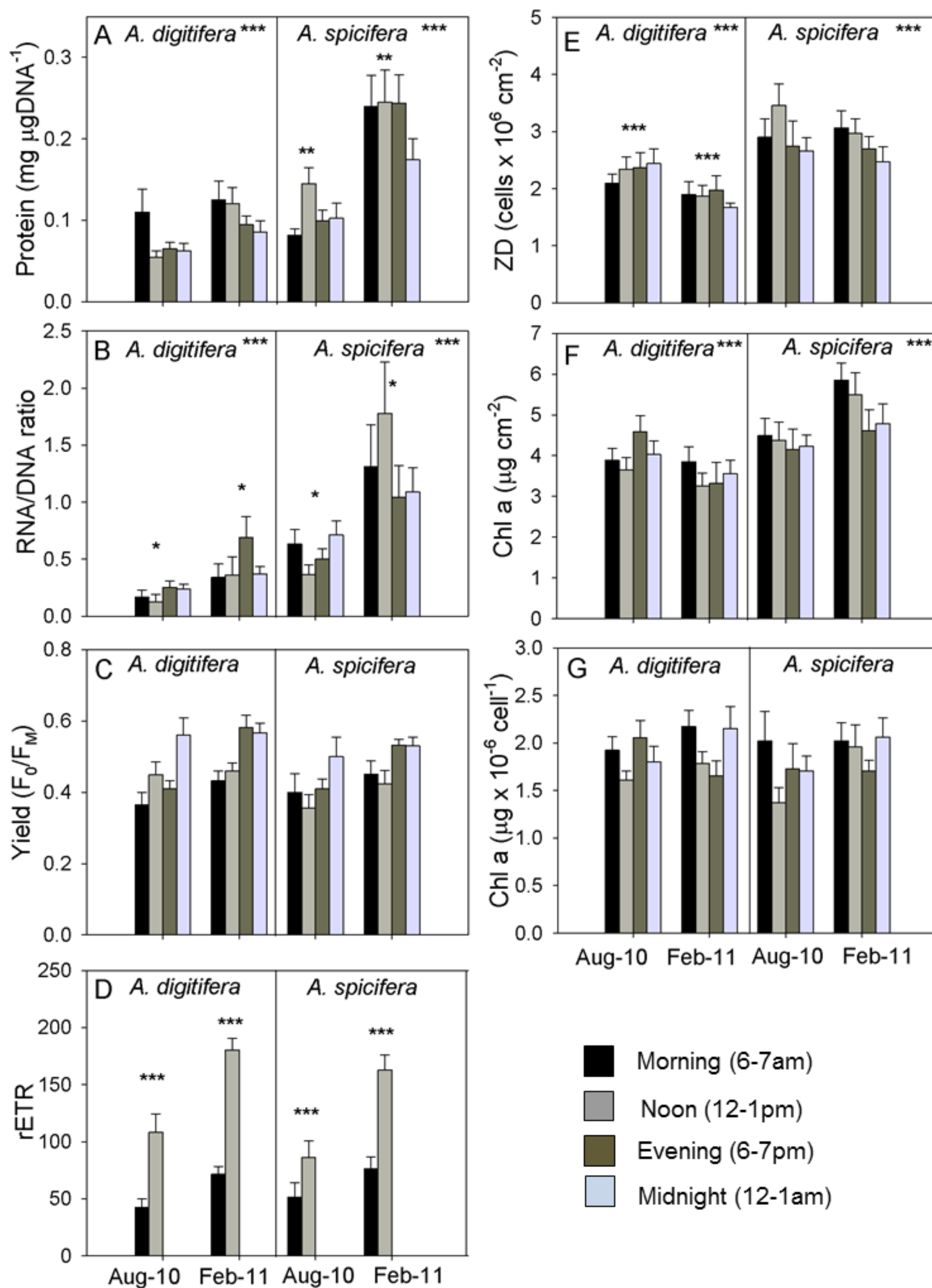


Figure 4-1: Diel pattern for health indices for *Acropora digitifera* and *Acropora spicifera* at Ningaloo. Values represent means ± standard error, n = 9 – 12 for metabolic indices (protein concentration, RNA/DNA ratio) and autotrophic indices (zooxanthellae density (ZD), chlorophyll *a* concentration (per surface area and per cell), yield and rETR)) at four different times per day in August 2010 (winter) and February 2011 (summer). Stars symbolize significant differences between species and seasons (* P < 0.05; ** P < 0.01; *** P < 0.001).

Correlations between health indices which changed on short-term basis showed that chl *a* per cell was positively correlated with yield values ($R = 0.17$, $p < 0.05$) and yield was also positively correlated with rETR ($R = 0.27$, $p < 0.001$). R-values increased when only those values measured during daytime were taken into account and night values excluded ($R = 0.34$, $p < 0.01$; $R = 0.54$, $p < 0.001$). No correlation was found between RNA/DNA ratio with chl *a* per cell, yield or rETR for either *A. digitifera* or *A. spicifera* within the whole day or only during daytime.

4.4.2 Environmental predictors for metabolic indices on a diel basis

Physico-chemical-biological factors (PAR), temperature, current speed, DIN, ON, chl *a* concentration, zooplankton, N-content of Picoplankton and C-content of Picoplankton) were tested for best prediction of coral metabolic indices (protein concentration, RNA/DNA ratio), separately for *Acropora digitifera* and *Acropora spicifera* during winter and summer. During winter changes in protein concentration during the day for *A. digitifera* were correlated with ON in the water, while RNA/DNA ratio showed strongest yet non-significant relationship with PAR. In summer, for *A. digitifera*, protein concentrations were not correlated with temperature or PAR, while RNA/DNA ratio were best explained by nitrogen provided by picoplankton and organic nitrogen (Table 4-1).

For *A. spicifera*, zooplankton and PAR were strongly (positively) correlated with protein concentrations, while temperature was negatively, and carbon supplied by picoplankton positively correlated with variability in RNA/DNA ratio. In summer light showed strongest but non-significant correlation with protein concentration while chl *a* concentration in the water column showed a negative correlation with RNA/DNA ratio of *A. spicifera* (Table 4-1).

Table 4-1: Physico-chemical-biological predictors for variation in protein concentration and RNA/DNA ratio in *A. digitifera* and *A. spicifera* during August 2010 and February 2011.Results are based on BIC tests (Primer). Bold letters symbolize negative correlations while other correlations were positive. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Non-significant correlations are in italic. Var = variability, tot = total.

Season	Health indices	Predictor	BIC	Pseudo-F	% var	% tot
	A. digitifera					
Aug-10	Protein (mg µgDNA^{-1})	**ON (µmol l^{-1}) ***	-271.73	6.59	13.6	13.6
	RNA/DNA ratio	*PAR (µE m^{-2} s^{-1})*	-163.92	3.85	8.4	8.4
Feb-11	Protein (mg µgDNA^{-1})	*Temp*	-250.74	3.43	7.7	
		DIN (µmol l^{-1})	-250.86	3.78	8	15.7
	RNA/DNA ratio	**Pico N (fg µl^{-1}) ***	-108.42	7.38	15.3	
		ON (µmol l^{-1}) *	-108.93	4.18	8	23.3
	A. spicifera					
Aug-10	Protein (mg µgDNA^{-1})	Zooplankton (mg l^{-1}) *	-278.08	3.99	8.1	
		PAR (µE m^{-2} s^{-1}) *	-278.67	4.36	8.3	16.4
	RNA/DNA ratio	**Temp ****	-139.01	7.89	14.9	
		Pico C (fg µl^{-1}) *	-139.7	4.46	7.8	22.8
Feb-11	Protein (mg µgDNA^{-1})	*PAR (µE m^{-2} s^{-1})*	-200.76	1.23	2.9	2.9
	RNA/DNA ratio	**Chl *a* (µg l^{-1}) ***	-79.198	10.66	20.6	20.6

4.4.3 Time-lag effect of light on health indices

Correlations between PAR and health indices showed that light at the time of sampling showed highest correlations for protein concentration, RNA/DNA ratios, rETR and chl *a* concentration (Fig. 4-2). However, when PAR was integrated over the previous three days from the time of sampling, PAR was significantly positively correlated with protein concentration, RNA/DNA ratio and rETR for both species and with chl *a* per surface area for *A. digitifera.* For *A. digitifera,* zooxanthellae density was correlated with PAR intensity one or more days prior to zooxanthellae sampling. However, for *A. spicifera* zooxanthellae density did not show any significant correlation with PAR. Overall, correlations coefficient for protein concentration, zooxanthellae density and chl *a* concentration with PAR history were weaker for *A. spicifera* then *A.*

digitifera (Fig. 4-2). Yield values only showed correlations with immediate PAR intensity (*A. digitifera*: R = 0.24, p < 0.05; *A. spicifera*: R = 0.26, p < 0.05).

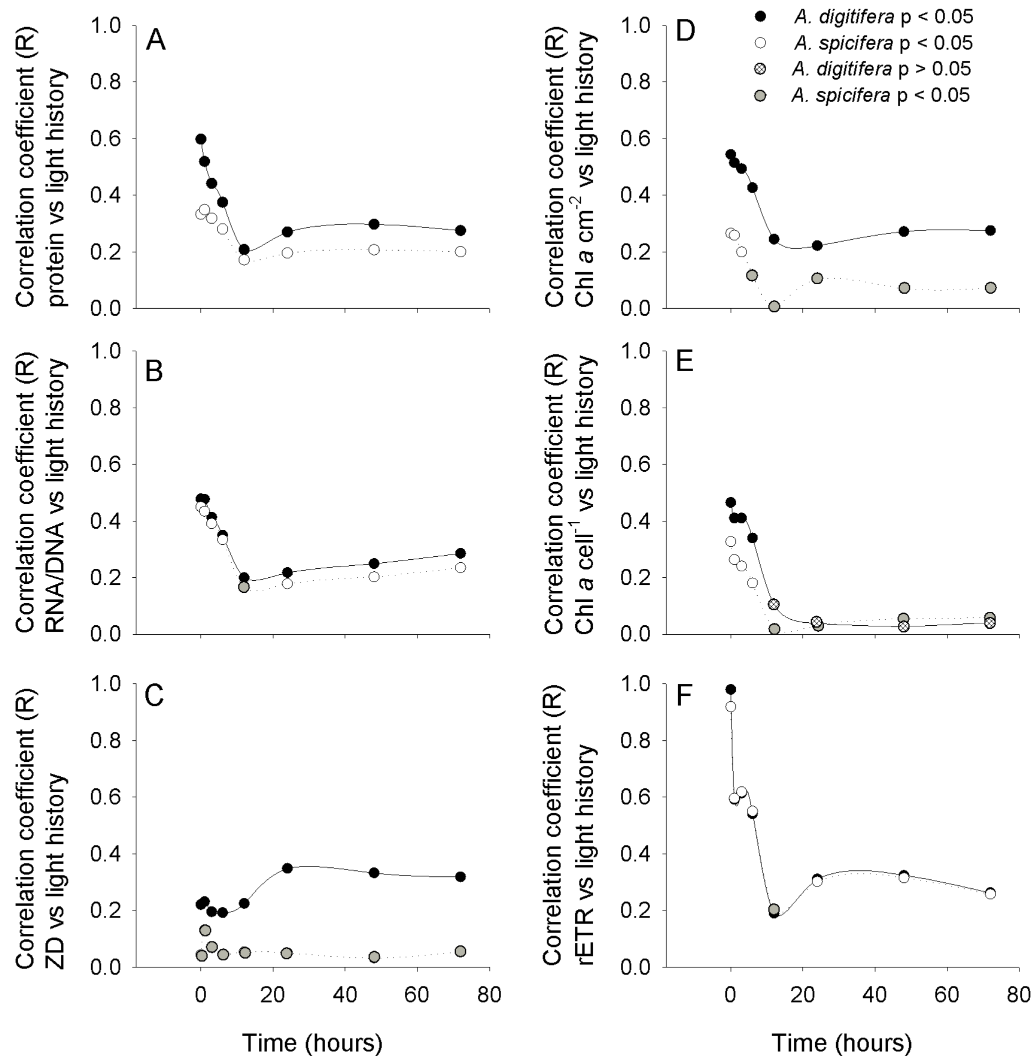


Figure 4-2: Correlation coefficient (R) between health indices and light (PAR) history. Correlation coefficients were determined for (A) protein concentration (mg $mgDNA^{-1}$), (B) RNA/DNA ratio, (C) zooxanthellae density (ZD) (cells cm^{-2}), (D) chlorophyll a per surface area (µg cm^{-2}), (E) chlorophyll *a* per cell (µg $cell^{-1}$) and F) relative electron transport rate (rETR) (Based on DISTLM).

4.4.4 Diel patterns of physico-chemical-biological factors

In general, averaged values for all physico-chemical-biological factors were lower in winter than in summer (Fig. 4-3). During winter 2010 and summer 2011, current speeds were highest at noon and lowest in the evening. Similar patterns occurred

for PAR and temperature during winter and summer, with highest values occurring at noon and lowest values occurring in the evening and at midnight. Picoplankton concentrations declined slightly in the evening in winter but showed comparable values throughout the day in summer. In summer, chl *a* concentration and zooplankton showed similar patterns with lowest concentrations occurring at noon and highest concentrations in the evening while in winter chl *a* concentration declined throughout the day and zooplankton peaked at noon. Highest DIN concentration was found in the evening for both seasons while ON showed lowest values in the evening for summer and no real change throughout the day in winter (Fig. 4-3).

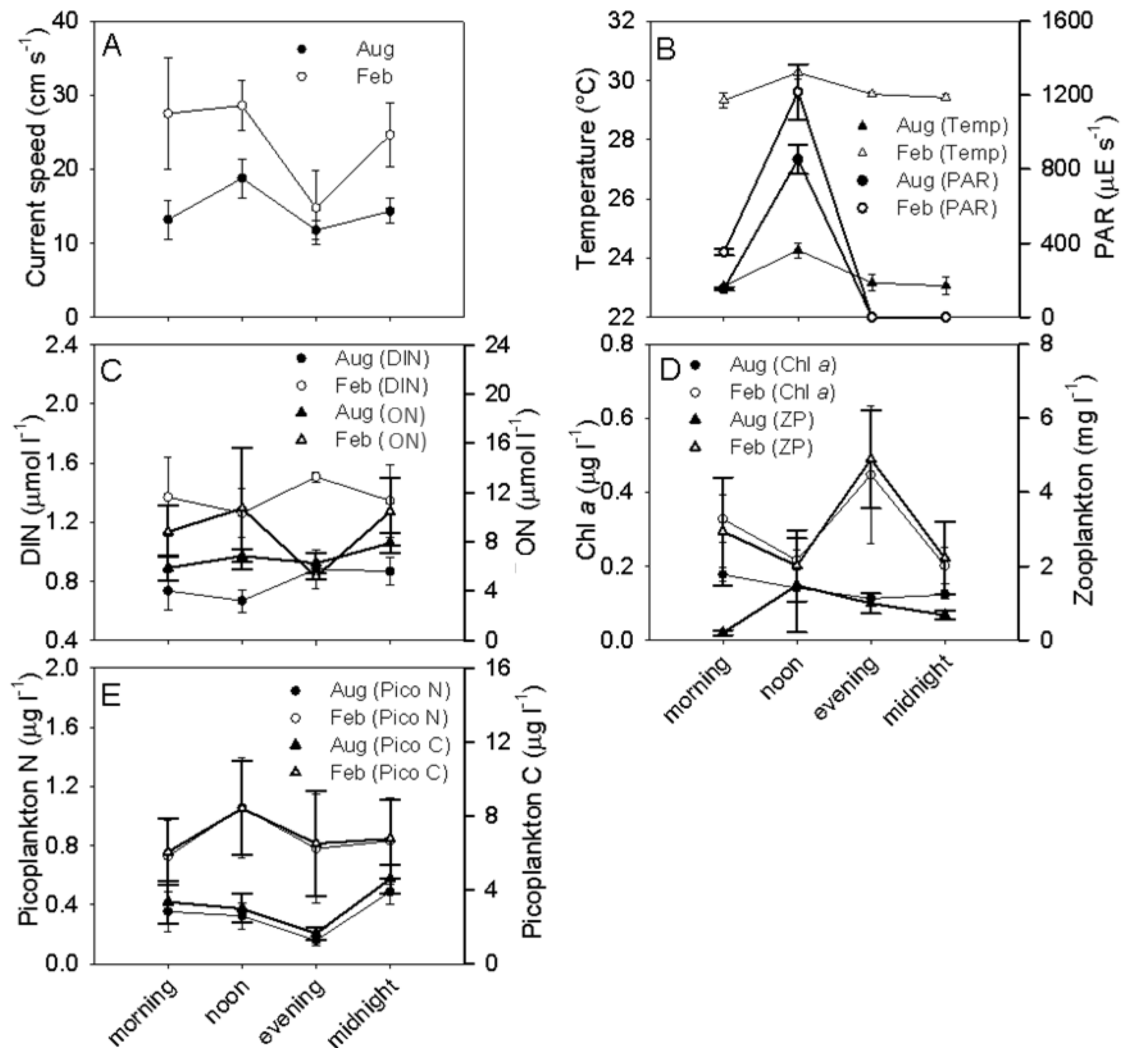


Figure 4-3: Diel patterns for physico-chemical-biological parameters at Ningaloo Reef. Patterns in (A) current speed (B) temperature and light intensity (PAR) (C) dissolved inorganic nitrogen (DIN) and organic nitrogen (ON), (D) chlorophyll *a* concentrations and zooplankton and (E) picoplankton carbon and nitrogen content at site 4 during August 2010 and February 2011. Data are means ± standard error (n=4).

4.5 Discussion

4.5.1 Temporal changes in health indices and suitability of indicators for monitoring

This is the first study to our knowledge, which determines how sensitive different metabolic and autotrophic indices are to the impact of long-term and short-term

changes in environmental factors. Previous work (Cooper et al. 2009) reviewed the suitability of different health indices for projects monitoring coral health based on a variety of studies, but no study so far investigated the full range of metabolic indices (protein concentration, RNA/DNA ratio) and autotrophic indices (zooxanthellae density and pigment concentration, yield and relative electron transport rate) simultaneously. Here we determine the diel, daily and seasonal patterns in the fluctuation of multiple indices. Since light is important for all coral indices even though light may not be their proximal driver (Chapter 3), we also determining how long it takes for health indices to react to light for a more sophisticated interpretation of diel, and daily, as well as seasonal, changes.

<u>*Protein concentration*</u>

Our results indicate that protein concentration is relatively stable on a diel basis, suggesting that this index could be used primarily to determine changes in seasonal and long-term coral health. This is in agreement with previous work which showed that protein concentration only shows detectable changes after weeks or months of perturbation, generally associated with changes in particulate food availability (Ferrier-Pages et al. 2003; Houlbrèque et al. 2003; Houlbrèque et al. 2004b) or extreme temperatures (Rodrigues and Grottoli 2007; Borell et al. 2008). The precise time it takes to build-up and use protein reserves seems to be species-specific and depends on individual metabolic rates (Rodrigues and Grottoli 2007; Borell et al. 2008). While metabolic activities of *A. spicifera* are generally higher than of *A. digitifera*, (Chapter 2); no recurrent diel or daily patterns were observed for either species. Significant correlations between protein concentration and short-term PAR exposure (seconds – minutes), as well as (slightly weaker) correlations between integrated PAR exposure over previous several days suggest that protein content does in fact respond to light, but

that corals effectively integrate energy across diel fluctuations in PAR intensity, such that heterotrophic processes including protein synthesis, remain relatively stable. For example, coral's protein synthesis is often dependent on DIN uptake, a light dependent process, which has been shown to express diel patterns (McAuley and Smith 1995). In fact, overall in our study, nitrogen was a primary physico-chemical-biological predictor for protein concentration in *A. digitifera* and for A. *spicifera* protein synthesis was correlated with light across seasons (Chapter 3). Positive correlations of protein concentration with PAR (seconds to days) might also be related to changes in the translocation rates of photosynthetic products from the zooxanthellae to the coral host which can be as rapid as 15 minutes, but might take 48 hours, at which time the products are finally integrated into coral tissue (Tremblay 2012). Overall, it is likely that a variety of physico-chemical-biological factors varying on a diel, daily and seasonal basis light, nitrogen concentrations and zooplankton concentrations (Chapter 3) will influence changes in protein concentrations in corals; however these changes are integrated via the longer metabolic time lines for protein synthesis. This index therefore emerges as a robust choice for the measurement of medium to long-term changes in coral health.

RNA/DNA ratio

RNA/DNA ratio showed significant differences between species, between seasons, between days and times of day. This index is therefore an appropriate indicator for short as well as long-term changes in metabolic activities of corals. Because daily and diel variations are smaller than seasonal differences, the RNA/DNA ratio also provides an index of coral health that integrates appropriately across time scales. Seasonal changes in RNA/DNA ratio have been seen for corals (Buckley and Szmant 2004) as well as changes within days when transplanted to different depths (Humphrey

2009). Diel patterns in our study were only recorded when data between species was pooled perhaps due to a too small sample size given the intra-specific variation. In general, there is a clear response of RNA/DNA ratio to diel physiological fluctuations (Cooper et al. 2009) as described for other species such as molluscs (Chicharo et al. 2001) and fish (Esteves et al. 2009). In our study, both species showed a winter pattern of lowest RNA/DNA ratio at noon and highest at midnight, while summer patterns were more variable, with highest RNA/DNA ratio in the day time, possibly since physico-chemical-biological factors showed stronger diel fluctuations in summer then winter. Given the apparent importance of PAR, plankton concentrations and dissolved nitrogen concentrations in driving RNA/DNA ratios (Buckley and Szmant 2004, Chapter 3), we suggest that this reflects corals' reliance on photosynthates transferred from the zooxanthellae to the coral host, as well as on energy gained through heterotrophic feeding. Short-term (seconds to minutes) PAR variation showed the strongest correlation with RNA/DNA ratio for both *Acropora* species but also integrated PAR level from up to three previous days were correlated with the RNA/DNA ratio. However, the lack of correlation between RNA/DNA ratio and rETR, an indicator for photosynthetic activity (Hoogenboom et al. 2006), is contradictory to the hypothesis that diel variations in RNA/DNA ratios are driven by photosynthetically derived carbon. However, even though no clear correlations exist, diel variations in photosynthesis are likely to be at least partly responsible for diel changes in RNA/DNA ratio potentially due to a time lag effect of the integration of translocation products into the tissue (Tremblay 2012) or an overestimation of rETR (Hoogenboom et al. 2006). Plankton and nitrogen concentration can also trigger diel and daily variations in coral health, since diel cycles are often set by the availability of demersal plankton food (Johannes and Tepley 1974; Alldredge and King 1977) and alternate sources of nitrogen (Fitt et al. 1995) which can change on a daily as well as seasonal basis. This time-lag in feeding

can be observed in the dynamics of coral food vacuoles, which appear in digestive cells within 2 hours of feeding and subsequently decrease in number per digestive cell and as the percentage of digestive cells with food vacuoles, only after 5-7 hours post-feeding (Fitt 2000). Overall, RNA/DNA ratio can be used as a short-term as well as long-term indicator even though further studies are needed. When used in monitoring projects it has to be kept in mind though, that RNA/DNA measurements should be taken at the same time each day to make data comparable.

Zooxanthellae densities and pigments

In general, zooxanthellae densities in our study fell within the range of previous studies, ranging between 0.5×10^6 and 5×10^6 cells per cm^2 (Drew 1972; Stimson 1997; Fagoonee et al. 1999). Zooxanthellae densities in corals in our study were surprisingly resilient to diel and daily changes in irradiance. Diel changes in zooxanthellae densities have been observed for some species due to synchronous division and degradation over a diel cycle (Hoegh-Guldberg 1994; Fitt 2000). However, this diel cycle in corals seems highly species specific and not always present (Stimson and Kinzie 1991; Titlyanov et al. 1996; Titlyanov et al. 2000). The results in our study suggest that if such cycles occur in zooxanthellae division, other processes regulating cell concentrations are likely to be operating at the same time, such as degradation (Titlyanov et al. 2001a), digestion and extrusion (Titlyanov et al. 1996) resulting in no significant change in zooxanthellae density.

The strongest correlation of incident PAR with zooxanthellae density occurred more than 24 hours prior to sampling in *A. digitifera*. Previous studies have shown that it takes 24 hours for zooxanthellae densities to be modified (Hoegh-Guldberg and Smith 1989a), since algal division rate is set to more than 24 hours prior to actual cytokinesis (Fitt 2000). Rhythmical changes in proliferation of zooxanthellae with periods of 3-6

days can certainly occur in addition to daily proliferation periodicity (Titlyanov et al. 2004) due to changes in light (Fitt 2000), as well as local supply of zooplankton and nutrient concentrations (Doyle and Poore 1974; Fitt 2000). The fact that for *A. spicifera* changes in zooxanthellae density do not seem to be directly driven by PAR history within either hours or days, suggests that zooxanthellae density is driven primarily by other factors such as nitrogen uptake (Chapter 3). Our results suggest that for certain species such as *A. digitifera* zooxanthellae densities can be used to describe long-term (seasonal) changes in coral health. This is in agreement with other studies which also observed seasonal changes in zooxanthellae densities (Fagoonee et al. 1999, Fitt et al. 2000). Daily changes in such physico-chemical-biological factors such as temperature, light and nutrient concentrations might have been not large enough to result in measurable changes in zooxanthellae density. Seasonal environmental variation is much greater, resulting in larger variation in symbiont densities. Temperature, nutrients and light were all observed to be important in driving measurable variation in coral health indices (Chapter 3).

We found no seasonal or diel difference for chl *a* per unit coral surface area, or for chl *a* per zooxanthellae. Our measured daily variations suggest chl *a* can be a good indicator for short-term (days) changes in physico-chemical-biological impacts on coral health. Daily changes in light intensity have also been known to impact coral chl *a* concentrations (Titlyanov et al. 2001b), but no significant diel patterns were observed in our study or a previous study (Levy et al. 2006). Our results do indicate that short term light levels have the strongest impact on chl *a* concentrations, suggesting there are no real time-lag effects. However, this is contradictory with the absence of diel patterns. The correlation between light history and chl a concentration show significant values but R values are only around 0.4, suggesting that other factors play a role and might add to the non-significant results in regard to diel patterns.

In regard to seasonal changes no significant variation was detected in this study in contrast with previous studies that have shown significant variations in chl *a* concentration throughout the year in the studied species (Fagoonee et al. 1999; Fitt et al. 2000; Ulstrup et al. 2008). Thus chl *a* might be suitable to describe seasonal changes in physico-chemical-biological factors in case these changes are big enough, however it should be kept in mind that daily changes might also influence these patterns.

Effective quantum yield and relative ETR

Diel patterns in photosynthetic yield of *A. spicifera* are in accordance with previous studies showing highest values at night, a decrease in the morning, low values around noon and an increase during afternoon towards evening (Brown et al. 1999b; Lesser and Gorbunov 2001; Winters et al. 2003). Down-regulation is attributed to dynamic photo-inhibition mediated by non-photochemical quenching in the reaction centre and antenna pigment bed (Hoegh-Guldberg and Jones 1999; Lesser and Gorbunov 2001). Yield depends on daily occurrence of peak solar radiation and is reversible and photo-protective (Gorbunov et al. 2001). The slightly different pattern for *A. digitifera*, with lowest values in the morning might be due to a different genetic type(s) of symbiodinium (Berkelmans and Van Oppen 2006; Warner et al. 2006), photo-protection trait in the tissue (Titlyanov and Titlyanova 2002) and tissue thickness (Hoegh-Guldberg 1999). In general yield values between 0.3 and 0.6 were within the range previously detected (Lesser and Goburnov 2001; Warner et al. 2002; Rowan et al. 2004; Hoogenboom et al. 2006). The fact that overall no species-specific differences occurred, suggest that both *Acropora* species' photosystems react in similar ways to variations in light levels. However, yield values showed only a low correlation with short-term PAR history (R = 0.24 and 0.26) suggesting that other physico-chemical-biological factors are more important for changes in yield. Despite previous studies

which showed seasonal changes in yield values due to temperature (Warner et al. 2002; Piniak and Brown 2009) our study did not observe seasonal variations even though temperature difference varied between 23 and 31°C between winter and summer. A previous study showed it can take months to recover yield values after a bleaching event (Rodrigues et al. 2008a) supporting our results that it is not a good long-term indicator. Overall for the studied *Acropora* species yield does not seem to be a good indicator for determining any seasonal differences, however yield appears more appropriate indicator for short-term changes since it reacts quickly to variations in light intensity. However since our study fell within a La Niña year and PAR values during summer were lower than normal (Chapter 3) further studies are needed to determine seasonal changes. In addition when used for short-term monitoring projects light intensities are needed and samples should be taken at the same time to make data comparable.

Seasonal, daily and diel changes in rETR were clearly observed in our study, suggesting this measure was a good short-term and long-term indicator of coral health. Higher values at noon than in the morning are in the same range as those seen in previous studies (Winters et al. 2003; Hoogenboom et al. 2006) for both species. Higher values during summer than in winter are also in accordance with previous work (Piniak and Brown 2009). Short-term light history had an overwhelming correlation with changes in rETR while also PAR from previous day showed a smaller impact. Our values are in the range of other studies for morning (50-150) and midday (100-200) (Hoogenboom et al. 2006) as well as with seasonal studies (Piniak and Brown 2009). Rates are influenced by phylotype and physiology (colour and tissue thickness) of resident *Symbiodinium* sp., colony morphology, animal behaviour (polyp extension and contraction) as well as the PAR history of a particular location from which fluorescence measurements are taken (Winters et al. 2003; Levy et al. 2004). However, photosynthetic productivity can be overestimated when interpreted only through rETR

(Hoogenboom et al. 2006) since rETR is non-linearly correlated with primary productivity and the relationship between biochemical and energetic assays of photosynthesis is influenced by photo-acclimatisation state of individual colonies (Hoogenboom et al. 2006). Thus the photosynthetic rates we estimated at noon when PAR was highest might be an overestimation.

4.5.2 Summary/ Conclusion

Overall, we conclude that zooxanthellae density and protein concentrations are good long-term indicators of changes in coral health since they express seasonal but not diel or daily changes, however species-specific variations must be taken into account. RNA/DNA ratios and rETR can be used as long-term as well as short-term indices of coral health. However diel changes in RNA/DNA ratios remain unresolved. Chl *a* concentration in coral tissue is a better indicator for daily changes and should be used carefully to describe seasonal changes in coral health, while photosynthetic yield is good indicator to describe diel and daily variations for the two studied *Acropora* species at Ningaloo Reef. This is in accordance with previous studies showing that chlorophyll fluorescence is a good short-term indicator to predict and monitor coral bleaching while yield values can take up to months to recover (Rodrigues et al. 2008a) and are therefore not recommended to describe long-term changes. The fact that chl *a* per cell is positively correlated with yield suggests that photo-adaptation processes in the antennae and photosystem II are related (Sheppard 2009). Species-specific differences in the dependence of light, in particular in regard to autotrophic indices, suggest that *A. digitifera* relies more strongly on autotrophy, which is in accordance with the earlier work (Chapter 3) and most likely related to differences in zooxanthellae clades (Rowan 2004), differences in morphology (Hoogenboom et al. 2008), photo-protection trait in the tissue (Titlyanov and Titlyanova 2002) and/or tissue thickness (Hoegh-Guldberg

1999). Further studies are needed for a better interpretation of diel changes in RNA/DNA ratios as well as seasonal changes during seasons which do not fall in La Niña years. This is particularly important since our study reflects the normal variation during winter within the corals; however, summer values were unexpected as shown previously (Chapter 2).

When the studied indices are used for monitoring corals under a management plan, a suitable time scale needs to be established for over which time, changes in coral health may occur and can be measured. As long-term indicators (weeks – months), zooxanthellae density and protein concentrations are recommended but also rETR and RNA/DNA ratios can be used, while for short-term indicators (minutes – hours), rETR as well as RNA/DNA ratio seem to be the most reliable indicators.

Chapter 5 General Discussion

We investigated seasonal, diel and spatial changes in metabolic indices, autotrophic indices and energy stores for two dominant *Acropora* species (*A. spicifera* and *A. digitifera)* at Ningaloo Reef. We determined the environmental variation in autotrophic and metabolic indices as well as energy stores, how the indices are correlated with each other, and how they correlate with physico-chemical-biological environmental predictors. We also investigated the effect of a record-strength La Niña event on health indices. Overall, we showed that seasonal and species- specific patterns drove a larger fraction of the environmental variability than local spatial variation.

5.1 Typical seasonal patterns of health indices and main physico-chemical-biological predictors for health indices of *A. digitifera* and *A. spicifera*

Seasonal patterns for autotrophic and metabolic indices for both *Acropora* species showed higher coral chl *a* concentration and zooxanthellae densities in winter than in autumn as a consequence of photoacclimation to lower light intensities (Dubinsky and Jokiel 1994; Titlyanov et al. 2001b). Previous studies have similarly shown that zooxanthellae densities and pigments are highest in winter and decrease during summer (Fagoonee et al. 1999; Fitt et al. 2000; Ulstrup et al. 2008). We emphasize the importance of understanding this variation in autotrophic indices as either neutral with respect to coral health, or an inverse coral health index, where the highest zooxanthellae densities and chl *a* content of corals occurs at the lowest light intensities and autotrophic growth rates (Dubinsky and Jokiel 1994; Titlyanov et al.

2001b). In short, it would be important not to interpret a small-scale reduction in chl *a* cm^{-2} as a reduction in coral health. We interpret this photoacclimation as being an effect quite distinct from and almost unrelated to bleaching, which is an extreme and toxic effect of high light and temperature, resulting in broad-scale expulsion of zooxanthellae (Sheppard 2009).

The highest values of RNA/DNA ratio and protein concentrations we measured at Ningaloo were observed in autumn, and the lowest in winter, (Buckley and Szmant 2004; Rossi et al. 2006), likely related to a decrease in respiration rates at low temperatures (Yamaguchi 1974; Howe and Marshall 2001), as well as lower availability of photosynthates due to lower light levels (Dubinsky et al. 1990) and/or utilization of energy and protein stores to maintain metabolic activity (Davies 1991). The highest concentrations of particulate nutrients in the water occurs in autumn as a consequence of a deepening of the mixed layer depths, partly due to the acceleration of the Leeuwin Current, resulting in an increased chl *a* concentration in winter (Rousseaux et al. 2011). We expected that greater availability of oceanic plankton would lead to increased heterotrophic feeding rates; however, energy stores and metabolic indices remained low during winter suggesting that any potential increased feeding rates did not fully compensate the corals for the energy lost through lower autotrophy.

Despite similar seasonal patterns for both *Acropora* species, there were clear species-specific differences in metabolic activity, autotrophic indices and energy stores between *A. digitifera* and *A. spicifera.* In addition, physico-chemical-biological predictors for these processes also differed between the two *Acropora* species. Results here suggested that *A. digitifera* relied on both autotrophy as well as heterotrophic feeding, with light, temperature and picoplankton concentrations emerging as strong predictors for autotrophic and metabolic indices. In particular, the largest picoplankton group picoeukaryotes were an important group for *A. digitifera*, most likely representing

an important source of nitrogen (Ribes et al. 2003; Houlbrèque et al. 2004a; Patten et al. 2011). Picoeukaryote concentration was also a good predictor for lipid stores; previous studies have similarly shown that heterotrophic feeding by corals resulted in increased lipid content inside corals such as *Galaxea fascicularis* (Linneaus, 1767) and *Turbinaria reniformis* (Bernhard 1896) (Al-Moghrabi et al. 1995; Treignier et al. 2008). Health indices for *A. spicifera* on the other hand were predicted to a stronger degree by nitrogen, temperature and zooplankton, suggesting that they needed to support their metabolism with sufficient nitrogen, supplied heterotrophically, (Grover et al. 2008) as well as carbon (Grottoli et al. 2006; Palardy et al. 2008). Overall, we concluded that both coral metabolism and energy stores were less dependent on autotrophically derived carbon-rich products, and that this species has higher metabolic activities than *A. digitifera* which are strongly dependent on temperature (Howe and Marshall 2001).

Investment into energy stores also differed between species. Lipid ratios for *A. spicifera* were largely constant throughout the year, possibly due to a constant top-up supply through heterotrophic feeding. The hypothesized decrease in lipid ratio after the spawning event (Leuzinger et al. 2003) did not occur in *A. spicifera*; our results suggest that spawning created only a minor perturbation in lipid stores against a backdrop of massive seasonal changes. However for *A. digitifera*, lipid ratios decreased from autumn to winter. A similar trend was observed for *Goniastrea aspera* (Verrill, 1905) and *Montipora digitata* (Dana, 1846) where lipid levels were higher during the spawning event then the following months (Oku et al. 2003b; Leuzinger et al. 2012). Since *A. digitifera* builds up and stores higher concentrations of lipids then *A. spicifera* (Chapter 2), *A. digitifera* might therefore rely more strongly on autotrophy to fulfil its energetic needs. We suggest that energy is invested directly into tissue growth rates instead of energy stores. High protein concentrations in *A. spicifera* seem to support this hypothesis; there is often a species-specific preference for protein vs. lipid accumulation

in coral tissue with increased food availability. Their storage of protein or lipid can also depend on whether the species relies more on autotrophy or heterotrophy for their energy supply, and their facility in shifting between feeding modes (Grottoli et al. 2004; Rodrigues and Grottoli 2007; Borell et al. 2008).

Differences in main physico-chemical-biological predictors for health indices of *A. digitifera* and *A. spicifera* might also be related to differences in coral morphology. Coral shape has been shown directly to affect flow patterns, turbulence and thickness of the boundary layer around corals, thus impacting uptake rates of particulate and dissolved nutrients (Atkinson et al. 1994; Reidenbach et al. 2006). Branching species rely at least to some extent on particulate matter (Anthony 1999) and have been shown to be better adapted to high light intensity (Muko et al. 2000; Hoogenboom et al. 2008), while plate-shaped corals encounter higher rates of fine suspended particles (Abelson 1993). These differences might partly explain why health indices in *A. digitifera* were better explained by light and phytoplankton concentrations in the water (potentially inversely correlated with light) while indices in plate-shaped *A. spicifera* were better explained by total nitrogen and ammonium concentrations in the water.

Since tested physico-chemical-biological parameters did not explain all of the variability in health indices, others factors such as bacterial uptake (Sorokin 1973; Patten et al. 2011) or nitrogen fixation through coral-associated bacteria (Rohwer et al. 2001) could be also important for the coral species and further investigation is needed here.

5.2 Impacts of La Niña on health condition for corals at Ningaloo Reef

Comparison between normal summer conditions and an event like La Niña is very instructive and allow us to predict what could be the impact of a long-term

climatic phenomenon on coral condition. The results presented here show that the response to such extreme events can vary according to the coral species and environmental conditions on the reef. We documented extreme values in health indices during the La Niña event, with high chl *a* concentration and zooxanthellae density and low metabolic indices for both *Acropora* species, and low lipid stores for *A. digitifera.* This is quite different than normal summer conditions, in which studies have observed lowest zooxanthellae densities and chl *a* concentrations (Fagoonee et al. 1999; Fitt et al. 2000), highest protein concentrations (Ferrier-Pages et al. 2003) and high lipid concentrations (Oku et al. 2003b). Our results therefore imply that extreme events have a significant impact on coral health. During La Niña we expected bleaching as a response to high temperatures as observed at this time at other reefs of Western Australia (Pearce et al. 2011). However, low light intensities due to high plankton concentrations, as well as high overall nutrient concentrations seems to have counteracted bleaching but resulted in low metabolic indices and lipid stores for both *Acropora* species. This suggests that extreme weather events have a negative impact on metabolic activities. Reasons for this are unclear but may include a shift of energy investment into growth of symbionts instead of translocation of photosynthate to the coral host (Clayton Jr and Lasker 1984; Ferrier-Pages et al. 2003; Houlbrèque et al. 2004b) resulting in a draw up of energy stores and/or a stronger dependence on heterotrophic feeding (Davies 1991; Rodrigues and Grottoli 2007; Borell et al. 2008). *A. digitifera* seemed to shift from dependence on light to picoeukaryotes and nitrogen (ammonium and total) concentrations as the main predictors of coral health, indicating a shift from an autotrophy/heterotrophy combination to mainly heterotrophic feeding. However, energy acquired during La Niña through heterotrophy seems to be insufficient to support metabolic activities and lipid synthesis, reflected in low metabolic indices and lipid ratios for *A. digitifera.* Low energy reserves as measured during La Niña were

not typical of other studies that have shown an increase in lipids (total and storage in % per tissue) throughout spring and constant values during summer (Oku et al. 2003b; Leuzinger et al. 2012), most likely linked with increased lipid biosynthesis due to oocyte development and/or temperature and light differences (Ward 1995; Oku et al. 2003b; Leuzinger et al. 2012). Thus extreme events such as La Niña may result in a decrease of energy available for reproduction.

For *A. spicifera*, during extreme events, autotrophy as well as heterotrophy becomes apparently important. We suggest that higher than optimal temperatures (~30 degrees) are likely responsible for low metabolic activities. A decline in protein concentration has already been shown for other coral species with abnormally high temperatures (Rodrigues and Grottoli 2007; Borell et al. 2008) and results of our studies showed that optimum temperatures for metabolic indices are between 26-28°C degrees, while temperatures above and below led to declined metabolic activities and in case of *A. digitifera* also a decline in lipid ratios.

In *A. spicifera* unusually low irradiances seem to be responsible for the large photoacclimatisation response, with relatively high zooxanthellae densities and coral chl *a* concentrations (Fitt and Cook 2001; Titlyanov and Titlyanova 2002), in contrast, nutrient concentrations seemed to drive variation in coral health indices for *A. digitifera*. This can possibly be explained by difference in morphology (Todd 2008), symbiont clades (Rowan 1997), photoprotection status of the tissue (Titlyanov and Titlyanova 2002) and tissue thickness (Hoegh-Guldberg 1999).

5.3 Implications for the suitability of coral health indices for monitoring projects

This is the first study investigating diel, daily and seasonal changes in health indices simultaneously for a variety of health indices in *A. spicifera* and *A. digitifera* to

determine which health indices are the most appropriate to describe metabolic and autotrophic changes for short-term as well as long-term monitoring projects. We suggest that RNA/DNA ratio, protein concentration and zooxanthellae density are suitable indices to describe changes on a seasonal basis, since diel and daily changes are smaller in scale than seasonal changes. Chl *a* concentrations within corals can potentially also be used as a long-term indicator but it has to be considered that daily variations may exceed seasonal ones and would need to be quantified. In addition, our data indicate that because chl *a* concentrations increase significantly at low light, they are inversely correlated with autotrophy. Daily changes in light intensity have been known to impact coral chl *a* concentrations (Titlyanov et al. 2001b), similar to daily changes observed in our study. Diel changes in effective quantum yield have been observed before (Brown et al. 1999b; Lesser and Gorbunov 2001; Winters et al. 2003) as well as diel changes of relative electron transport rates (Hoogenboom et al. 2006) and seasonal variations (Piniak and Storlazzi 2008). However, previous studies showed significant seasonal variations in coral chl *a* concentration (Fagoonee et al. 1999; Fitt et al. 2000; Ulstrup et al. 2008) which we did not observe.

To study the effect of short-term changes (diel, daily) in physico-chemical-biological factors on coral health, we determined that effective quantum yield, RNA/DNA ratios and relative electron transport rate are suitable indices. In contrast, protein concentrations varied over weeks or months of perturbation, generally due to changes in particulate food availability (Ferrier-Pages et al. 2003; Houlbrèque et al. 2004b) or extreme temperatures (Rodrigues and Grottoli 2007; Borell et al. 2008). Zooxanthellae densities also have been observed to change in a timescale of weeks due to changes in physico-chemical-biological conditions such as temperature (Fagoonee et al. 1999; Fitt et al. 2000; Rodolfo-Metalpa et al. 2006b), light intensity (Titlyanov et al. 2001b) as well as nutrient concentrations, especially nitrogen (Hoegh-Guldberg and

Smith 1989b). Seasonal changes in RNA/DNA ratios have been seen previously for corals (Buckley and Szmant 2004) as well as rapid changes (within days) when transplanted to different depths (Humphrey 2009), similar to the daily and seasonal changes shown in our study. However more data is needed to resolve diel changes in RNA/DNA ratios; this would allow us to gain a better understanding of how metabolic activity changes throughout day/night cycles. Our results together with those from previous studies therefore suggest the suitability of the described health indices for coral species on other coral reefs as short-term and long-term indicators of coral health. However, in order to undertake meaningful monitoring work, as a first step, baseline monitoring is required to understand how health indices vary for each investigated species. It can be expected that slow-growing *Porites* species differ in their metabolic and autotrophic indices from fast-growing *Acropora* species. Additional points, which need to be considered when choosing coral health indices for monitoring programs, include the time required for undertaking analytical techniques and gaining the subsequent results, the cost involved in the analysis and the training required. For example, the costs to determine RNA/DNA ratios are relatively high, while measuring rETR is quick and cheaper if using a PAM. In general also other parameters might be good coral health indicators such as growth rates and should be taken into account. Within the scope of this study it was unfortunately not possible to include more health indices.

5.4 Implications for Ningaloo reef for future scenarios

Overall our results suggest that changes in hydrodynamic regimes, temperature, light and availability of particular and dissolved nutrients due to climate change are likely to impact coral health and possibly reproductive success and coral composition at Ningaloo Reef.

Despite the low energy storage capacity in *A. spicifera*, which might increase this species' vulnerability to bleaching events (Borell et al. 2008), the higher feeding rates and protein concentration will most likely counteract this and result in faster recovery after bleaching events (Grottoli et al. 2006). This might be a longer-term advantage for this species. Predicted changes such as sea-level rise, resulting in lower light levels and a reduction in local current speed (Taebi et al. 2011), will most likely impact metabolic activities in corals negatively, in particular for more autotrophy-dependent species such as *A. digitifera*. This has been shown previously for *Porites compressa,* a species which are largely dependent on photosynthetically fixed carbon and drained their stored energy reserves during bleaching until rates of photosynthesis returned, while species that are able to increase heterotrophy as e.g., *Montipora capitata* have been shown to be able to keep up their energy reserves (Grottoli et al. 2004; Grottoli et al. 2006; Rodrigues and Grottoli 2007).

Dependence of both *Acropora* species on particulate food, including live phytoplankton, is in accordance with findings for the Ningaloo Reef community (Wyatt et al. 2010; Patten et al. 2011). A gradual slowdown in the Leewin current as well as ENSO-related events will result in change in the broad current patterns, thermocline depth, and marine productivity off Western Australia coast (Feng et al. 2003; Feng et al. 2009). This may have serious consequences for coral health at Ningaloo since it will most likely result in a decline of nutrients and plankton delivered to the reef. Also, a reduction of local current speed due to sea-level rise (Taebi et al. 2011) may impact both *Acropora* species by lowering available nutrients and plankton in the water. *A. spicifera* seems to be particularly vulnerable to changes in current patterns, indicated by high percentage cover of plate *Acropora* in areas with higher long-term current speed. This is possibly related to the strong dependence on dissolved nutrients (ammonium and total nitrogen) and zooplankton for *A. spicifera* since uptake mechanisms and

availability of particular nutrients as well as boundary layer around corals and thus diffusion of dissolved nutrients into the corals are affected by current speed (Atkinson et al. 1994; Ribes and Atkinson 2007). In addition acute high stress events such as cyclones and storm wave have been shown to impact plate-shape corals such as *Acropora hyacinthus* (similar to *A. spicifera*) more than caespito-corymbose ones such as *Acropora digitifera* (Hughes and Connell 1999), reflected in our study in the disproportional percentage cover of plate *Acropora* at station one which likely to be exposed to these events (Lough 1998). A gradual temperature rise (Anthony and Marshall 2012) as well as extreme temperatures during ENSO-related events (Feng et al. 2003; Feng et al. 2009) are likely going to impact metabolic activities, autotrophic indices and in case for *A. digitifera* also energy stores with a decline in metabolic activity and energy stores when temperatures exceed 28°C.

In general, results suggest that if climate-change occurs as predicted in worst-case scenarios, a decline in coral health at Ningaloo will be seen in *A. digitifera* and *A. spicifera*.

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APPENDICES

Appendix A

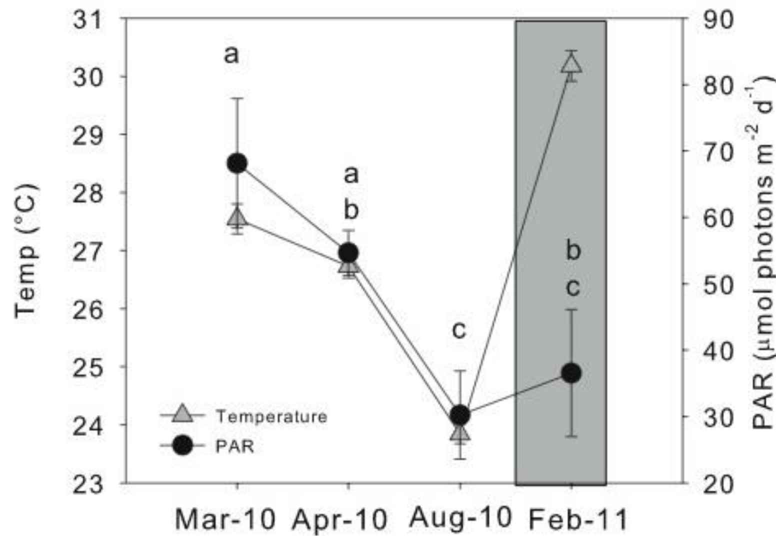

Figure 1: Light and temperature values during autumn (March and April 2010), winter (August 2010) and summer (February 2011). Means ± standard error, n=6. Temperature values were all significantly different. Same letters indicate no significant difference for light values between seasons based on pair-wise analysis (Euclidian distance) of PERMANOVA using Type III (partial) sum of squares with unrestricted permutation of raw data (9999 permutations).

Table 1: Pair-wise PERMANOVA (p-values) for protein concentration, RNA/DNA ratio, lipid ratio, zooxanthellae density (ZD), chl *a* concentration per surface area and per cell, compares two species (*A. digitifera* and *A. spicifera*) during four months (March and April 2010 (autumn), August 2010 (winter) and February 2011 (summer) at Sandy Bay, Ningaloo Reef. Species and season were fixed effects. PERMANOVA was based on Euclidean distance after log-transformation. Type I (sequential) sum of squares was used with permutation of residuals under a reduced model (9999 permutations). Bold letters indicate significant differences.

Months	*A. digitifera*	*A. spicifera*	*A. digitifera*	*A. spicifera*
	Protein (mg µgDNA^{-1})		**RNA/DNA ratio**	
Mar, Apr	**0.000**	0.830	0.978	0.393
Mar, Aug	**0.000**	**0.000**	**0.000**	**0.042**
Mar, Feb	**0.000**	**0.000**	**0.000**	**0.000**
Apr, Aug	**0.000**	**0.000**	**0.000**	0.137

Apr, Feb	**0.000**	**0.000**	**0.000**	**0.001**
Aug, Feb	**0.002**	0.141	**0.023**	0.128
	Lipid ratio		**ZD (cells cm^{-2})**	
Mar, Apr	0.129	0.976	0.486	0.910
Mar, Aug	**0.000**	0.816	**0.000**	**0.013**
Mar, Feb	**0.000**	0.606	**0.001**	**0.003**
Apr, Aug	**0.000**	0.810	**0.000**	**0.005**
Apr, Feb	**0.002**	0.547	**0.006**	**0.002**
Aug, Feb	0.223	0.725	**0.004**	0.783
	Chl *a* (µg cm^{-2})		**Chl *a* (µg cell^{-1})**	
Mar, Apr	**0.022**	0.189	0.053	0.053
Mar, Aug	**0.000**	**0.000**	**0.000**	**0.000**
Mar, Feb	**0.000**	**0.000**	**0.000**	**0.000**
Apr, Aug	**0.000**	**0.000**	**0.000**	**0.000**
Apr, Feb	**0.000**	**0.000**	**0.000**	**0.000**
Aug, Feb	0.116	0.050	0.081	**0.001**

Table 2: Pair-wise PERMANOVA (p-values) for RNA/DNA ratio, lipid ratio, zooxanthellae density (ZD), chl *a* concentration per surface area and per cell, comparing two species (*A. digitifera* and *A. spicifera*) during four months (March and April 2010 (autumn), August 2010 (winter) and February 2011 (summer) at six different stations at Sandy Bay, Ningaloo Reef. Species, season and station were fixed effects. PERMANOVA was based on Euclidian distance after log-transformation. Type I (sequential) sum of squares was used with permutation of residuals under a reduced model (9999 permutations). Bold letters indicate significant differences.

	A. digitifera				***A. spicifera***			
Stations	**Mar-10**	**Apr-10**	**Aug-10**	**Feb-11**	**Mar-10**	**Apr-10**	**Aug-10**	**Feb-11**
A) RNA/DNA ratio								
1, 2	0.20	0.12	0.11	0.14	0.30	0.63	0.91	
1, 3	**0.03**	0.58	0.35	0.14	0.76	0.62	0.27	
1, 4	0.78	0.22	0.61	0.47	0.21	0.98	0.88	
1, 5	0.24		**0.04**	**0.02**	0.55		0.39	
1, 6	0.45	0.06	**0.04**	**0.03**	0.60	0.40	0.18	
2, 3	0.19	0.26	0.42	1.00	0.67	0.98	0.16	0.32
2, 4	0.21	0.85	0.56	0.62	0.84	0.36	0.97	**0.04**
2, 5	0.81		**0.01**	0.17	0.58		0.21	0.86
2, 6	0.50	0.86	**0.03**	0.86	0.08	0.50	0.06	0.33
3, 4	**0.02**	0.43	0.97	0.53	0.41	0.33	0.25	0.11
3, 5	0.11		**0.04**	0.44	0.79		**0.03**	0.80
3, 6	0.06	0.11	0.06	1.00	0.33	0.57	0.01	0.11
4, 5	0.28		0.07	0.42	0.42		0.30	0.32
4, 6	0.63	0.63	0.06	0.49	**0.04**	0.18	0.10	**0.02**
5, 6	0.62		0.83	**0.04**	0.20		0.64	0.43

B) Lipid ratio								
1, 2	0.05	0.09	0.37	0.89	0.90	0.30	0.74	
1, 3	**0.03**	0.76	0.45	0.57	0.12	0.50	0.47	
1, 4	**0.01**	0.37	0.07	0.21	0.18	0.59	0.67	
1, 5	0.31	0.33	0.17	1.00	0.33	0.68	0.93	
1, 6	0.17	0.93	0.08	0.45	0.22	0.23	0.44	
2, 3	**0.00**	0.13	0.18	0.42	**0.03**	**0.03**	0.66	0.75
2, 4	**0.01**	0.82	0.07	0.07	0.14	0.05	0.95	0.99
2, 5	**0.02**	**0.03**	0.06	0.80	0.39	**0.04**	0.64	0.71
2, 6	**0.03**	0.07	0.07	0.35	0.09	**0.00**	0.17	0.80
3, 4	0.09	0.45	0.26	0.39	**0.02**	0.86	0.69	0.74
3, 5	0.28	0.17	0.45	0.46	**0.04**	0.67	0.44	0.97
3, 6	0.87	0.66	0.32	0.90	0.50	0.32	0.07	0.59
4, 5	**0.03**	0.16	0.58	0.05	0.59	0.82	0.59	0.81
4, 6	0.36	0.33	0.74	0.42	**0.01**	0.18	0.14	0.74
5, 6	0.43	0.30	0.71	0.37	0.05	0.09	0.51	0.59
C) Zooxanthellae density (cells cm^{-2})								
1, 2	0.67	0.97	0.34	0.52	0.89	0.93	0.70	
1, 3	0.67	0.57	0.48	0.26	0.11	0.81	0.35	
1, 4	0.33	0.69	0.95	0.57	0.66	0.39	0.80	
1, 5	0.77	0.42	0.77	0.78	0.73	0.24	0.84	
1, 6	0.73	0.19	1.00	0.12	0.86	0.24	0.71	
2, 3	0.96	0.48	0.97	0.05	0.09	0.81	0.27	0.08
2, 4	**0.01**	0.57	0.33	0.90	0.78	0.46	0.63	0.80
2, 5	0.10	0.26	0.30	0.52	0.59	0.30	0.66	0.58
2, 6	0.15	0.11	0.26	0.15	0.95	0.54	0.16	0.17
3, 4	**0.04**	0.90	0.55	**0.03**	0.13	0.53	0.07	0.06
3, 5	0.18	0.77	0.39	0.06	0.11	0.32	0.08	0.33
3, 6	0.21	0.37	0.50	**0.00**	**0.05**	0.20	**0.01**	**0.02**
4, 5	0.10	0.66	0.84	0.54	0.43	0.66	1.00	0.45
4, 6	0.17	0.34	0.89	**0.02**	0.68	0.07	0.17	0.18
5, 6	0.98	0.50	0.80	**0.02**	0.48	**0.05**	0.19	0.12
D) Chl *a* (µg cm^{-2})								
1, 2	0.53	0.74	0.51	0.41	0.40	0.26	0.92	
1, 3	0.05	**0.00**	0.73	0.44	0.70	0.50	0.17	
1, 4	0.11	**0.02**	0.97	0.76	0.11	**0.01**	0.86	
1, 5	**0.02**	0.10	0.58	0.37	0.06	0.72	0.78	
1, 6	0.26	**0.01**	0.50	0.29	0.73	0.43	0.28	
2, 3	0.22	**0.00**	0.72	0.95	0.57	0.09	**0.01**	0.72
2, 4	0.46	**0.02**	0.54	0.55	0.43	**0.00**	0.66	0.13
2, 5	0.37	0.10	0.22	0.97	0.15	0.46	0.81	0.69
2, 6	0.94	**0.01**	0.26	0.06	0.40	**0.03**	0.07	**0.02**
3, 4	0.78	**0.02**	0.86	0.61	0.17	0.05	0.05	0.33
3, 5	0.60	**0.01**	0.34	0.98	0.05	0.34	**0.02**	0.86
3, 6	0.11	0.06	0.36	0.08	0.82	0.92	**0.00**	**0.01**
4, 5	0.91	0.54	0.55	0.48	0.13	**0.01**	0.56	0.51

4, 6	0.19	0.95	0.63	0.21	0.06	**0.00**	0.07	**0.00**
5, 6	**0.02**	0.42	0.73	**0.04**	**0.01**	0.30	0.17	**0.01**
E) Chl *a* (µg cell^{-1})								
1, 2	0.42	0.71	0.55	**0.04**	0.40	0.14	0.75	
1, 3	**0.01**	**0.01**	0.41	0.17	0.12	0.67	0.21	
1, 4	0.45	0.09	0.74	0.09	0.05	**0.00**	0.31	
1, 5	**0.01**	0.27	0.84	0.15	0.07	0.74	0.59	
1, 6	0.49	0.13	0.35	0.92	0.40	0.71	0.86	
2, 3	0.21	**0.00**	0.71	**0.00**	0.64	0.10	0.21	**0.04**
2, 4	0.95	**0.01**	0.49	0.57	0.43	**0.00**	0.34	**0.02**
2, 5	0.49	0.17	0.64	0.46	0.27	0.05	0.88	0.81
2, 6	0.69	0.06	0.53	0.65	0.64	**0.02**	0.68	0.06
3, 4	0.18	0.15	0.42	**0.01**	0.64	**0.04**	0.70	**0.00**
3, 5	0.18	**0.03**	0.51	**0.00**	0.32	0.86	0.22	**0.02**
3, 6	**0.03**	**0.05**	0.71	0.45	0.23	0.84	0.07	0.78
4, 5	0.40	0.21	0.85	0.82	0.52	**0.00**	0.39	**0.00**
4, 6	0.76	0.36	0.48	0.91	0.13	**0.00**	0.16	**0.00**
5, 6	0.07	0.77	0.43	0.93	0.06	1.00	0.53	**0.04**

Appendix B

Table 1: Pair-wise correlations (p-values) for diel variations of A) effective quantum yield and B) relative electron transport rate. Values were pooled together for both *Acropora spicifera* and *Acropora digitifera*. Analysis was done with PERMANOVA (Primer). e.n.m = evening not measured. Bold letters indicate significant differences.

Season	Aug-10			Feb-11		
Days	1	2	3	1	2	3
A		**Effective quantum yield**				
midnight, noon	0.69	**0.003**	0.643	0.699	**0.012**	**0.021**
midnight, morning	**0.002**	**0.016**	0.52	0.471	**0.01**	0.07
midnight, evening	**0.003**	**0.012**	0.288	0.058	0.67	e.n.m
noon, morning	**0.003**	**0.006**	0.829	0.325	0.77	0.626
noon, evening	**0.009**	**0.003**	0.769	0.109	**0.006**	e.n.m
morning, evening	**0.029**	0.858	0.952	0.058	**0.003**	e.n.m
B		**relative electron transport rate**				
midnight, noon	**0.003**	**0.002**	**0.001**	**0.003**	**0.002**	**0.003**
midnight, morning	**0.003**	**0.003**	**0.002**	**0.001**	**0.001**	**0.002**
midnight, evening			determinator both 0			
noon, morning	**0.003**	0.072	**0.002**	**0.003**	**0.002**	**0.002**
noon, evening	**0.003**	**0.002**	**0.002**	**0.002**	**0.002**	e.n.m
morning, evening	**0.002**	**0.002**	**0.002**	**0.001**	**0.003**	e.n.m

Table 2: Pair-wise correlations (p-values) for diel variations for RNA/DNA ratios. Values were pooled together for both *Acropora spicifera* and *Acropora digitifera*. Analysis was done with PERMANOVA (Primer). e.n.m = evening not measured. Bold letters indicate significant differences.

RNA/DNA ratio							
Season	Aug-10				Feb-11		
Days	1	2	3	4	1	2	3
midnight, noon	0.157	0.962	**0.042**	**0.013**	**0.012**	0.06	**0.049**
midnight, morning	0.31	0.996	**0.036**	0.27	0.479	0.027	0.391
midnight, evening	0.361	0.084	0.07	0.948	0.23	0.053	e.n.m
noon, morning	0.785	0.929	0.694	**0.011**	0.055	0.837	**0.047**
noon, evening	0.621	0.192	0.777	**0.044**	0.523	0.511	e.n.m
morning, evening	0.62	0.126	0.959	0.297	0.461	0.352	e.n.m