The effects of ocean warming and acidification on Corallinaceae coralline algae

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This thesis is presented for the degree of Doctor of Philosophy of The University of Western Australia

School of Earth and Environment
Declaration of Authorship

The work contained herein is entirely my own, except where otherwise stated. For all work that has been published or prepared for publication with other authors, I have the permission of all co-authors to include this work in my thesis and my contributions, as well as those of my co-authors are clearly indicated.

Student Signature

Coordinating Supervisor Signature

Cover images: Clockwise from top left; Sampling Lithophyloideae rhodoliths at Basile Is., Houtman Abrolhos Islands (Photo: Taryn Foster), Hydrolithoideae crustose coralline algae with *Ecklonia radiata* kelp in Marmion Lagoon, Experimental setup at Coral Bay (Photo: Taryn Foster).
Publications arising from this thesis and statement of candidate contribution

This thesis is presented as a series of papers, in accordance with standards set by The University of Western Australia. For experimental chapters which have been published or submitted to be published in peer reviewed journals, my contributions, as well as those of my co-authors are outlined below.

**Short, J., Foster, T., Falter, J., Kendrick, G.A., McCulloch, M.T. 2014.** Coralline algal growth, calcification and mortality following a marine heatwave in Western Australia. Under review in *Continental Shelf Research* (Chapter 2)

The work is primarily my own, including planning and carrying out fieldwork, data analysis, and writing; Taryn Foster has been included as a co-author because she helped with experimental design and fieldwork. Jim Falter and Malcolm McCulloch assisted with experimental design and carbonate chemistry measurements and all co-authors provided advice on data interpretation and provided feedback on the manuscript.


This work is primarily my own, including planning, experimental design and setup, collection and analysis of data and writing; Gary Kendrick, James Falter and Malcolm McCulloch have been included as co-authors as they provided advice on experimental design, seawater measurements and feedback on the manuscript.

**Short, J., Pedersen, O., Kendrick, G.A. 2014.** Turf algal epiphytes metabolically induce local pH increase, with implications for underlying coralline algae under ocean acidification. Under review in *Estuarine, Coastal and Shelf Science* (Chapter 4)

This work is primarily my own, including planning, experimental design and setup, collection and analysis of data and writing. Ole Pedersen has been included as a co-author because he provided valuable assistance with microsensor use. Gary Kendrick has also been included as a co-author as he assisted with planning and experimental design. Both co-authors also provided assistance with data interpretation and feedback on the manuscript.
Abstract

Coralline algae (Rhodophyta, Corallinaceae) are a ubiquitous group of calcifying red macroalgae, which form an integral component of a diverse range of ecosystems worldwide. The effects of environmental change on ecologically important taxa, such as the coralline algae, have major implications for the future fate of marine ecosystems. Understanding how particular habitats will be affected by environmental change is currently of great interest, as this knowledge will contribute to the effective protection and management of the marine environment in the coming decades.

Coralline algae are sensitive to the increases in seawater temperature and CO$_2$ predicted with global climate change due to the negative effects of these changes on the process of calcification. In contrast, fleshy (non-calcifying) algal species can respond in a positive manner to ocean warming and acidification. As such, a shift in dominance from calcifying to fleshy algal species is predicted for many marine communities. Since coralline and fleshy algae occupy many of the same benthic habitats, understanding how differential responses to environmental change will affect the interaction between them is important for predicting future changes in community structure. Thus, the general aims of this thesis were to assess the effects of ocean warming and acidification on 1) coralline algal metabolism and 2) the interaction between coralline algae and fleshy algae.

In 2010-11 there was a marine heatwave off Western Australia, which had lasting impacts on many marine organisms. In chapter two of this thesis growth, calcification and mortality rates were measured for three sub-families within the Corallinaceae (Porolithoideae and Lithophylloideae rhodoliths and Hydrolithoideae crustose coralline algae (CCA)) along ~8° of latitude for 9 to 24 months following the heatwave. High rates of mortality during summer and a lack of seasonal pattern in rates of coralline algal growth and calcification were indicative of thermal stress, however the extent of this was temporally and spatially variable as coralline
algae were influenced by additional environmental factors and community-specific ecological interactions.

Due to the importance of ecological interactions in determining species’ response to environmental change, chapter three investigated the effects of ocean acidification on the interaction between the CCA and an overgrowing assemblage of filamentous turf algae. A factorial aquarium setup was used to test the effects of turf algal overgrowth on coralline algal calcification, photosynthesis and mortality under ambient and elevated CO₂ conditions. Coralline algae alone responded negatively to elevated CO₂, but in the presence of turf algae this response was temporally variable. At times, turf algae facilitated coralline algal calcification under acidified conditions, but overgrowth had negative effects on coralline algal photosynthesis for the majority of the experimental period.

Filamentous turf algae have demonstrated enhanced rates of photosynthesis under acidified conditions, which presumably increases the rate of local CO₂ removal. Chapter four tested the hypothesis that overgrowth by turf algae modifies seawater chemistry within the diffusive boundary layer (DBL) above underlying coralline algal crusts, affecting coralline algal calcification under ocean acidification conditions as observed during the previous experiment. Microsensors were used to measure the effect of turf algae on seawater chemistry within the DBL above Hydrolithoideae crusts under ambient and elevated CO₂. Overgrowth by turf algae significantly modified pH and O₂ in the DBL, resulting in local chemical conditions favouring coralline algal calcification during the day, despite ocean acidification conditions in the surrounding seawater.

Overall, this thesis demonstrates that coralline algae (Rhodophyta, Corallinaceae) are vulnerable to ocean warming and acidification. However, predicting the extent of their susceptibility to these changes is complex as it must be considered together with additional environmental and ecological factors. The studies presented herein highlight the importance of ecological interactions in determining species response to environmental change.
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The sea, once it casts its spell, holds one in its net of wonder forever.

- Captain Jacques-Yves Cousteau
Chapter 1 General Introduction

Sampling Hydrolithoideae crustose coralline algae in Marmion Lagoon, Perth, Western Australia (Photo: Taryn Foster)
1.1 Introduction

Coralline algae (Rhodophyta, Corallinaceae) are a ubiquitous group of red calcifying macroalgae, occurring from the tropics to the poles and from shallow coastal habitats to depths of >200 m (Adey and Macintyre, 1973). They are ecologically vital in a diverse range of benthic marine habitats such as coral reefs and maerl beds, increasing biodiversity by providing substrata as well as chemical cues promoting settlement of other species of algae and invertebrates (Morse et al., 1988; Steneck and Testa, 1997; Adey, 1998; Heyward and Negri, 1999; Vermeij and Sandin, 2008; Peña et al., 2014). They also provide important nursery areas for a range of benthic invertebrates, many of which are commercially important (Kamenos et al., 2004a; b; c). More fundamentally, they contribute to carbonate accretion, and provide structural complexity (Steneck and Adey, 1976; Adey, 1978; Nelson, 2009). Changes in the physical and chemical properties of seawater are predicted in the coming decades due to anthropogenic global climate change, and this will have major impacts on marine organisms. Due to their importance in many benthic communities, coralline algal response to environmental change will play a crucial role in the persistence of such habitats in the near future. This thesis aims to untangle some of the physical, chemical and biotic drivers of coralline algal metabolism, in order to contribute to our understanding of how this group, as well as the habitats in which they occur, will respond to ocean warming and acidification as predicted from global change modeling.

1.2 Climate change

Anthropogenic carbon dioxide (CO₂) emissions are currently affecting the marine environment by inducing changes in the chemistry and temperature of seawater. Approximately one third of atmospheric CO₂ is absorbed by the world’s oceans, inducing shifts in the seawater carbonate equilibrium system (Feely et al., 2004; Orr et al., 2005). The resulting increase in the partial pressure of CO₂ (pCO₂), bicarbonate (HCO₃⁻) and hydrogen (H⁺) ion concentrations in seawater and concomitant reductions in pH and carbonate (CO₃²⁻) ion concentrations are
collectively known as ocean acidification (Caldeira and Wickett, 2003). In addition, the rise in atmospheric CO₂ has caused temperatures at the Earth’s surface to rise (Mitchell, 1989). Marked changes in the seawater carbonate equilibrium system and sea surface temperatures have occurred during the 20th century, with declines of ~30 μmol kg⁻¹ and 0.1 units in mean global seawater CO₃²⁻ concentration and pH, respectively (Karl and Trenberth, 2003), and increases in mean global sea surface temperatures of ~0.7 °C (Raven et al., 2005). Ocean acidification and warming are predicted to accelerate in the near future as atmospheric CO₂ rises at an unprecedented rate from the current level of 396 ppm (2013 annual mean (NOAA, 2014a)) to ~750 ppm in the IPCC A2 scenario by 2100 (Solomon et al., 2007). Understanding the effects of ocean acidification and warming on marine organisms is currently the focus of a large body of research, as it has major implications for the persistence of many ecologically important marine habitats.

1.3 The effects of environmental change on marine organisms

Research to date has shown that the effect of ocean acidification on marine organisms is variable, predicting shifts in the structure and function of marine ecosystems. Decreased rates of growth and calcification, and increased mortality rates have been observed for marine organisms including some species of macroalgae (Connell and Russell, 2010; Price et al., 2011), invertebrates (Gazeau et al., 2007; Ries et al., 2009; Brennand et al., 2010; Barton et al., 2012) and vertebrates (Munday et al., 2009). In contrast, enhanced rates of growth under ocean acidification have been observed for some species of macroalgae (Gao et al., 1999; Kübler et al., 1999), cyanobacteria (Fu et al., 2008; Kranz et al., 2009) and seagrasses (Palacios and Zimmerman, 2007; Hall-Spencer et al., 2008). In addition, the increase in available inorganic carbon can facilitate increased rates of photosynthesis for some marine macrophytes under elevated CO₂ (Borowitzka, 1981a; Semesi et al., 2009; Kroeker et al., 2010; Koch et al., 2013). Such variable responses to ocean acidification may lead to shifts from less tolerant to more tolerant species, resulting in shifts in community structure (Kroeker et al., 2013).
As with ocean acidification, differential susceptibilities to ocean warming among benthic organisms have been observed with increases in seawater temperature, and this can have community-wide implications. As seawater temperatures increase, many species exhibit increased rates of growth and photosynthesis, at least within a normal seasonal range (Adey and McKibbin, 1970; Buddemeier and Kinzie, 1976; Coles and Jokiel, 1978). However, due to warming in the last century, many organisms currently exist at or near their upper thermal limits (Jentsch et al., 2007). As a result, temperatures exceeding normal summer maxima have negative effects on several marine species, including important habitat-forming taxa such as corals (Hoegh-Guldberg and Bruno, 2010), seagrasses (Short and Neckles, 1999) and macroalgae (Agegian, 1985; Anthony et al., 2008). Field studies investigating the effects of extreme high temperature events such as marine heatwaves, have shown that mixed responses to high temperatures between species can lead to shifts in community composition (Seddon et al., 2000) or extensive range contractions (Smale and Wernberg, 2013), which can have permanent effects on ecosystem structure and function.

1.4 Marine calcifiers

Marine calcifiers such as hard corals, calcifying algae, coccolithophores, foraminifera, pteropods, as well as some species of mollusks, arthropods and echinoderms require a specific chemical environment in order to precipitate their mineral skeletons. ‘Calcification’ is the production of biogenic calcium carbonate (CaCO₃) used to build hard skeletons, and relies on the availability of calcium ions (Ca²⁺), CO₃²⁻ and HCO₃⁻, as well as chemical conditions which favour its precipitation (Langdon and Atkinson, 2005; Hurd et al., 2009) (Eq.1). The uptake of atmospheric CO₂ by the oceans reduces the calcium carbonate saturation state (Ω) in seawater. Calcifying organisms are particularly sensitive to ocean acidification because a decrease in carbonate saturation state increases the amount of free energy required for the precipitation of CaCO₃ (Broecker and Takahashi, 1966). As a result, declines in the abundance of calcareous
species have been observed with a decrease in ambient pH in naturally acidified marine waters (Porzio et al., 2011; Kroeker et al., 2012; Kroeker et al., 2013).

\[
\text{(1.1) } \text{Ca}^{2+} + \text{CO}_3^{2-} \leftrightarrow \text{CaCO}_3(s)
\]

Despite the importance of seawater chemistry in regulating calcification rates, this relationship is not always direct due to the influence of additional abiotic and biotic factors. Some marine calcifiers, such as the scleractinian corals, have metabolic control over internal pH, and this can facilitate higher rates of biogenic calcification when external pH or $$\Omega_{Ar}$$ is low (Al-Horani et al., 2003; Cohen and McConnaughey, 2003; McCulloch et al., 2012). Indeed, numerous studies have shown that corals maintain their calcifying fluids at a higher pH than surrounding seawater, buffering their internal environment against external changes (Al-Horani et al., 2003; Trotter et al., 2011; Venn et al., 2011). Laboratory studies in which corals and coralline algae have been subjected to elevated CO$_2$ have shown a higher sensitivity in the coralline algae (Jokiel et al., 2008; Ries et al., 2009). Although it is thought that coralline algae also have the physiological ability to regulate pH at the site of calcification within the cell wall (Borowitzka and Larkum, 1987; McConnaughey and Whelan, 1997), evidence suggests that this is much more limited than it is in the corals.

In addition to the ability to regulate internal pH, coupling between metabolic processes may affect the outcome of environmental change on some marine organisms. For the calcifying autotrophs such as the calcifying algae, or calcifiers harbouring photosynthetic symbionts such as the scleractinian corals, foraminiferans and mollusks, environmental control of metabolism is particularly complex. For example, CO$_2$ produced during calcification may be used for photosynthetic carbon fixation (Gattuso et al., 1999), while photosynthetic production of CO$_2$ can maintain saturating levels of CaCO$_3$ as well as an oxic environment, facilitating calcification (Goreau, 1959; Rands et al., 1992). Indeed, within normal diurnal and seasonal ranges, the increase in photosynthetic rates associated with increases in light and temperature drives a corresponding increase in rates of calcification (Gattuso et al., 1999).
The coupling between calcification and photosynthesis has important implications for marine calcifying autotrophs, as well as the communities in which they occur, in the context of climate change. High levels of irradiance and seawater temperatures cause bleaching in some taxa, such as corals and coralline algae, such that photosynthesis is either inhibited or reduced (Brown, 1997; Irving et al., 2004; Anthony et al., 2008). In turn, reductions in photosynthesis may lead to corresponding decreases in rates of calcification. The processes of calcification and photosynthesis are also closely linked on a community scale, with a strong correlation between net rates of community calcification and photosynthesis (Barnes and Devereux, 1984; Gattuso et al., 1996; Falter et al., 2012; Albright et al., 2013), with the extent of each dependent on the particular community (Gattuso et al., 1999). As such, the overall outcome of environmental change on a single organism appears to depend on the complex interaction between tightly coupled metabolic processes, and this may have important ramifications on a community scale.

### 1.5 Coralline algal response to environmental change

Coralline algae are among the most sensitive marine calcifiers to ocean acidification, and this may be in part due to the solubility of their high-magnesium calcite skeletons compared to that of the aragonite and calcite skeletons produced by other calcifying marine taxa (Feely et al., 2004; Morse et al., 2006; Dupont et al., 2008). Indeed, reductions in coralline algal growth and/or calcification (Agegian, 1985; Anthony et al., 2008; Semesi et al., 2009; Gao and Zheng, 2010; Büdenbender et al., 2011; Hofmann et al., 2012; James et al., 2014; Johnson et al., 2014) and photosynthesis (Gao and Zheng, 2010; Hofmann et al., 2012; Martin et al., 2013) have been observed in the laboratory under elevated CO₂ for multiple species in a range of habitats. Declines in coralline algal recruitment (Kuffner et al., 2008) and increased mortality (Diaz-Pulido et al., 2012a) have also been observed for this group under ocean acidification. However, this response is species-specific and temporally variable with increases in calcification and photosynthesis also reported (Borowitzka, 1981a; Semesi et al., 2009; Martin et al., 2013). Furthermore, increases in seawater temperature within a normal seasonal range generally leads to increased rates of coralline algal photosynthesis and calcification (Martin et
al., 2006; Büdenbender et al., 2011), while seawater temperatures exceeding normal summer maxima result in decreased metabolic rates (Noisette et al., 2013a). Most consistently, the combination of elevated seawater temperature and CO$_2$ have a negative interactive effect on coralline algal metabolism, such that the prognosis for this group under predicted ocean warming and acidification is generally negative (Anthony et al., 2008; Martin and Gattuso, 2009; Diaz-Pulido et al., 2012a; Martin et al., 2013). The variability observed in coralline algal response to ocean warming and acidification suggests that additional local-scale factors may be important in driving species response to environmental change.

1.6 Filamentous turf algae

Like the coralline algae, filamentous turf algae are widespread, occurring in shallow marine habitats worldwide (Virgilio et al., 2006). The term “turfing filamentous algae” has been used to describe a low-lying layer of fleshy algae, however there has been some confusion regarding this term throughout the literature (Connell et al., 2014). Here, I will use the term ‘turf algae’ to describe a multispecies assemblage of green filamentous fleshy algae, ~1-10 cm in height. Turf algae play an important role in the early succession (Irving and Connell, 2006) and degradation of coral reefs and kelp forests (Airoldi, 2003; Diaz-Pulido and McCook, 2003; Gorman et al., 2009; Perkol-Finkel and Airoldi, 2010) and thus, can shape benthic distribution patterns both locally (Virgilio et al., 2006) and regionally (Connell and Irving, 2008). In contrast to the coralline algae, turf algae may benefit from ocean acidification, with the increase in carbon enhancing rates of photosynthesis, growth and reproduction (Connell and Russell, 2010; Kroeker et al., 2010; Connell et al., 2013; Johnson et al., 2014).

1.7 Interactions between turf and coralline algae

Coralline and turf algae often occur within the same marine habitats, interacting with one another in several ways. Coralline algae are frequently overgrown by turf algae and this has mixed implications for the former. In some environments, coralline algae are light limited under
epiphytic turfs (Williams and Carpenter, 1990), and this can result in reduced rates of coralline
algal growth as well as increased rates of mortality (Adey and Macintyre, 1973; Steneck, 1982).
Kendrick (1991) observed a strong negative interaction between crustose coralline algae and
filamentous turf algae in the tropics and the loss of coralline algae was reported as being a
product of turf algal overgrowth and the subsequent building up of sediments. In contrast,
overgrowth by turf algae or shading by macroalgal canopy species is beneficial for coralline
algae under some environmental conditions. Positive effects of overgrowth by Ulva epiphytes
on coralline crusts have been observed in intertidal environments where there is a risk of
desiccation or burial by sediments (Figueiredo et al., 2000). Similarly, shading by kelp canopies
can be beneficial for underlying coralline algae in shallow environments with high levels of
irradiance (Melville and Connell, 2001; Irving et al., 2004). Thus, the nature of the interaction
between coralline and turf algae can be both competitive and facilitative, and this appears to
depend primarily on environmental conditions.

In the context of ocean acidification, the interaction between turf and coralline algae
may be modified by the potential influence of turf algae on micro-environment seawater
chemistry. The diffusive boundary layer (DBL) is a discrete layer of seawater which buffers
underlying organisms, such as coralline algal crusts, from surrounding seawater (Vogel, 1994).
Chemistry within the DBL differs from that of the surrounding seawater and this has
implications for organisms under ocean acidification, particularly the calcifiers. Macroalgae,
including the coralline algae, are known to metabolically modify seawater chemistry within the
DBL, with increases in DBL pH associated with photosynthesis while decreases in pH are
driven by calcification and respiration (Hurd et al., 2011; Cornwall et al., 2013; Cornwall et al.,
2014). However, this has not been investigated on a community scale. Understanding how
organisms such as epiphytic turf algae may modify seawater chemistry within the DBL above
understorey CCA has implications for CCA calcification, and thus for their persistence under
ocean acidification.
1.8 Implications of this work

Coralline algae are extremely important in marine habitats across the globe. Their response to near-future environmental change will have key implications for the resilience of dependent communities and assemblages. This group is currently of particular interest as they appear to be among the most sensitive groups of calcifiers to increases in seawater temperature and $pCO_2$ corresponding to near-future climate scenarios (Jokiel et al., 2008). Despite this, relatively few studies have investigated coralline algal response to *in situ* environmental disturbances such as heatwaves, or naturally acidified seawater (Porzio et al., 2011), such that it is difficult to predict the effects of climate change on the coralline algae in their natural environment.

In addition, the perception of how environmental change affects coralline algae in the laboratory is difficult to extrapolate to their natural environment without knowledge of how such changes will affect ecological interactions, which are paramount in shaping benthic communities. With differential susceptibilities to environmental change among taxa, species interactions may also be affected, modifying the effects of ocean acidification and warming on individual organisms. For this reason species interactions are a recent topic of interest in the context of climate change (Porzio et al., 2011; Johnson and Carpenter, 2012; Kroeker et al., 2012; Connell et al., 2013; Cornwall et al., 2013; James et al., 2014); but additional research is required in this area for a complete understanding of the effects of environmental change on coralline algae and, by proxy, the benthic habitats in which they occur. This project will contribute to our understanding of coralline algal response to environmental change, particularly with respect to the effects of warming in its natural habitat, as well as the effects of ocean acidification on interactions between coralline algae and other members of the benthic community.
1.9 Aims

This thesis aims to contribute to a more complete understanding of the impacts of ocean warming and acidification on the metabolism and ecology of coralline algae. Specifically I aimed to:

- Assess the impacts of a marine heatwave and a period of prolonged warming thereafter on seasonal patterns in coralline algal growth, calcification and mortality, in order to predict the effects of warming on this group in their natural environment (chapter two).
- Examine how the nature of the ecological interaction between coralline algae and an overgrowing assemblage of filamentous turf algae will be affected by ocean acidification (chapter three).
- To closely investigate how overgrowth by turf algae affects the chemical micro-environment within the diffusive boundary layer (DBL) above coralline algal crusts under ambient and elevated CO$_2$ (chapter four).

1.10 Thesis structure

This thesis comprises a general introduction (chapter one) followed by three experimental chapters presented in manuscript format (chapters two through four) and a general discussion (chapter five). The effects of ocean change on coralline algal metabolism and ecology studied in this thesis are outlined in the conceptual model in Fig. 1.1. A large-scale field study is presented in chapter two. This study examines the response and recovery of dominant sub-families of Western Australian Corallinaceae coralline algae (Porolithoideae, Lithophylloideae and Hydrolithoideae) for two years following a marine heatwave along a ~8° latitudinal gradient from the tropical north to the warm temperate south. This study aimed to measure coralline algal response to an in situ heating event in an effort to increase our understanding of how this group will respond to future ocean warming in the natural environment. Calcification patterns were highly variable within and between study locations.
across the latitudinal gradient investigated, inferring local drivers of calcification were as important as the change in oceanographic variables. This led to the laboratory experiment presented in chapter three, which focuses on the possible influence of ecological interactions on coralline algal response to environmental change.

Chapter three investigates the effects of ocean acidification on rates of calcification, photosynthesis, and mortality in the encrusting Hydrolithoideae species investigated in the field study both in the presence and absence of overgrowing filamentous turf algae. The aim of this experiment was to investigate the impacts of ocean acidification on the ecological interaction between the coralline and turf algae. One outcome from this chapter—a positive effect of turf algal overgrowth on coralline algal growth and calcification—was studied in detail in a second laboratory experiment. Chapter four focuses on unraveling the mechanism behind the change in the interaction between Hydrolithoideae crustose coralline algae (CCA) and turf algae under acidified conditions. In this chapter, pH and oxygen microelectrodes were used to describe the chemical environment within the DBL above coralline algal crusts in the presence and absence of epiphytic turf algae. This chapter aimed to investigate the effect of turf algal epiphytes on local seawater chemistry in an effort to understand how this might affect coralline algal metabolism under acidified conditions. Finally, chapter five, the General Discussion, integrates the scales and effects from the previous chapters and discusses the relative importance of species interactions in modifying the effects of environmental change on nearshore temperate reef systems with a focus on the interaction between crustose coralline algae and filamentous turf algae.
Fig. 1.1 Conceptual model of this thesis, summarising how the chapters fit together to describe the role of a series of environmental and ecological drivers of coralline algal response to ocean warming and acidification.
Chapter 2
Coralline algal growth, calcification and mortality following a marine heatwave in Western Australia


Porolithoideae rhodoliths deployed on experimental units at Coral Bay, Western Australia
**Conceptual model** – Chapter two investigates the effects of a marine heatwave together with additional environmental factors on three Corallinaceae sub-families in their natural habitats along the Western Australian coastline.
2.1 Abstract

Coralline algae within the Rhodophyta are ubiquitous components of many benthic habitats and play a critical ecological role by contributing significantly to their structural complexity and diversity. During the austral summer of 2010 - 2011 a “marine heatwave” severely impacted the tropical and temperate reefs along the coast of Western Australia (WA). The heatwave was characterised by anomalously high seawater temperatures throughout that summer and, to a lesser extent, for the two summers thereafter. To investigate coralline algal response to heating we measured growth, calcification and mortality rates for three subtidal Corallinaceae sub-families (Porolithoideae and Lithophylloideae rhodoliths and Hydrolithoideae crustose coralline algae (CCA)), which were the most abundant sub-families at three locations, respectively, spanning ~ 8 degrees of latitude along the WA coastline from winter 2011 to winter 2013, directly following the 2010-2011 heatwave. Seawater carbonate chemistry was also characterized at each study location to facilitate a more complete understanding of the various drivers of coralline algal growth and calcification over the latitudinal range investigated.

Coralline algal growth and calcification were generally within the expected range, however, normal seasonal patterns were not observed. Instead, Porolithoideae and Lithophylloideae growing as rhodoliths had calcification rates that declined substantially during summer and there was a distinct lack of seasonality in temperate Hydrolithoideae CCA growth rates. Together with high mortality during summer these patterns suggested that coralline algae at all locations were experiencing thermal stress. Our results indicate that Western Australian coralline algae are susceptible to prolonged heating events, and the extent of their vulnerability may be dependent on species-specific tolerance levels as well as additional ecological and environmental factors.

2.2 Introduction

Coralline algae are essential components of many benthic habitats such as coral and temperate reefs and form ecologically and economically important maerl beds. They increase
diversity in these habitats by providing substrata, inducing settlement and providing nursery areas for several groups of benthic invertebrates (Morse et al., 1988; Johnson et al., 1991; Lasker and Kim, 1996; Daume et al., 1999; Heyward and Negri, 1999; Steller et al., 2003; Kamenos et al., 2004a; b; c). More fundamentally they also contribute significantly to carbonate production and accretion, and reef consolidation (Adey, 1998; Chisholm, 2003; Nelson, 2009). Habitats dominated by calcareous organisms are sensitive to the effects of ocean warming and acidification on biomineralization. Coralline algal response to climate change is therefore of great importance for the future fate of a diverse range of benthic habitats from the tropics to the poles.

The metabolic rates of most benthic organisms are partially controlled by the physical and chemical properties of seawater. Growth of marine macroalgae is affected by temperature, with optimal growth occurring within the temperature range to which they are most acclimated and sub-optimal growth occurring outside of this range (Fortes and Lüning, 1980). Temperature can also have an interactive effect on macroalgal growth with additional environmental parameters, such as light (Macchiavello et al., 1998). Similarly, rates of growth and calcification in coralline algae are affected by seawater temperature, pH and light intensity (Adey, 1970; Hall-Spencer et al., 2008; Kuffner et al., 2008; Kamenos and Law, 2010). Temperature, in particular, plays a central role in determining the geographic range of coralline algal species (Wilson et al., 2004) and drives temporal and spatial variability in calcification rates (Adey and McKibbin, 1970; Kuffner et al., 2008). In the field, tropical species tend to grow faster than those at higher latitudes (Leukart, 1994) and both encrusting and free-living coralline algal species have been shown to grow faster in summer than in winter (Adey, 1970; Blake and Maggs, 2003; Martin et al., 2006; Kamenos and Law, 2010; Burdett et al., 2011). However, if summer temperature maxima are exceeded, calcification rates can decrease (King and Schramm, 1982; Agegian, 1985). This is important for coralline algae in coral reef habitats, given that many of these already exist at their upper thermal tolerance limit due to recent ocean warming (Hoegh-Guldberg et al., 2007), which makes these habitats vulnerable to extreme climatic events such as heatwaves (Jentsch et al., 2007). Such events can cause range
contractions and shifts in dominance, significantly altering the structure and function of an ecosystem (Aronson et al., 2004; Smale and Wernberg, 2013). Given the importance of the coralline algae in many benthic habitats, their response to heating events will potentially play a major role in the ability of these habitats to persist in the coming decades.

During summer 2010-11 a “marine heatwave” occurred along the coastline of Western Australia (WA) due to strong La Niña conditions across the Indo-Pacific (Feng et al., 2013). As a result, sea surface temperatures from Ningaloo Reef in the north to Cape Naturaliste in the south (Fig. 1) were 3 to 5°C above average (Feng et al., 2013). This caused wide-spread bleaching of coral along the WA coastline (Evans and Bellchambers, 2011; Moore et al., 2012; Depczynski et al., 2013), and temporarily extended the southerly latitudinal range of many tropical and subtropical organisms (Smale and Wernberg, 2013). During the subsequent summers of 2012 and 2013 sea surface temperatures along the WA coast were still above the climatological means, but only by 1 to 2°C.

The main objectives of the present study were 1) to investigate the response of the most abundant coralline algal sub-families within the Corallinaceae at three locations spanning from 23°S to 32°S along the coast of WA to seasonal changes in temperature following the 2010-11 marine heatwave, and 2) to characterize seawater chemistry over the latitudinal and temporal range investigated to understand some of the additional drivers of coralline algal calcification rates at our study locations.
Fig. 2.1 Sea surface temperature anomalies extending from Ningaloo Reef (A) to Cape Naturaliste (B) along the Western Australian coastline from 21 Feb. to 6 March 2011 at the peak of the marine heatwave. Study locations are indicated. Satellite image modified from Feng et al. (2013).

2.3 Materials and methods

2.3.1 Field locations and study organisms

We measured growth and mortality rates for three sub-families of coralline algae within the Corallinaceae (Porolithoideae and Lithophyloideae rhodoliths and Hydrolithoideae crustose coralline algae (CCA)) at three locations, respectively, along the WA coastline; each of these representing a different latitudinal regime (Fig. 2.1). Coral Bay (S23°08’ E113°46’), on the Ningaloo Reef Tract on the northwest coast of WA represented a tropical environment. Our study site at Coral Bay was located at a depth of ~1-2 m just inside the reef break in the fringing reef-lagoon system. For a sub-tropical environment we chose a platform reef at Basile Island in the Southern Group of the Houtman Abrolhos Islands (S28°53’ E113°59’, herein referred to
simply as the Abrolhos Is.), located ~ 60 km off the coast of central WA. This site was located at ~4 m depth just off the reef platform. Finally, Marmion Lagoon (S31°48', E115°42'), a relic fringing reef-lagoon system located on the southwestern coast of WA was selected to represent a temperate environment. The study site at Marmion was located at a depth of ~5 m on the algal-dominated temperate reef. During summer this location became overgrown by the kelp _Ecklonia radiata_, which shaded much of the benthic environment for this period. The three reef systems differ in the degree to which they are influenced by the Leeuwin current (a warm, oligotrophic, and southward flowing boundary current) as well as by latitudinal variations in light and temperature (Hatcher, 1991). Study sites were landward of the surf zone at all locations, protecting them from direct exposure to large waves while still being relatively well-flushed by wave-driven circulation. Corallinaceae sub-families at each location were identified via amplification of the psbA gene at the University of Auckland and are the first known coralline algae from this region to be genetically identified. Because of this, identification to species level was not possible. Porolithoideae and Lithophylloideae rhodoliths were the most abundant Corallinaceae sub-families at Coral Bay and the Abrolhos Is., respectively, while a Hydrolithoideae CCA was the most abundant sub-family at Marmion Lagoon.

### 2.3.2 Coralline algal calcification, growth and mortality rates

We measured calcification rates from changes in skeletal mass using the buoyant weight technique (Jokiel et al., 1978). A total of 16 replicates of the most abundant Corallinaceae sub-family (whole rhodoliths or pieces of CCA ~ 6-12 cm$^2$) were collected from each study location at the beginning of the experiment. However, at both Coral Bay and the Abrolhos Is., there were two morphotypes of the most abundant sub-family. We therefore used 16 replicates of each morphotype in our investigations at both of these locations. Coralline algal replicates were haphazardly collected from the same depth within each study location (~1-2 m at Coral Bay, ~4 m at the Abrolhos Is., and ~5 m at Marmion Lagoon).
Replicates were fixed to plexiglass tiles (64 cm$^2$) using a marine epoxy (Z-spar®, A-788 Splash Zone compound). The replicate tiles were attached to high-density polyethylene (HDPE) plates and mounted on cement blocks, which were haphazardly positioned with respect to one another at the study locations. At approximately four-month intervals between July 2011 and June 2013, the experimental units were collected using SCUBA or by free diving and transported to onshore laboratories in bins of aerated seawater. All visible encrusting and epiphytic organisms were removed and replicate tiles were weighed while freely suspended in seawater below an Ohaus SP402 electronic balance ($\pm$0.01 g). After weighing, the experimental units were redeployed at the same study locations as they were originally collected. Surface areas of algal replicates were determined using a combination of image analysis of overhead photos and the aluminium foil technique as in Marsh (1970). Image analysis was used for CCA during the initial growth intervals since growth was primarily two-dimensional along the experimental tiles. For rhodoliths and for CCA during the later growth intervals the aluminium foil technique was used since growth was three-dimensional. Image analysis was carried out using the software Image J. Due to logistical constraints, our sampling program was cut short at the Abrolhos Is., resulting in calcification measurements for approximately nine months at this location; in contrast to the 21-24 months investigated at Coral Bay and Marmion Lagoon.

We deployed a second cohort of CCA replicates at Marmion Lagoon during summer 2012 to 1) test the effects of initial deployment on rates of growth and calcification by comparing initial growth rates of the new cohort to those of the original cohort, and 2) because by Oct 2012 the first cohort of CCA at Marmion Lagoon had overgrown our experimental tiles. Thus, the second CCA cohort was also used for calculations of growth and calcification rates throughout summer 2012-13. To test the effects of initial deployment on Corallinaceae rhodoliths we deployed a second cohort of Porolithoideae replicates at Coral Bay during summer 2013. In contrast to the CCA species at Marmion Lagoon, the coralline algal sub-families at both Coral Bay and the Abrolhos Is. were free-living rhodoliths and thus did not overgrow the experimental tiles.
Calcification rates were calculated from buoyant weights as in Short et al. (2014). Average marginal extension rates of CCA at Marmion Lagoon were calculated as geometric average rates of radial extension using:

\[
(2.1) \quad E = \frac{\left(\frac{S_f}{\pi}\right)^2 - \left(\frac{S_i}{\pi}\right)^2}{\tau}
\]

Where \(\tau\) is the number of months in a particular growth interval, and \(S_i\) and \(S_f\) are the initial and final surface areas, respectively.

Finally, we calculated percentage mortality at each study location for each Corallinaceae sub-family following each growth period. A replicate was counted as dead if there was no living tissue remaining (complete loss of original pigment) and dead replicates were excluded from all prior and subsequent calculations of growth and calcification.

2.3.3 Environmental variables

Temperature (°C) was measured at each study location every 10 minutes using Hobo U22-002 temperature sensors (Onset Computer Corporation, Water Temp Pro v2), which were attached to the cement blocks containing the experimental replicates ~20 cm above the substrate. To calculate summer temperature anomalies at our study locations, we compared monthly average sea surface temperature (SST) from 2000-2010 (excluding the 2010-2011 marine heatwave) to monthly average SST measured offshore of each study location over the experimental period (NOAA (2014b) 50 km satellite SST data).

Seawater pH was measured on the total scale (pHT) at each study location during each sampling period using a Schott Handylab pH 12 equipped with a Blueline Elektrode. The electrode and meter were calibrated before each field sampling against both seawater (‘Tris’) and NBS buffers. Seawater samples for the analysis of Total Alkalinity (TA ±5 μeq kg \(^{-1}\)) were taken at the same time and measured using the spectrophotometric method of Yao and Byrne (1998). Salinity was estimated from the data provided by Zhang et al. (2012), Lourey &
Kirkman (2009) and Lourey et al. (2006), for Coral Bay, the Abrolhos Is. and Marmion Lagoon, respectively. All other carbonate chemistry parameters derived from our measurements of pH\(_T\), TA and salinity were calculated from these defined input conditions using the CO2SYS program (Lewis and Wallace, 1998) based on pre-defined dissociation and solubility constants (Murray and Riley, 1971b; Dickson and Millero, 1987; Dickson, 1990a; Dickson, 1990b).

To infer the ambient mineral saturation state of high-magnesium calcite (\(\Omega_{Mg\text{calcite}}\)), we measured the skeletal mole fraction of MgCO\(_3\) for five coralline algal samples collected from our study locations during summer 2012 on a Thermo Fisher Scientific (Bremen, Germany) X Series II quadrupole inductively coupled plasma mass spectrometer at the University of Western Australia. Coralline skeletal mineralogy can vary with ambient seawater Mg/Ca and temperature (Ries, 2006; Caragnano et al., 2014). Therefore, we compared coralline algal skeletal mole fraction of MgCO\(_3\) between five samples collected during summer and five samples collected during winter. This comparison was investigated at Marmion Lagoon, as this location experiences the largest seasonal changes in temperature. \(\Omega_{Mg\text{calcite}}\) was then calculated according to:

\[
\Omega_{Mg\text{calcite}} = \left( \frac{\prod_{x} \left\{ \left( \prod_{y} \left[ \left[ Mg^{2+} \right]^{y} \left[ Ca^{2+} \right]^{1-x} \right] \right) \right\} \gamma_{Mg^{2+}} \gamma_{Ca^{2+}} \gamma_{CO_{3}^{2-}} \gamma_{Mg,Ca,CO_{3}^{2-}} }{k_{Mg,Ca,CO_{3}^{2-}}} \right)
\]

where \(x\) is the mole fraction of magnesium versus calcium in the calcite crystal; \(\gamma_{Mg^{2+}}, \gamma_{Ca^{2+}},\) and \(\gamma_{CO_{3}^{2-}}\), are the total ion activity coefficients of magnesium, calcium, and carbonate, respectively (Millero and Pierrot, 1998), and \(k_{Mg,Ca,CO_{3}^{2-}}\) is the ion solubility product of biogenic high-magnesium calcite (Plummer and Mackenzie, 1974).

### 2.3.4 Data analysis

To explore seasonal changes in rates of growth and calcification for coralline algal sub-families at Coral Bay and Marmion Lagoon we used repeated measures ANOVA with time.
(three to four intervals, corresponding to season) as a fixed factor. Post hoc tests were carried out using the Bonferroni correction. A Greenhouse-Geisser correction was used when the assumption of sphericity was violated. Shapiro-Wilk’s tests were used to test the assumption of normality. Calcification rates at the Abrolhos Is. were not normally distributed (Shapiro-Wilk’s test, p < 0.05). Therefore, seasonal changes in Lithophyloideae calcification rates were explored at this location using a Friedman test with Bonferroni-corrected Wilcoxon signed-rank tests for post hoc analysis. Seawater chemistry data were not normally distributed (Shapiro-Wilk’s test, p < 0.05). Kruskal-Wallis tests were therefore used to compare pH, $pCO_2$ and $\Omega_{Mg\text{-calcite}}$ between seasons within each study location. All statistical analyses were completed using SPSS version 20.0.

2.4 Results

2.4.1 Environmental variables

Seasonal ranges of monthly average seawater temperature were 22.3 – 28.3, 20.1 – 25.1 and 16.7 – 24.3 °C at Coral Bay, the Abrolhos Is. and Marmion Lagoon, respectively (Fig. 2.2). Sea surface temperatures were anomalously high during summer 2011-12 at the Abrolhos Is. and during both summer 2011-12 and 2012-13 at Coral Bay and Marmion Lagoon. In summer 2011-12 the largest anomaly was measured at the Abrolhos Is. (+2.3 °C) compared to Marmion Lagoon and Coral Bay, where anomalies of +1.9 and 1.7 °C, respectively, were recorded. In summer 2012-13 temperatures anomalies were +1.7 °C and +2.8 °C at Marmion Lagoon and Coral Bay, respectively (Fig. 2.2). There was some offset between offshore SST and seawater temperature measured at our study sites (1-2°C), likely the result of finer-scale spatial differences in nearshore thermal forcing and climatology (Zhang et al., 2013; Falter et al., 2014). Nonetheless, both offshore and nearshore temperatures followed similar trends and were likely dominated by the same regional-scale ocean warming phenomena (Feng et al., 2003).
Fig. 2.2 Monthly average seawater surface temperature (logger data; coarse dotted line) and NOAA satellite monthly average sea surface temperature (SST; fine dotted line) during the experimental period, as well as long term monthly average SST (NOAA 2000-2010; solid line) at Coral Bay, the Abrolhos Islands and Marmion Lagoon. Grey bars indicate monthly temperature anomalies at all study locations for the duration of the experimental period. Grey rectangles highlight summer growth intervals.
There were no significant differences in pH, $p$CO$_2$, or $\Omega_{\text{Mg calcite}}$ between seasons at any of the study locations. There was, however, a period during late summer 2011-12 at the Abrolhos Is. when ambient pH was 7.93, well below values in equilibrium with the atmosphere (~8.05, Table 2.1). Furthermore, calculated $\Omega_{\text{Mg calcite}}$ were $>1$ at all sites and in all seasons apart from this same period of low pH at the Abrolhos Is. during late summer 2011-12. Thus, ambient seawater was generally saturated with respect to high-magnesium calcite across the latitudinal gradient investigated.

### 2.4.2 Growth and mortality

At Coral Bay, Porolithoideae rhodolith net calcification rates ranged from -0.36 to 0.57 mg cm$^{-2}$ d$^{-1}$ and were highest during early summer 2011-12 (Fig. 2.3A, Table 2.2). Net calcification rates then decreased significantly during late summer 2011-12 with net dissolution rates of $-0.15 \pm 0.07$ mg cm$^{-2}$d$^{-1}$ (mean ± 1 s.e., n= 10, $t(29) = -2.266$, $p = 0.03$) for the remainder of the year (Fig. 2.3A).

At the Abrolhos Is., net calcification rates of Lithophylloideae rhodoliths ranged from -0.09 to 0.90 mg cm$^{-2}$d$^{-1}$ and the highest calcification rates were measured during winter 2011. Net calcification rates then decreased significantly from winter 2011 to summer 2012, remaining constant and not significantly different from zero throughout summer 2011-12 ($t(29) = -0.44$, $p = 0.67$) (Fig. 2.3B, Table 2.2).

At Marmion Lagoon, Hydrolithoideae CCA net calcification rates ranged from 0.34 to 1.30 mg cm$^{-2}$ d$^{-1}$ and were highest during winter 2011. Net calcification rates ($\pm 1$ s.e., n=7) decreased from 1.30 ±0.09 mg cm$^{-2}$ d$^{-1}$ in winter 2011 to 0.45 ±0.05 mg cm$^{-2}$ d$^{-1}$ in early summer 2011-12 and remained relatively stable for the remainder of 2012 (Fig. 2.3C; Table 2.2).
Table 2.1 Seasonal seawater measurements made at Coral Bay, the Abrolhos Islands and Marmion Lagoon including temperature (T, °C) salinity (S), total alkalinity (TA, μmol kg⁻¹), and pH on the total scale. Also shown are calculated values of dissolved inorganic carbon (DIC, μmol kg⁻¹), carbonate ion concentration (CO₃²⁻, μmol kg⁻¹), partial pressure of carbon dioxide (pCO₂, μatm) and high-magnesium calcite saturation state (Ω₅₆₅₉₆).

<table>
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<th>Location</th>
<th>Season</th>
<th>T</th>
<th>S</th>
<th>TA</th>
<th>pH₆</th>
<th>DIC</th>
<th>[CO₃²⁻]</th>
<th>pCO₂</th>
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<td>Early summer 2011-12</td>
<td>23.0</td>
<td>36.1</td>
<td>2331</td>
<td>8.25</td>
<td>1900</td>
<td>297</td>
<td>217</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>Late summer 2011-12</td>
<td>22.8</td>
<td>36.0</td>
<td>2328</td>
<td>8.16</td>
<td>1962</td>
<td>255</td>
<td>303</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>Winter 2012</td>
<td>19.2</td>
<td>35.4</td>
<td>2303</td>
<td>8.12</td>
<td>1999</td>
<td>214</td>
<td>328</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>Early summer 2012-13</td>
<td>20.6</td>
<td>35.6</td>
<td>2306</td>
<td>8.18</td>
<td>1969</td>
<td>235</td>
<td>276</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>Late summer 2012-13</td>
<td>23.5</td>
<td>36.2</td>
<td>2326</td>
<td>8.12</td>
<td>1979</td>
<td>242</td>
<td>324</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>21.3</td>
<td>35.8</td>
<td>2316</td>
<td>8.16</td>
<td>1971</td>
<td>240</td>
<td>294</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>2.0</td>
<td>0.3</td>
<td>14</td>
<td>0.05</td>
<td>40</td>
<td>34</td>
<td>42</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Fig. 2.3 Seasonal variation in average calcification rates (mg CaCO₃ cm⁻² day⁻¹ ± 1 s.e.) of Corallinaceae sub-families at Coral Bay (A, n = 10), the Houtman Abrolhos Islands (B, n = 15) and Marmion Lagoon (C, n = 5/7) from winter 2011 through late summer 2013. Letters on top of bars indicate statistically significant differences as indicated by Tukey’s post hoc tests at the α = 0.05 level.
Marginal extension rates at this location ranged from 0.97 to 4.34 mm month\(^{-1}\) and, in contrast to calcification rates, were greatest during early summer 2011-12, rather than during winter 2011, stabilising thereafter (Fig. 2.4) (Table 2.2). Significantly higher growth rates were measured in the second, relative to the first deployment of Hydrolithoideae CCA at Marmion Lagoon during late summer 2011-12 (calcification: \(t(4.2) = -4.12, p = 0.01\); extension: \(t(10) = -2.796, p = 0.02\)). No differences in mean growth rates were detected between deployments during winter 2012 (calcification: \(t(10) = -1.28, p = 0.23\); extension: \(t(10) = -0.026, p = 0.98\)) such that the second Hydrolithoideae deployment was representative of normal growth from winter 2012 and thereafter. There were no significant differences in rhodolith calcification rates between deployments at Coral Bay during summer 2012-13 (\(t(12) = -0.8, p = 0.44\)).

Coralline algal mortality rates were variable at all study locations. At Coral Bay there was no apparent seasonal pattern but at both the Abrolhos Is. and Marmion Lagoon mortality occurred only during summer (Table 2.3).
Average coralline algal skeletal mole fraction of magnesium calcite (± 1 s.e., n = 5) was 18.62 ± 0.75%, 18.23 ± 1.56% and 19.53 ± 2.71% at Coral Bay, the Abrolhos Is. and Marmion Lagoon, respectively. No significant seasonal variation in skeletal magnesium calcite was detected (as tested for CCA at Marmion Lagoon: t(13) = -0.711, p = 0.490).

2.5 Discussion

Western Australian coralline red algae were impacted by recent warming both during the 2010-11 marine heatwave and for at least two years thereafter. Calcification and growth rates did not follow expected seasonal patterns throughout the study period and mortality rates were highest when temperature anomalies were most pronounced. Together, these observations are strongly indicative of thermal stress for all of the Corallinaceae sub-families investigated. The data presented herein allow for inferences to be made with respect to the effects of ocean warming on coralline algae along the Western Australian coast, and are of particular interest as they represent the first in situ measurements from the eastern Indian Ocean.

The in situ measurements of coralline algal growth and calcification measured in the current study were generally within the expected range based on previous observations in similar environments. In the tropical reef system at Coral Bay the upper calcification rates measured for Porolithoideae rhodoliths were comparable to those measured previously in the field for a tropical Porolithon sp. rhodolith (0.85 mg cm\(^{-2}\)d\(^{-1}\)) (Johansen, 1981). However, rhodoliths at this location also experienced extensive net dissolution towards the end of the experiment. Within the sub-tropical reef system at the Abrolhos Is., calcification rates measured for Lithophyloideae rhodoliths were at times faster than previously measured rates for temperate Lithothamnion spp. (0.01 to 0.39 mg cm\(^{-2}\)d\(^{-1}\)) (Bosence, 1980; Freiwald and Henrich, 1994). Faster rates of calcification would be expected in the sub-tropical environment compared to those previously measured in cooler temperate locations given the dependence of coralline algal growth rates on temperature (Adey, 1970; Leukart, 1994). Nonetheless, net dissolution
was also observed at the Abrolhos Is. during summer. Similarly, calcification rates for the
temperate Hydrolithoideae CCA at Marmion Lagoon were close to previously reported rates for
a shallow water tropical *Hydrolithon onkodes* (~ 1 to 3 mg cm\(^{-2}\)d\(^{-1}\)) and a deeper-dwelling
tropical *Neogoniolithon* sp. (~0.1 mg cm\(^{-2}\)d\(^{-1}\)) (Chisholm, 2000; Payri, 2000). Furthermore,
extension rates measured in Marmion Lagoon were comparable to those measured for a range of
CCA species in a shallow forereef environment in Japan (2.9 to 3.9 mm month\(^{-1}\)) (Matsuda,
1989) and a shallow Caribbean back reef (0.9 to 1.4 mm month\(^{-1}\)) (Adey and Vassar, 1975).

Despite rates of growth and calcification within the expected range, coralline algal growth rates
can be highly variable between species, independent of environmental conditions (Dethier and
Steneck, 2001). Thus, the rates of growth and calcification reported do not necessarily indicate
whether these algae were or were not experiencing significant environmental stress.

Within a normal seasonal range, rates of coralline algal growth and calcification generally
increase when temperature rises, both in the field (Adey, 1970; Adey and McKibbin, 1970;
King and Schramm, 1982; Potin et al., 1990; Leukart, 1994; Blake and Maggs, 2003; Martin et
al., 2006) and in controlled laboratory experiments (Martin and Gattuso, 2009; Noisette et al.,
2013a). Therefore, under a normal seasonal temperature regime growth rates would be
expected to be highest in summer when both light and temperature are maximal. In contrast, we
observed a decrease in rhodolith calcification rates from winter to summer at Coral Bay and the
Abrolhos Is., with some instances of summertime net dissolution. CCA growth and calcification
rates at Marmion Lagoon exhibited no distinct seasonal pattern; however, net calcification was
maintained for the duration of the field study.
Table 2.2 Results of statistical tests performed to compare rates of Corallinaceae growth and calcification over time at each study location. Matching letters indicate no significant differences and contrasting letters indicate significant differences in growth rates between seasons, respectively. NA indicates that no measurements were taken at a particular location during that season. Tests significant at the $\alpha = 0.05$ level are indicated with asterisks.

<table>
<thead>
<tr>
<th>Location</th>
<th>Corallinaceae sub-family</th>
<th>N</th>
<th>df</th>
<th>F/X^2</th>
<th>P</th>
<th>Post hoc comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Winter 2011 Early summer 2011-12 Late summer 2011-12 Winter 2012 Early summer 2012-13 Late summer 2012-13</td>
</tr>
<tr>
<td>Coral Bay</td>
<td>Porolithoideae</td>
<td>10</td>
<td>1.8, 16.5</td>
<td>14.88</td>
<td>&lt;0.001*</td>
<td>NA a b b</td>
</tr>
<tr>
<td>Abrolhos Is.</td>
<td>Lithophylloideae</td>
<td>15</td>
<td>2</td>
<td>23.02</td>
<td>&lt;0.001*</td>
<td>a b b NA</td>
</tr>
<tr>
<td>Marmion</td>
<td>Hydrolithoideae cohort 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcification</td>
<td>7</td>
<td>3, 18</td>
<td>80.69</td>
<td>&lt;0.001*</td>
<td>a b b b NA</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>7</td>
<td>1.4, 8.6</td>
<td>8.69</td>
<td>0.012*</td>
<td>a b ab a NA</td>
</tr>
<tr>
<td></td>
<td>Hydrolithoideae cohort 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcification</td>
<td>5</td>
<td>3,12</td>
<td>10.44</td>
<td>0.001*</td>
<td>NA NA a b b b</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>5</td>
<td>1.77</td>
<td>0.206</td>
<td></td>
<td>NA NA c c c c</td>
</tr>
</tbody>
</table>
Coralline algal calcification rates have been shown to decline with increasing temperature when a thermal optimum is exceeded (King and Schramm, 1982; Agegian, 1985). Given the context of anomalously high temperatures for three summers along the WA coast, counter-seasonal trends in rhodolith calcification rates at Coral Bay and the Abrolhos Is., as well as the lack of seasonal changes in CCA calcification rates at Marmion Lagoon suggest that the corallines were responding to temperatures well above their thermal optima. Furthermore, mortality rates were highest during the summer when absolute temperatures as well as temperature anomalies were the most pronounced. Thus, the marked decline in calcification rates from winter to summer combined with high rates of mortality during summer were indicative of thermal stress at all study locations, particularly at the Abrolhos Is., where temperature anomalies were highest.

Temperature anomaly alone does not fully explain the lack of seasonal trends in coralline algal growth rates measured in the current study, and this is most likely due to the influence of additional environment- or species-specific factors, which may have amplified or ameliorated the stressful effects of higher summer temperatures. Although there was little variation in seawater chemistry over the seasonal and latitudinal gradients investigated, there was one period of relatively low pH and undersaturation of magnesium calcite (7.93 and 0.92, respectively) during late summer 2011-12 at the Abrolhos Is. when net dissolution was observed. Significant

### Table 2.3 Percentage mortality of coralline algae at each study location from winter 2011 through summer 2012-13.

<table>
<thead>
<tr>
<th>Location</th>
<th>Corallinaceae sub-family</th>
<th>Winter 2011</th>
<th>Early summer 2011-12</th>
<th>Late summer 2011-12</th>
<th>Winter 2012</th>
<th>Early summer 2012-13</th>
<th>Late summer 2012-13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coral Bay</td>
<td>Porolithoideae</td>
<td>NA</td>
<td>18.8</td>
<td>19.2</td>
<td>28.6</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Abrolhos Is.</td>
<td>Lithophyloideae</td>
<td>0</td>
<td>25.0</td>
<td>29.2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Marmion</td>
<td>Hydrolithoideae</td>
<td>0</td>
<td>12.5</td>
<td>35.7</td>
<td>0</td>
<td>0</td>
<td>37.5</td>
</tr>
</tbody>
</table>
net dissolution of coralline algal rhodoliths has been observed under similar pH conditions in the laboratory for mixed communities of *Lithophyllum, Hydrolithon* and *Porolithon* spp. (Ries et al., 2009). Although low pH can have a negative and synergistic effect with anomalously high temperatures on coralline algal calcification rates (Martin et al., 2013), such conditions were applicable only to the Abrolhos Is. and only in the late summer of 2011-12.

It is possible that interactions with other members of the benthic community ameliorated the stressful effects of heating on the coralline algae in the current study, and this was likely location-specific. In addition to interactions between environmental controls on coralline algal growth, ecological factors such as species interactions can influence individual response to environmental change. For example, in macroalgal communities understorey species can benefit from the presence of canopy-forming species, which can protect them from the harmful effects of high light and temperature (Figueiredo et al., 2000; Melville and Connell, 2001). However, the nature of this interaction is dependent on environment-specific, small-scale processes and reductions in understorey species richness and biomass associated with macroalgal canopies have also been observed (Toohey et al., 2004; Toohey and Kendrick, 2008). Garrabou & Ballesteros (2000) did not see a clear seasonal pattern in calcification rates in the Mediterranean crustose corallines *Mesophyllum alterans* and *Lithophyllum frondosum* due to the shading of coralline crusts by epiphytic filamentous algae in spring and summer, presumably due to a dampening of seasonal variation in light reaching the coralline algal thalli. An increase in epiphytic algae was not observed at Marmion lagoon, but experimental units were shaded during summer by extensive beds of the canopy-forming kelp *Ecklonia radiata*, which can facilitate growth and survival of understorey encrusting coralline algae (Melville and Connell, 2001). Therefore, the presence of kelp canopies may have allowed for normal rates of crustose coralline algal growth in Marmion Lagoon, despite the unusually warm temperatures. Neither shading by macroalgae nor overgrowth by epiphytic turf algae were evident at the other study locations, which may explain the counter-seasonality observed in rates of Porolithoideae and Lithophylioideae calcification at Coral Bay and the Abrolhos Is.
We have observed unexpected seasonal growth patterns and high mortality rates in Western Australian red coralline algae during periods of anomalously high summer temperatures from 2011-2013. The lack of a clear seasonal pattern in rates of growth and calcification stands in stark contrast with other field measurements as well as expectations based on laboratory experiments. Furthermore, the high rates of mortality of mid-latitude rhodoliths and higher-latitude CCA during summer suggest that physiological stress due to summer temperature anomalies was at least partially responsible. Thus, our results indicate a negative effect of recent heating on Western Australian coralline algae within the Corallinaceae, but additional measurements taken during normal temperature years are required for a full understanding of the magnitude of this effect, and the relative influence of additional factors such as irradiance and interactions with other members of the benthic community. Regardless, \textit{in situ} studies provide the essential first steps towards an accurate assessment of response to environmental change. Given the critical ecological role of coralline algae, our results suggest that continued ocean warming and associated heating events may have major consequences for the sustainability of reef habitats worldwide.
Chapter 3

Interactions between filamentous turf algae and coralline algae are modified under ocean acidification

J. Short, G. A. Kendrick, J. Falter and M.T. McCulloch

Hydrolithoideae crustose coralline algae in experimental setup at Fisheries Research Laboratories, Hillarys, Western Australia (Photo: Liza Roger)
Conceptual model – During the field study in chapter two, it was hypothesized that coralline algal response to environmental disturbance was modified by interactions with other species of macroalgae. Chapter three investigates the importance of ecological interactions in determining coralline algal response to environmental change in the laboratory by exploring the effect of elevated CO$_2$ on the interaction between the temperate Hydrolithoideae crustose coralline alga from Marmion Lagoon and an overgrowing assemblage of green filamentous turf algae.
3.1 Abstract

Ocean acidification is a decrease in seawater pH and carbonate ion concentration due to sustained uptake of anthropogenically derived atmospheric carbon dioxide by the world’s oceans. This has major implications for many marine organisms, particularly the calcifiers. Crustose coralline algae (CCA) are among the most sensitive calcifying organisms to ocean acidification. In contrast, filamentous turf algae, which compete with CCA for space on the substratum, could benefit from high CO$_2$ conditions, suggesting that the negative effects of filamentous turf on coralline algae may be amplified in a high CO$_2$ environment. The effect of ocean acidification on the growth of coralline algae, however, has rarely been investigated in combination with ecological interactions such as competition with filamentous turfing algae. Here we tested the combined effects of ocean acidification and overgrowth by filamentous turf algae on CCA calcification, photosynthetic capacity and quantum yield of photosynthesis. We observed a positive effect of algal turfs on CCA calcification but a negative effect on photosynthesis in the high CO$_2$ treatment; however, these effects were variable over time. Our results have demonstrated the importance of investigating how inter-species interactions such as competition will modify the impacts of ocean acidification on individuals.
3.2 Introduction

Coralline algae are ubiquitous components of euphotic benthic communities from the tropics to the poles and from the intertidal to the deepest recorded depths for a photosynthetic organism. They require seawater conditions favouring the precipitation of calcium carbonate during the process of biogenic calcification for normal growth and production, and as the partial pressure of carbon dioxide ($pCO_2$) in seawater increases, the saturation state of carbonate minerals decreases, adversely affecting the metabolic process of calcification (Hoegh-Guldberg et al., 2007). As such, higher $pCO_2$ levels negatively affect coralline algal recruitment, growth, mortality, productivity and calcification (Anthony et al., 2008; Kuffner et al., 2008; Diaz-Pulido et al., 2012a; Martin et al., 2013). Indeed, ocean acidification threatens the growth of these important organisms more so than other calcifying organisms such as aragonite-forming corals (Anthony et al., 2008; Jokiel et al., 2008; Ries et al., 2009), potentially due to the high solubility of their magnesium calcite skeletons (Feely et al., 2004; Morse et al., 2006) and possibly their biological response to changing seawater pH (McCulloch et al., 2012).

Crustose coralline algae (CCA) provide substrata for the settlement and growth of many marine invertebrates and seaweeds (Borowitzka et al., 1978; Morse, 1996; Adey, 1998). Space on primary substratum in benthic marine environments is often limiting (Sebens, 1986) and competition for this space, or its associated resources, leads to the exclusion of all but a small number of competitively dominant species (Connell, 1961). Overgrowth is a frequent mechanism of interference between space-limited organisms (Sebens, 1986; Olson and Lubchenco, 1990) and this is presumed to be a disadvantage for underlying species since epibionts may reduce light, $O_2$ and nutrients available to the underlying host, resulting in reduced growth, fecundity or death (Seed and O'Connor, 1981; D'Antonio, 1985; Stevens, 1987; Dittman and Robles, 1991; Harris, 1996). Indeed, recruitment and growth of CCA can be heavily impacted by overgrowth of filamentous turfs (Underwood, 1980; Sebens, 1986; Kendrick, 1991), and CCA are often considered subordinate in their capacity to compete for space (Littler and Littler, 1980; Dethier, 1994; Steneck and Dethier, 1994). In contrast, when
irradiance levels are high or when there is a risk of desiccation (in intertidal environments), overgrowth by turf algae or shading by macroalgal canopy species can be beneficial for underlying corallines, providing protection from harmful environmental conditions (Figueiredo et al., 2000; Melville and Connell, 2001). Thus, the nature of the interaction between turf and coralline algae is variable, depending primarily on environmental conditions.

Despite the implications, few studies have investigated the potential effects of ocean acidification on ecological interactions (Kroeker et al., 2010; Johnson and Carpenter, 2012), which may define the fate of many species in the coming decades. In contrast to the CCA, the growth and metabolism of filamentous turf algae may increase under high pCO₂ conditions (Levitan et al., 2007; Kuffner et al., 2008; Tribollet et al., 2009; Diaz-Pulido et al., 2012a). As such, the effects of filamentous turf on coralline algae may be synergistic with those associated with a high pCO₂ environment. Here we examined the effects of ocean acidification in combination with overgrowth by filamentous turf algae on calcification, photosynthesis and mortality of a temperate Hydrolithoideae crustose coralline alga. We hypothesized that in the absence of competitive interactions, CCA calcification and productivity would decrease and mortality rates would increase under elevated pCO₂. In a light-limited environment representative of a temperate fringing reef during autumn and early winter, we expected a negative effect of overgrowth by filamentous turf on coralline algae, thus, in the presence of filamentous turf we anticipated that the negative effects of elevated pCO₂ would be amplified.

3.3 Materials and methods

3.3.1 Field site and sample collection

In April 2013, CCA ‘chips’ were collected using a hammer and chisel at ~5 m depth at Whitford Rock (S31°48’, E115°42’) at Marmion Lagoon, a relic fringing reef-lagoon system located on the southwestern coast of Western Australia. CCA chips collected were members of the Corallinaceae sub-family Hydrolithoideae, which is the dominant Corallinaceae sub-family
at Marmion Lagoon. Ambient $pCO_2$ at Whitford Rock ranges from ~210 - 400 ppm seasonally and sea surface temperatures range seasonally from ~16 - 24 °C (Smale and Wernberg, 2009).

CCA chips collected from the reef were transported in bins of seawater to an onshore laboratory, where they were cut into pieces ~3 to 15 cm$^2$ and cleaned of all visible epiphytes. CCA chips were then allowed to acclimate in aquaria under ambient conditions for three weeks prior to the application of experimental treatments. At the end of the acclimation period epiphytes were removed from all CCA chips.

### 3.3.2 Experimental design

To explore the potentially interactive effects of ocean acidification and filamentous turfing algae on CCA, a flow-through aquarium system was set up at the Department of Fisheries Research Laboratories in Hillarys, WA. The system comprised of twelve 25-L aquaria, each containing 20 CCA chips, receiving seawater from two 200-L header tanks (Fig. 3.1). Seawater entering the system was pumped directly from Marmion Lagoon and filtered to 30 μm. Turnover time of water within each treatment aquarium was ~ 90 minutes. Independent variables were (1) $pCO_2$ (ambient: ~300 ppm & high: ~900 ppm, chosen to represent the SRES worst-case scenario (RCP8.5) by 2100) and (2) the presence or absence of filamentous turf algae. The result was a fully factorial set up with four distinct treatments of three replicate aquaria each: (i) Ambient $pCO_2$ without filamentous turf (300), (ii) Ambient $pCO_2$ with filamentous turf (300 + F), (iii) High $pCO_2$ without filamentous turf (900) and (iv) High $pCO_2$ with filamentous turf (900 + F).

A CO$_2$ control system (Aqua Medic) was used to increase $pCO_2$ in the ‘high $pCO_2$’ treatments by direct injection of pure (food grade) CO$_2$ gas. To control the level of filamentous turf algae, all visible epiphytes were removed from CCA chips twice weekly with a soft brush in
Fig. 3.1 Experimental setup with two $pCO_2$ conditions (300 and 900 ppm) and two levels of filamentous turf algae (F; present and absent). Arrows indicate the direction of water flow from mixing tanks into treatment aquaria and exiting the system. Each aquarium contained 20 crustose coralline algal chips.

6 of the 12 treatment tanks, while filamentous turf was allowed to colonise CCA chips in the remaining six tanks over the course of the experiment. Filamentous turf algae established during this period were a mixture of thin upright filaments of ~80% *Cladophora* sp. and ~20% *Ulva* sp. (J. Huisman, personal communication), ~1-10 cm tall, resembling Figure 2E in Connell et al. (2014). We conducted the experiment for 10 weeks during late autumn - winter 2013 (May through July).

### 3.3.3 Seawater measurements

Downwelling PAR irradiances of ~ 100 μmol photons m$^{-2}$ s$^{-1}$ were maintained using Maxspect s-series LED lights on a diurnal cycle with 10 hours of light and 14 hours of darkness to reflect the low-end of light conditions and natural day length during winter at Marmion Lagoon. Ambient temperature was maintained in all treatment tanks over time. In order to
represent natural seasonal variation, temperature was allowed to fluctuate over time, mimicking that at Marmion Lagoon. pH on the total scale ($pH_T$) was measured three times weekly in each of the treatment aquaria and header tanks just prior to midday using a Schott Handylab pH 12 equipped with a Blueline Elektrode. The electrode and meter were calibrated before each sampling day against seawater (‘Tris’) and NBS buffers. Salinity ($\pm 1$) was measured once per week in both header tanks using a portable refractometer. Samples for the analysis of total alkalinity (TA $\pm 5$ μmol kg$^{-1}$) were taken once per week in each treatment aquarium. These were filtered using glass fibre filters with 0.7 μm nominal pore size (Whatman GF/F), collected in Nalgene HDPE containers and stored on ice. These samples were transported back to the laboratory at the University of Western Australia and analysed using a modified version of the spectrophotometric approach developed by Yao & Byrne (1998). All other carbonate chemistry parameters, including $pCO_2$ and saturation state of aragonite ($\Omega_{Ar}$; because the solubility of aragonite is closer to that of high magnesium calcite than calcite), were derived from our measurements of TA and $pH_T$ and calculated from defined input conditions using the CO2SYS program (Lewis and Wallace, 1998) based on the dissociation and solubility constants of (Murray and Riley, 1971a; Dickson and Millero, 1987; Dickson, 1990a; Dickson, 1990b).

3.3.4 Biological response variables

3.3.4.1 Calcification and growth

Skeletal growth rates were measured for 5 of the 20 CCA chips from each treatment using a modification of the buoyant weight technique (Jokiel et al., 1978). This technique assumes that the density of organic (non-skeletal) material approaches that of seawater, such that, in the current study, buoyant weights are a measure of CCA skeletal weight. CCA chips were weighed while freely suspended in a twine basket in seawater below an Ohaus SP402 electronic balance (±0.01 g). Skeletal weights were calculated according to:

$$M_{CaCO_3} = M_{sw} \left( \frac{\rho_{CaCO_3}}{\rho_{CaCO_3} - \rho_{sw}} \right)$$
Where $M'_{sw}$ is the mass of a replicate in seawater (buoyant weight), $\rho_{sw}$ is the density of seawater calculated from temperature and salinity, and $\rho_{\text{CaCO}_3}$ is the density of the dominant skeletal mineral (2.71 g cm$^{-3}$ for high-magnesium calcite). Calcification rate was then calculated according to:

$$G_{\text{net}} = \frac{2\Delta M_{\text{CaCO}_3}}{\tau(S_i+S_f)}$$

Where $\tau$ is the number of days between buoyant weight measurements, and $S_i$ and $S_f$ are the initial and final bioactive surface area of the chip. Surface areas were measured using image analysis at the beginning and end of the experimental period and linearly interpolated in between. Calcification rate was measured weekly on the same five replicates from each aquarium, for a total of 15 replicates per treatment.

### 3.3.4.2 Photosynthetic performance

Measurements of photosynthetic performance were carried out during weeks 5 and 9 on five haphazardly selected CCA chips from each treatment aquarium using a diving PAM fluorometer (Walz, Germany). Measurements of the quantum yield of photosystem II (PSII) were determined as in Genty et al. (1989):

$$Y = (F'/m - F)/F_m$$

These measurements were made after dark-adapting the CCA chips for a minimum of 20 minutes. Quantum yield is useful for evaluating reductions in coralline algal PSII activity caused by environmental stressors (Wilson et al., 2004). Rapid light curves were also generated and used to calculate maximum electron transport rate, or photosynthetic capacity (ETR$_{\text{max}}$), which is linearly correlated with photosynthetic oxygen evolution in crustose corallines (Kühl et al., 2001). For CCA chips in 300 + F and 900 + F treatments, a small area of epiphytic turf algae
was cleared from the surface of the CCA immediately before measurements were taken such that CCA photosynthesis alone was considered in all treatments.

3.3.4.3 Partial tissue mortality

Partial tissue mortality of CCA chips was assessed after terminating the experiment following week 10. The five CCA chips from each treatment tank used for calcification measurements were cleaned of all visible epiphytes and photographed. Mortality rates were determined using image analysis and were defined as the percentage of CCA tissue that was completely white. All image analysis was carried out using ImageJ.

3.3.4.4 Epiphyte cover

Percent cover of filamentous turf algae was assessed from a subsample of 12 haphazardly selected CCA chips from 900 + F and 300 + F treatments during weeks 5 and 10. The software Coral Point Count (Kohler and Gill, 2006) was used to overlay 15 randomly positioned points onto an in situ photo of each CCA chip investigated. The group of algae (CCA chip or filamentous turf) under each point was recorded.

3.3.5 Data analysis

For CCA calcification, mortality and photosynthetic performance a nested ANOVA was initially used with treatment as a fixed factor, treatment tanks as replicates and CCA chips nested within tanks. Because the tank factor was insignificant, tanks were pooled in subsequent analyses and CCA chips were used as replicates in order to increase the power of analysis. The effects of $p$CO$_2$ together with the presence of filamentous turf algae on calcification of CCA chips over the course of the experimental period were assessed using repeated measures ANOVA with $p$CO$_2$ (ambient and high), level of filamentous turf (present or absent) and time (five time periods) as fixed factors. Growth intervals of interest were further explored using ANOVA and significant differences between treatment means within a single growth interval were further examined with Tukey’s HSD post hoc tests. The effects of $p$CO$_2$ and filamentous
turf on CCA mortality rates were tested using ANOVA. Measures of photosynthetic performance were analysed using ANOVA with $pCO_2$ and level of filamentous turf as fixed factors for each time point (weeks five and nine). When necessary, data were transformed to meet the assumptions of normality and homogeneity of variance as indicated by Shapiro-Wilk’s and Levene’s tests, respectively. For repeated measures ANOVA, a Greenhouse-Geisser correction was applied if the assumption of sphericity was not met, as indicated by Mauchly’s test of sphericity. Calcification rates were not normally distributed and normality was not achieved through transformation (Shapiro-Wilk’s test, $P < 0.05$). Nonetheless, we carried out ANOVA analysis on these data since ANOVA is robust to deviations from normality (Zar, 1999). For CCA calcification, homogeneity of variance was not fully achieved through transformation (Levene’s test, $\alpha < 0.05$). To account for this, we adopted a more conservative $\alpha$ ($\alpha_{critical} = 0.01$) for these analyses. Due to equipment failure issues in our experimental system during weeks 3-4 and 6-7, wherein pump failure resulted in briefly elevated temperature and pH in all treatments, and skewed CCA calcification response for those periods, we did not consider these time intervals in any of our analyses. All statistical analyses were conducted using SPSS (version 20.0).

3.4 Results

3.4.1 Seawater properties

Total alkalinity did not vary significantly between treatments (ANOVA, $F(3,120) = 0.729, P = 0.537$) and ranged from 2303 to 2344 $\mu$mol kg$^{-1}$ over the course of the study (Fig. 3.2A). Similarly, pH was maintained at relatively constant value of $7.7 \pm 0.02$ (mean $\pm$ 1 s.d) in the high $pCO_2$ treatments and $8.1 \pm 0.05$ in the ambient $pCO_2$ treatments (Fig. 3.2B). Thus, we were able to maintain $pCO_2$ of $927 \pm 62$ and $\Omega_{Ar}$ of $1.53 \pm 0.1$ in the high $pCO_2$ treatments, and $pCO_2$ of $327 \pm 48$ ppm and $\Omega_{Ar}$ of $3.27 \pm 0.2$ in the ambient $pCO_2$ treatments throughout the course of the experiment (Fig. 3.2C, D).
**Fig. 3.2** Seawater carbonate chemistry observed just prior to midday. Total alkalinity (A), pH (B), $p$CO$_2$ (C) and aragonite saturation state ($\Omega_{Ar}$) (D) in the four experimental treatments are shown. Alkalinity samples were taken from each treatment aquarium once per week and linearly interpolated in between for $p$CO$_2$ and $\Omega_{Ar}$ calculations. Data are means ± 1 s.d. (n = 3 tanks).
Temperature did not vary significantly between experimental treatments (ANOVA, F(3, 355) = 0.061, P=0.980) and ranged from 22.3 to 16.5°C, cooling by ~3°C on average over the course of the experimental period in accordance with seasonal changes in seawater temperature at Marmion Lagoon (R^2 = 0.62, P <0.001) (Fig. 3.3).

3.4.2 Biological response to experimental treatments

3.4.2.1 Filamentous turfs

Percent cover of filamentous turfs increased over time in 300 + F and 900 + F treatments. After five weeks, epiphyte cover was significantly higher on CCA chips in 900 + F aquaria, with 43 ± 4% compared to 24 ± 5% cover in 300 + F treatment aquaria (t(22) = 2.914, P= 0.008). By week 10 of the experiment there was no significant difference in epiphyte cover between 900 + F (69 ± 5 %) and 300 + F (70 ± 7 %) treatment aquaria (t(22) = -0.064, P = 0.95).
3.4.2.2 CCA calcification, growth and partial tissue mortality

Mean net calcification rates of CCA chips ranged from -1.32 to 1.27 mg CaCO$_3$ cm$^{-2}$ day$^{-1}$ (Fig. 3.4). Net calcification of CCA chips in ambient $p$CO$_2$ tanks was recorded for the duration of the experimental period while net calcification of CCA chips in high $p$CO$_2$ treatments was recorded for the first two weeks of the experimental period only, with net dissolution measured thereafter (Fig. 3.4). A significant negative effect of high $p$CO$_2$ on calcification was detected over the course of the experiment, and mean net calcification rate ($\pm$ 1 s.e.) of CCA chips in the ambient $p$CO$_2$ aquaria was 0.44 ± 0.08 compared to -0.12 ± 0.09 mg CaCO$_3$ cm$^{-2}$ day$^{-1}$ in the high $p$CO$_2$ aquaria. In contrast, the effect of filamentous turf algae on CCA calcification was variable over time (Fig. 3.4, Table 3.1).

**Fig. 3.4** Average calcification rates (mg CaCO$_3$ cm$^{-2}$ day$^{-1}$ $\pm$ 1 s.e., n= 15 chips) of crustose coralline algal chips in four experimental treatments measured every 2 weeks* for 10 weeks during late autumn – winter 2013. *weeks 6-7 and 3-4 removed due to equipment failure.
Filamentous turf algae had no significant effect on coralline algal calcification rates for the first three weeks of the experimental period, but from weeks four through six there was a positive effect on coralline calcification rates in high $pCO_2$ tanks only ($F(3.59) = 8.972$, $P < 0.001$, Tukey’s HSD post hoc: $900+F > 900$). For the remainder of the experiment there was no significant effect of filamentous algae on calcification rates (Fig. 3.4).

Partial tissue mortality was $11.6 \pm 3.9 \%$ and $3.2 \pm 0.9 \%$ in 300 and 300 + F treatments, respectively, and $13.9 \pm 6.5 \%$ and $9.6 \pm 1.9 \%$ in 900 and 900 + F treatments, respectively. There were no significant differences in CCA mortality rates between treatments (Table 3.1).

### 3.4.2.3 CCA Photosynthetic performance

During week five, average quantum yield was significantly higher for CCA in all aquaria excluding filamentous algae (300, 900 treatments) relative to those with filamentous algae included (300 + F, 900 + F), with average quantum yield values ($\pm$ 1 s.e.) of $0.30 \pm 0.01$ and $0.37 \pm 0.02$, respectively (Fig. 3.5, Table 3.1). There were no significant effects of $pCO_2$ on the quantum yield of photosynthesis and no significant differences in photosynthetic capacity ($ETR_{max}$) between treatments at this time (Table 3.1). During week nine there was no significant effect of $pCO_2$ level alone on the measured photosynthetic parameters but there was a significant interactive effect of the level of filamentous algae with $pCO_2$ on both quantum yield and $ETR_{max}$ (Table 3.1). For both weeks five and nine CCA photosynthetic parameters increased in ambient aquaria when filamentous algae were present compared to when the latter were absent; whereas the opposite was observed when CO$_2$ was high, with reductions in photosynthetic parameters when filamentous algae were present compared to CCA alone (Figs. 3.5 & 3.6).
Table 3.1 Summary of results and conclusions from ANOVAs used to test the effect of $pCO_2$ and filamentous turf algae (turf) on crustose coralline algal calcification, photosynthesis and mortality. Data transformations are indicated. Insignificant interaction terms were excluded from the final statistical model.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>N</th>
<th>df</th>
<th>$F$</th>
<th>$P$</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calcification</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pCO_2$</td>
<td>1</td>
<td></td>
<td>20.182</td>
<td>&lt;0.001*</td>
<td>Ambient $pCO_2$ &gt; high $pCO_2$.</td>
</tr>
<tr>
<td>Turf</td>
<td>1</td>
<td></td>
<td>0.022</td>
<td>0.882</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>4</td>
<td></td>
<td>7.786</td>
<td>&lt;0.001*</td>
<td>Calcification rate decreased over time.</td>
</tr>
<tr>
<td><strong>Quantum yield of photosynthesis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 Week 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pCO_2$</td>
<td>30</td>
<td>1</td>
<td>2.448</td>
<td>0.123</td>
<td>Turf absent &gt; turf present.</td>
</tr>
<tr>
<td>Turf</td>
<td>1</td>
<td></td>
<td>20.993</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td><strong>Week 9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pCO_2$</td>
<td>30</td>
<td>1</td>
<td>&lt;0.001</td>
<td>0.995</td>
<td>Turf absent &gt; turf present.</td>
</tr>
<tr>
<td>Turf</td>
<td>1</td>
<td></td>
<td>0.046</td>
<td>0.830</td>
<td>When $pCO_2$ is high: Turf absent &gt; turf present; when $pCO_2$ is ambient: Turf absent &lt; turf present.</td>
</tr>
<tr>
<td>$pCO_2$ x Turf</td>
<td>1</td>
<td></td>
<td>12.548</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td><strong>Photosynthetic capacity</strong> (ETR$_{max}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pCO_2$</td>
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<td>1</td>
<td>0.066</td>
<td>0.798</td>
<td>Turf absent &gt; turf present.</td>
</tr>
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<td>1</td>
<td></td>
<td>4.769</td>
<td>0.033*</td>
<td></td>
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<tr>
<td><strong>Week 9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>$pCO_2$</td>
<td>30</td>
<td>1</td>
<td>2.111</td>
<td>0.152</td>
<td>Turf absent &gt; turf present.</td>
</tr>
<tr>
<td>Turf</td>
<td>1</td>
<td></td>
<td>1.398</td>
<td>0.242</td>
<td>When $pCO_2$ is high: Turf absent &gt; turf present; when $pCO_2$ is ambient: Turf absent &lt; turf present.</td>
</tr>
<tr>
<td>$pCO_2$ x Turf</td>
<td>1</td>
<td></td>
<td>7.21</td>
<td>0.01*</td>
<td></td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pCO_2$</td>
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<td>1.422</td>
<td>0.238</td>
<td>No differences.</td>
</tr>
<tr>
<td>Turf</td>
<td>1</td>
<td></td>
<td>3.048</td>
<td>0.087</td>
<td></td>
</tr>
</tbody>
</table>

Data transformations:  
1. $\log (x + 3.2)$  
2. $\log (x)$  
3. $\sqrt{x}$
Fig. 3.5 Average quantum yield of photosynthesis (± 1 s.e., n= 15 chips) for crustose coralline algal chips in the four experimental treatments measured during weeks five and nine of the experimental period.

3.5 Discussion

We observed a significant response to $p$CO$_2$ level, with CCA calcification rates consistently lower in high $p$CO$_2$ treatments, and dissolution only occurring when $p$CO$_2$ was elevated. Thus, the effect of higher $p$CO$_2$ had a significant negative impact on CCA calcification, a finding that is consistent with previous studies (Gao et al., 1993; Anthony et al., 2008; Jokiel et al., 2008; Semesi et al., 2009; Gao and Zheng, 2010; Büdenbender et al., 2011;
Diaz-Pulido et al., 2012a; Johnson and Carpenter, 2012; Noisette et al., 2013a; Noisette et al., 2013b). However, in the presence of other photosynthetic members of the benthic community this effect can become dynamic and complex. The presence of filamentous turfs altered the relationship between $pCO_2$ level and CCA calcification in a manner that was not consistent over time. Unexpectedly, the main effect of overgrowth by filamentous turf algae was not entirely negative and, at times, the interaction between high $pCO_2$ and filamentous turfs indicated a positive effect of algal turfs on CCA calcification.

For two weeks in the middle of the experimental period (weeks four through six) there was a significant increase in calcification rates in the 900 + F treatment. Filamentous algae may increase local pH, via CO$_2$ removal through photosynthesis (Levitan et al., 2007; Tribollet et al., 2009). This mechanism may have facilitated higher rates of CCA calcification by effectively increasing the carbonate saturation state of the local seawater environment. In contrast, CCA recruitment and growth can be reduced when overgrown by filamentous turf algae (Underwood, 1980; Sebens, 1986; Kendrick, 1991), and CCA are known to be poor competitors for space in the rocky intertidal and subtidal (Littler and Littler, 1980; Dethier, 1994; Steneck and Dethier, 1994). CCA dissolution during the final weeks of the experimental period was exacerbated by the presence of filamentous turf algae, indicating that competition for space-associated resources such as light ultimately became more detrimental as turf algal cover increased, presumably beyond some critical threshold. We have observed a variable, temporally dependent effect of filamentous turf on CCA calcification, which appears to depend on the amount of epiphytic overgrowth as well as the $pCO_2$ of seawater. These results indicate a shift in the interaction between filamentous turf and coralline algae as seawater becomes more acidic, which may obscure, or at times ameliorate the effects of higher $pCO_2$ alone on CCA calcification.
Fig. 3.6 Average photosynthetic capacity (ETR\textsubscript{max}) ($\mu$mol e$^{-1}$ m$^{-2}$ s$^{-1}$) (± 1 s.e. n= 15 chips) for crustose coralline algal chips in the four experimental treatments measured during weeks five and nine of the experimental period.

It is possible that CCA calcification rates were affected by the general decline in ambient temperature over the course of our experimental period. Prior work has shown that CCA calcification is directly related to temperature in temperate regions (Martin et al., 2006; Martin and Gattuso, 2009; Martin et al., 2013). Furthermore, an increase in temperature can have interactive and detrimental effects on CCA calcification when paired with higher $p$CO$_2$, but only when temperature is increased beyond the normal summer maximum (Martin and Gattuso, 2009; Martin et al., 2013). Within a normal seasonal range, temperature has no interactive effect with higher $p$CO$_2$ on CCA calcification (Noisette et al., 2013a). The temperature changes observed in our experiment were driven by the natural decline in ambient
temperature at Marmion Lagoon over the winter period (Smale and Wernberg, 2009). As such, it is likely that the decline in CCA calcification over time was partly a response to natural seasonal temperature decrease. Furthermore, since the temperature decrease was within the normal seasonal range for Marmion Lagoon, the effect of temperature on CCA calcification rates was presumably independent of $p$CO$_2$ and would therefore reflect the conditions under which competition between filamentous turf and CCA normally occur.

Photosynthetic performance in crustose corallines has previously been shown to be negatively affected by elevated $p$CO$_2$ (Anthony et al., 2008; Gao and Zheng, 2010; Martin et al., 2013). However, increases in $p$CO$_2$ may also enhance productivity in some marine algae by alleviating carbon limitation, particularly when $p$CO$_2$ levels are below 1000 ppm (Bowes, 1993; Iglesias-Rodriguez et al., 2008), and this has been observed in coralline algae grown under high $p$CO$_2$ conditions (Ries et al., 2009; Semesi et al., 2009). Our results indicated no effect of higher $p$CO$_2$ alone on the measured photosynthetic parameters. As in the case of CCA calcification, the presence of filamentous turf algae had an interactive effect with $p$CO$_2$ level on maximum electron transport rate, which is a proxy for photosynthetic rate (Kühl et al., 2001). The main effect of overgrowth by filamentous turf algae on CCA photosynthetic performance in the 900 + F treatment was negative, while the reverse was observed in the 300 + F treatment. Quantum yield of photosynthesis is an effective indicator of how environmental stress due to high irradiance, UV radiation, temperature, etc., affects the photosynthetic apparatus of marine macroalgae (Hanelt et al., 1997; Gómez et al., 2001; Wilson et al., 2004). By week nine we observed an ~20% decline in CCA quantum yield in 900 + F, relative to 300 + F treatments, indicating photoinhibition and stress in the 900 + F treatment. As such, overgrowth by filamentous turf algae appears to interact synergistically with high levels of $p$CO$_2$ on algal stress levels; the result of which was reduced quantum yield in high $p$CO$_2$ treatments when filamentous turf algae were present. This was not entirely surprising given that overgrowth by epiphytes and burial by sedimentation impede primary production through reductions in incident light (Hall-Spencer and Moore, 2000; Grall and Hall-Spencer, 2003). During week
five of our experiment, turf algal cover exceeded 20% in ambient aquaria and 40% in high $p$CO$_2$ aquaria, most likely decreasing the irradiance available to CCA for photosynthesis, an effect that was exacerbated in high $p$CO$_2$ tanks at this time due to the relatively high level of turf algal overgrowth. Overgrowth by filamentous turf is thus more detrimental to CCA photosynthetic performance in higher $p$CO$_2$ conditions; potentially due to the adverse effects of competition in an already stressful environment, or the faster growth of filamentous turf leading to greater reductions in light available to the CCA. Most importantly, these results provide further evidence that species interactions can be dependent on $p$CO$_2$ level.

We have observed a variable effect of competition with filamentous turf algae on CCA, the nature of which interacts with $p$CO$_2$ level. Unexpectedly, filamentous turf algae may have been beneficial at times for CCA calcification under higher $p$CO$_2$, an effect which is potentially linked to the modification of local-scale $p$CO$_2$ conditions. In contrast, the competitive interaction with filamentous turf algae amplified the negative effects of high $p$CO$_2$ on CCA photosynthetic performance, an effect which may be related to light limitation through overgrowth. The relationship between $p$CO$_2$ level and CCA metabolism appears clear and reproducible when considering a single variable, as is often the case in laboratory studies, but rarely in the natural environment. Our results have demonstrated that the effects of ocean acidification on single species or groups must be considered in parallel with the effects of high $p$CO$_2$ on ecological interactions, as these may not remain constant with environmental change.
Chapter 4

Turf algal epiphytes metabolically induce local pH increase, with implications for underlying coralline algae under ocean acidification.

J. Short, O. Pedersen and G. A. Kendrick

pH and O\textsubscript{2} microsensors in the diffusive boundary layer above a Hydrolithoideae coralline algal crust overgrown by filamentous turf algae (Photo: Ole Pedersen)
Conceptual model – The study presented in chapter four investigates the mechanism behind a facilitative interaction between turf algae and coralline algae under ocean acidification observed during the 10-week tank experiment in chapter three. Specifically, I explored the capacity of turf algae to modify seawater chemistry within the diffusive boundary layer above Hydrolithoideae crusts, potentially modifying calcification in the latter when $pCO_2$ is elevated.
4.1 Abstract

The presence of epiphytic turf algae may modify the effects of ocean acidification on coralline algal calcification rates by altering seawater chemistry within the diffusive boundary layer (DBL) above coralline algal crusts. We used microelectrodes to measure the effects of turf algal epiphytes on seawater pH and the partial pressure of oxygen ($pO_2$) within the DBL at the surface of Hydrolithoideae coralline algal crusts under ambient (36 Pa) CO$_2$ and an ocean acidification scenario with elevated CO$_2$ (200 Pa). Turf algae significantly increased the mean diel amplitude of pH and $pO_2$, and this effect was more pronounced under elevated (200 Pa) CO$_2$. Specifically, under elevated CO$_2$, mean amplitudes of pH and $pO_2$ were 1.40 and 76 kPa, respectively, when turf algae were present. In contrast, in seawater with ambient levels of CO$_2$, mean ranges of pH and $pO_2$ were 0.80 and 43 kPa, respectively, when turf algae were present. In addition, for elevated CO$_2$ treatments with a mean pH of 7.48, pH in the DBL in the light exceeded ambient levels when turf algae were present, with a mean maximum pH of 8.63, while pH remained low for coralline algal crusts in the absence of turf algae, with a mean maximum pH of 7.60. In the elevated CO$_2$ treatments in the dark, pH within the DBL was similar in both the presence and absence of turf algae. We attribute the effect of turf algal overgrowth on DBL chemistry under elevated CO$_2$ to an increase in turf algal biomass. Thus, the effect of epiphytic turf algae on microscale pH is striking, and will likely affect coralline algal response to ocean acidification, highlighting the importance of understanding the effects of environmental change on species interactions.
4.2 Introduction

Atmospheric carbon dioxide is currently being taken up by the world’s oceans, causing ocean acidification (Caldeira and Wickett, 2003). This is predicted to have significant impacts on the metabolism and survival of many marine species, which will likely have major, broad-scale ecological consequences (Kroeker et al., 2010). Macroalgal communities are extremely important, as they perform a range of ecosystem services in shallow coastal systems. The benthic photoautotrophs, which dominate these communities, have exhibited contrasting responses to ocean acidification. Crustose coralline algae (CCA) are of fundamental ecological importance, providing substrata for settlement, as well as chemical settlement cues for other species of seaweeds and invertebrates (Morse et al., 1988; Heyward and Negri, 1999; Vermeij and Sandin, 2008). Moreover, they provide food and contribute significantly to carbonate accretion in the habitats where they occur (Steneck and Adey, 1976; Adey, 1978).

The increase in seawater CO$_2$ has negative effects on the metabolic process of calcification such that CCA are highly sensitive to ocean acidification (Orr et al., 2005). Indeed, reductions in coralline algal growth, recruitment, calcification and photosynthesis have been observed under ocean acidification conditions in the laboratory (Anthony et al., 2008; Kuffner et al., 2008; Gao and Zheng, 2010). In contrast, filamentous turf algae – fleshy seaweeds which form low-lying and extensive mats in benthic communities – may benefit from ocean acidification, with the increase in inorganic carbon enhancing rates of photosynthesis and growth (Kroeker et al., 2010; Connell et al., 2013).

Differential susceptibilities to ocean acidification between taxa within a single community illustrate the potential for shifts in community structure due to shifts in ecological interactions under environmental change (Hall-Spencer et al., 2008; Porzio et al., 2011). Increased susceptibility to grazing in some species of algae (Johnson and Carpenter, 2012) and shifts in dominance from calcareous to fleshy algal species as a result of altered competitive interactions under ocean acidification have been observed (Porzio et al., 2011; Kroeker et al.,
Thus, community response to environmental change is more complex than simple extrapolation from the response of single sensitive species, and understanding the effects of environmental change on species interactions will allow for a better prediction of the state of marine communities in the near future.

CCA and filamentous turf algae compete for resources and the outcome of this interaction is variable. CCA are often overgrown by the faster growing turf algae (Adey, 1970; Steneck and Paine, 1986; Figueiredo et al., 1997), which generally has negative effects on the growth and survival of the former due to a limitation of available resources such as light, CO₂, nutrients and space on the substrate (Adey and Macintyre, 1973; Steneck, 1982; Kendrick, 1991). In contrast, when irradiance is high, CCA may benefit from turf algal overgrowth, which can provide protection by shading underlying coralline algal crusts from the harmful effects of a high light environment (Figueiredo et al., 2000). Furthermore, this interaction may vary with environmental change, with unexpected positive effects of turf algal overgrowth on CCA calcification rates in acidified seawater (Short et al., 2014). The mechanism behind the enhanced calcification has not yet been investigated. However, it has been hypothesized that increases in turf algal photosynthesis are likely to alter seawater chemistry in the diffusive boundary layer (DBL) above coralline algal crusts, facilitating CCA calcification under elevated CO₂ (Short et al. 2014). CCA and other photosynthetic organisms have been shown to alter the chemical micro-environment within the DBL and the extent of this is dependent on the ecological and environmental context (Hurd et al., 2011; Cornwall et al., 2013; Cornwall et al., 2014). Understanding the interaction between turf algae and CCA in this context will increase our understanding of how macroalgal communities may respond to near-future environmental change. Therefore, we examined the effects of turf algal overgrowth on pH, CO₂ and O₂ in seawater within the DBL above coralline algal crusts under ambient and elevated CO₂ conditions in order to understand the capacity of turf algal epiphytes to modify local seawater chemistry in the context of ocean acidification.
4.3 Materials and methods

4.3.1 Field site and sample collection

In October 2013, 60 ‘chips’ of an encrusting Hydrolithoideae coralline alga were collected by SCUBA using a hammer and chisel from approximately 5 m depth at Whitford Rock (S31°48', E115°42') in Marmion Lagoon, a fringing reef-lagoon system located on the southwestern coast of Western Australia. Ambient $p\text{CO}_2$ at Whitford Rock ranges from ca. 21-41 Pa (210 - 400 μatm) seasonally and sea surface temperatures range seasonally from ca. 16 - 24 °C (Smale and Wernberg, 2009). CCA chips collected from the reef were transported in bins of seawater to an onshore laboratory, where they were cut into pieces ~ 4-12 cm$^2$ and cleaned of all visible epiphytes. These were then allowed to acclimate in aquaria under ambient $p\text{CO}_2$ conditions for one week prior to the application of experimental treatments.

4.3.2 Experimental design

A flow-through aquarium system was set up to establish epiphytic turf algae on CCA chips under two $p\text{CO}_2$ regimes. The system comprised six 25-L aquaria receiving ambient seawater filtered to 30 μm from two 200-L header tanks. Turnover time of water within each treatment aquarium was approximately 90 minutes. Independent variables were (1) dissolved $p\text{CO}_2$ (ambient: 36 Pa (350 μatm), and high: 203 Pa (2000 μatm)), and (2) the presence or absence of filamentous turf algae. Three of the treatment aquaria contained seawater with ambient levels of CO$_2$ while the remaining three aquaria contained seawater with elevated CO$_2$ levels. Each of the six treatment aquaria contained 10 CCA chips; five overgrown by turf algae and five free of epiphytes. The result was a fully factorial set up with four distinct treatments, each with 15 CCA chips: (i) ambient $p\text{CO}_2$ without filamentous turf ($\text{Ambient CO}_2$), (ii) ambient $p\text{CO}_2$ with filamentous turf ($\text{Ambient CO}_2 + \text{turf}$), (iii) high $p\text{CO}_2$ without filamentous turf ($\text{High CO}_2$) and (iv) high $p\text{CO}_2$ with filamentous turf ($\text{High CO}_2 + \text{turf}$). The $p\text{CO}_2$ level in the high $p\text{CO}_2$ treatments was set higher than predictions for near future scenarios (ex. ca. 71 Pa (700 μatm) by 2100 (Solomon et al., 2007)) so that the effects of turf algae on seawater...
chemistry within the DBL above the coralline algal crusts would be clearly demonstrated under our experimental conditions.

A pH stat system (Aqua Medic) was used to control $pCO_2$ in the three ‘high $pCO_2$’ treatment aquaria by direct injection of pure (food grade) CO$_2$ gas. To control the level of filamentous turf algae, all visible epiphytes were removed from 5 of the 10 CCA chips in each treatment aquarium twice weekly with a soft brush, while filamentous turf algae were allowed to colonise the remaining 5 CCA chips. CCA were allowed to grow, and filamentous turf algae to establish in these treatments for four weeks. Filamentous turf algae established during this period were a mixture of ~80% *Cladophora* spp. and ~20% *Ulva* spp. (J. Huisman, personal communication). Filaments were ~5-10 cm long and formed turfs overgrowing the CCA chips, resembling those described in Fig. 2E. in Connell (2014).

A small-scale, closed aquarium system was established to carry out chemical measurements within the DBL. This system comprised two 25-L aquaria receiving seawater from two separate mixing tanks; the first with ambient (ca. 36 Pa) and the second with high (ca. 203 Pa) $pCO_2$. Both treatment aquaria contained 20 CCA chips; 10 overgrown by filamentous turf algae and 10 cleaned of all epiphytes, which were transported from the original aquarium system immediately following the four-week growth period. Turnover time of water in these aquaria was approximately 12 hours. To minimise gas exchange with ambient air, the high $pCO_2$ treatment aquarium was covered with clear plastic. CCA were maintained in the smaller-scale system for approximately one week. Aquaria were kept in a temperature controlled room at 20°C. In both the large and small-scale systems, downwelling PAR of approximately 250 μmol photons m$^{-2}$ s$^{-1}$ was maintained using Maxspect s-series LED lights on a diurnal cycle with 12 hours of light and 12 hours of darkness, which reflected the natural irradiance levels and day length at 5 m depth in Marmion Lagoon during spring.
4.3.3 Seawater measurements

In the large-scale aquarium system, temperature and pH on the total scale (pHT) were measured three times weekly in each of the treatment aquaria and header tanks just prior to midday using a Schott Handylab pH metre equipped with a Blueline Elektrode. In the small-scale aquarium system temperature and pH were measured twice daily in both treatment aquaria. The electrode and pH meter were calibrated before each sampling day against seawater (‘Tris’) and NBS buffers. In both the large and small-scale aquarium systems salinity (± 1) was measured weekly in both mixing tanks using a portable refractometer. Samples for the analysis of total alkalinity (TA ±5 μmol kg$^{-1}$) were taken weekly from each mixing tank and analysed as in Short et al. (2014). Additional carbonate chemistry parameters, including pCO$_2$ and saturation state of aragonite ($\Omega_{Ar}$; because the solubility of aragonite is closer to that of high magnesium calcite than calcite), were derived from our measurements of temperature, salinity, TA and pHT, and calculated from defined input conditions using the CO2SYS program (Lewis et al., 1998) based on the dissociation and solubility constants of (Murray and Riley, 1971a; Dickson and Millero, 1987; Dickson, 1990a).

4.3.4 CCA calcification

During the four-week growth period in the large-scale aquarium system skeletal growth rates were measured for the 15 CCA chips from each treatment using a modification of the buoyant weight technique (Jokiel et al., 1978) as in Short et al. (2014).

4.3.5 Overgrowth by filamentous turf algae

The software Coral Point Count (Kohler and Gill, 2006) was used to determine percent cover of filamentous turf algae overgrowing coralline algal chips. This was assessed at the end of the four-week growth period from photographs of 12 randomly selected CCA chips from High CO$_2$ + turf and Ambient CO$_2$ + turf treatments.
4.3.6 pH and partial pressure of oxygen in the DBL

pH and partial pressure of O$_2$ ($p$O$_2$) in the DBL at the surface of CCA chips were measured using pH and oxygen microelectrodes. Whole CCA chips for Ambient CO$_2$ and High CO$_2$ treatments, or whole CCA chips overgrown by filamentous turf algae for Ambient CO$_2$ + turf and High CO$_2$ + turf treatments were considered individual replicates and three replicate samples were analysed from each treatment. For each replicate, microelectrode measurements were made every 10 s at a distance to the CCA surface of 10 to 50 µm using a micromanipulator (MM50, Unisense A/S, Denmark) and a boomstand microscope (Nikon SMZ-10) to position the electrodes. For pH measurements, a 100 µm microelectrode (PH100, Unisense A/S, Denmark) was used whilst oxygen measurements were taken with a 25 µm microelectrode (OX25, Unisense A/S, Denmark); both were connected to a multimeter (Multimeter, Unisense A/S, Denmark).

For clean CCA chips boundary layer pH and $p$O$_2$ were measured for one hour in full irradiance, followed by one hour in the dark, followed by a second one hour period of full irradiance. For CCA chips overgrown by turf algae, in order to account for the hypothesized effects of turf algae on seawater chemistry within the DBL, pH and $p$O$_2$ were measured for at least nine hours, with 4.5 hours of full irradiance and 4.5 hours of darkness in contrast to 1h intervals for CCA chips alone. Due to time constraints, measurements were taken for an additional 4.5 hour period of full irradiance for only one of the replicates from each of the High CO$_2$ + turf and Ambient CO$_2$ + turf treatments. Microelectrode measurements were made in a 4 L glass aquarium, with replicates mounted on a glass pedestal. The aquarium was illuminated with a 12V white halogen light source with a photon flux of approximately 250 μmol photons m$^{-2}$ s$^{-1}$.

4.3.7 Oxygen balance: photosynthesis and dark respiration

Oxygen balance in light and dark conditions was determined for CCA and turf algae using closed incubation chambers with injection ports for oxygen-sensitive electrodes as
described in Pedersen et al. (2013). Two 83 mL incubation chambers were run concurrently using 500 µm oxygen microelectrodes (OX500, Unisense A/S, Denmark) connected to a multimeter (PA2000, Unisense A/S, Denmark). For the CCA, whole clean chips, and for filamentous turf, ~ 50 cm$^3$ of loosely packed filaments removed from a single CCA chip, were considered individual replicates. $pO_2$ in the incubation chambers was measured for 0.5-2 hours, or until there was a minimum $pO_2$ change of 10% from initial values in both the light and dark for each replicate. Five replicate samples were analysed for CCA in high and ambient CO$_2$ as well as filamentous turf grown in high and ambient CO$_2$. The chambers were illuminated with a 12V white halogen light source with a photon flux on each chamber of ~250 µmol photons m$^{-2}$ s$^{-1}$.

Net photosynthesis ($P_N$) and dark respiration ($R_d$) were calculated as:

$$P_N \ (or \ R_d)(kPa \ O_2 \ mg \ Chl \ a^{-1} \ (or \ g \ DM^{-1}) \ s^{-1}) = \frac{O_2 \ slope \ (kPa \ O_2 \ L^{-1} \ s^{-1}) \ V_{vial} \ (L)}{Chl \ a \ (mg)(or \ DM \ (g))}$$

where $O_2$ $slope$ is the change in oxygen concentration inside the incubation chamber per unit time, $V_{vial}$ is the individual volume of the incubation chamber, Chl $a$ is the total chlorophyll $a$ content of the particular sample, and DM is the total dry mass of the particular sample. CCA chip volume was determined to be 2.05 ±0.28 cm$^3$, or 2.5 ±0.3% (means ± 1 s.e.) of the volume of the incubation chamber, for a separate sub-sample of 10 CCA chips. Therefore, for oxygen budget calculations it was assumed that the volume of the CCA chip in the incubation chamber was negligible. Oxygen balance was normalized to both Chl $a$ content and dry mass for each of the replicates investigated.
4.3.8 Chlorophyll a pigment analysis

All algal replicates used for DBL pH and oxygen measurements, as well as oxygen balance measurements were stored in a conventional freezer at -18 °C immediately after measurements were taken. Before freezing, overgrowing turf algae were removed from CCA using tweezers and stored separately. All replicates were then dried in a VirTis Benchtop 2K series freeze-drier for 4.8 d. Total chlorophyll a (Chl a) content was determined for all samples using spectrophotometry with methanol as the solvent, as described in Wellburn (1994).

4.3.9 Data analysis

The effect of filamentous turf algae and CO₂ level on the average range of pH, \( pO_2 \) and \( pCO_2 \) in the DBL at the surface of a CCA chip between full irradiance and dark conditions were assessed using ANOVA for the four experimental treatments. Significant differences between treatment means were further examined with Tukey’s HSD post hoc tests. The effects of \( pCO_2 \) together with the presence of filamentous turf algae on calcification of CCA chips over the course of the four-week growth period were assessed using 2-way ANOVA with \( pCO_2 \) (ambient and high) and level of filamentous turf (present or absent) as fixed factors. When necessary, data were transformed to meet the assumptions of normality and homogeneity of variance as indicated by Shapiro-Wilk’s and Levene’s tests, respectively. All data met the assumptions of normality and heterogeneity following transformation. Data in text and figures are presented as mean ± s.d.

4.4 Results

4.4.1 Seawater chemistry within the DBL

Seawater in the microsensor aquarium was maintained at a mean temperature of 19.7 ± 0.4 °C (n = 15) and a mean pH of 8.03 ±0.02 for ambient and 7.48 ±0.02 for acidified treatments. Correspondingly, \( pCO_2 \) was 43 ±2 for ambient and 181 ±8 Pa for acidified treatments. Fluctuations in DBL pH, \( pCO_2 \) and \( pO_2 \) between light and dark conditions were
greater when turf algae were present in both CO\textsubscript{2} treatments, and this was most pronounced when CO\textsubscript{2} was elevated (Figs. 4.1 & 4.2). In the High CO\textsubscript{2} + turf treatment, pH reached a mean maximum value of 8.63 ±0.11 in the light and decreased to 7.22 ±0.08 in the dark. In contrast, in the High CO\textsubscript{2} treatment, pH reached a mean maximum value of 7.60 ±0.15 in the light and decreased to minimum values of 7.24 ±0.03 in the dark. For the Ambient CO\textsubscript{2} + turf treatment, pH reached mean maximum values of 8.42 ±0.17 and minimum values of 7.62 ±0.02 in the dark.

Fig. 4.1 Representative time series of CO\textsubscript{2}, O\textsubscript{2} and pH measured in the diffusive boundary layer 10 to 50 μm from the surface of a single coralline algal chip in each of the four experimental treatments. Measurements were taken over a period of full irradiance, followed by a period of darkness (grey rectangles), followed by a second period of full irradiance.
In the Ambient CO$_2$ treatment, mean pH was 8.23 ±0.05 in the light and 7.59 ±0.28 in the dark. The pH range between light and dark conditions was four-fold greater in the High CO$_2$ + turf treatment than in the High CO$_2$ treatment, corresponding to pCO$_2$ and pO$_2$ ranges of ~1.8 and ~2.5-fold greater in the High CO$_2$ + turf treatment relative to the High CO$_2$ treatment (Fig. 4.2).

### 4.4.2 Photosynthesis and dark respiration

Seawater temperature in the oxygen incubation chambers was maintained at a mean of 19.5 ±0.7 °C ($n = 10$) and mean pH was 8.04 ±0.07 ($n = 10$) for ambient and 7.43 ±0.01 for acidified treatments. For CCA, oxygen consumed in the dark via respiration was significantly greater than that produced in the light by algal photosynthesis in both CO$_2$ treatments (Fig. 4.3a, b), which was likely due to the influence of bacterial biofilms occurring on the surface of the CCA, especially on the back of the chip (Garland et al., 1985; Lewis et al., 1985; Johnson and Sutton, 1994), or to endolithic algae, which are common on CCA crusts (Diaz-Pulido et al., 2012b). Such biofilms would increase the rate of community respiration, such that caution should be exercised when interpreting dark respiration measurements for the CCA. Significantly more oxygen was required for respiration in the high compared to the ambient CO$_2$ treatment, however this was only the case when oxygen balance in the dark was normalized to Chl. $a$ content (Fig. 4.3a). For oxygen balance normalized to dry mass, there was no significant difference in the amount of oxygen consumed between CO$_2$ treatments in the dark (Fig. 4.3b). No differences were detected in photosynthetic oxygen production in the light between CO$_2$ treatments (Fig. 4.3a, b).
Fig. 4.2 Average $pO_2$ (kPa) (a), $pCO_2$ (Pa) (b) and pH (c) ranges (± 1 s.e., $n = 3$) between light and dark conditions, measured in the diffusive boundary layer 10 to 50 µm from the surface of coralline algal chips in each of the four experimental treatments. Letters above bars denote statistical differences between treatments as indicated by Tukey’s HSD post hoc tests at the $\alpha = 0.05$ level. For the $pO_2$ data a log($x$) transformation was applied.
Fig. 4.3 Rates of dark respiration and net photosynthesis (± 1 s.e., n = 5) normalized to chlorophyll a (top panel) and dry mass (bottom panel) for coralline algae (left panel) and turf algae (right panel) in ambient and high pCO₂ conditions. Asterisks indicate significant differences between CO₂ treatments as indicated by Mann-Whitney tests at the α = 0.05 level.

For filamentous turf algae, relative changes in oxygen balance between treatments and light conditions were consistent for calculations normalized to Chl. a content as well as those normalized to dry mass (Fig. 4.3c, d). Oxygen consumed in the dark was significantly less than that produced in the light for turf algae in both the ambient and high CO₂ treatments. Dark
respiration was greater in the ambient relative to the high CO$_2$ treatment, but no differences were detected in net photosynthesis between CO$_2$ treatments (Fig. 4.3c, d).

### 4.4.3 Seawater properties

For the four-week growth period in the large scale aquarium system prior to boundary layer and oxygen balance measurements, pH was maintained at relatively constant values of 7.40 ±0.03 in the high $p$CO$_2$ and 8.08 ±0.03 ($n = 13$) in the ambient $p$CO$_2$ treatment tanks. Thus, we were able to maintain a $p$CO$_2$ of 209 ±13 Pa and an Ω$_{Ar}$ of 0.80 ±0.07 in the high $p$CO$_2$ treatment tanks, and a $p$CO$_2$ of 35 ±3 Pa and an Ω$_{Ar}$ of 3.18 ±0.27 in the ambient CO$_2$ treatment tanks for the course of the four-week growth period did not vary significantly between experimental treatment aquaria (Kruskal-Wallis: $\chi^2 = 0.706, P=0.983$) and ranged from 20.8 – 22.2°C, increasing by ~1.4°C on average over the course of the 4-week growth period, mimicking natural seasonal temperature change in Marmion Lagoon during spring (Appendix B).

For the fifth week of the experimental period in the small scale aquarium system, pH was maintained at 7.57 ±0.13 ($n = 14$) in the high $p$CO$_2$ treatment tank and 8.05 ±0.07 in the ambient $p$CO$_2$ treatment tank. Thus, a $p$CO$_2$ of 147 ±41 Pa and an Ω$_{Ar}$ of 1.20±0.37 were maintained in the high CO$_2$ treatment tank, and a $p$CO$_2$ of 41 ±8 Pa and an Ω$_{Ar}$ of 2.96±0.41 were maintained in the ambient CO$_2$ treatment tank. Carbonate chemistry values were more variable in this system and lower on average than in the large-scale system due to the lack of fresh seawater supply at this facility. Nonetheless, all of the measured parameters of carbonate chemistry were significantly different between CO$_2$ treatments in the small scale system (Mann Whitney U test: $p$CO$_2$: $Z = -6.029, P<0.001$; pH: $Z = -6.029, P<0.001$; Ω$_{Ar}$: $Z = -6.03, P <0.001$). Temperature in this system varied slightly between treatments with mean temperatures of 19.1 ± 0.62 ($n =14$) and 19.8 ±0.38 in the ambient and high CO$_2$ treatment aquaria, respectively (Appendix B). Total alkalinity did not vary significantly between CO$_2$
treatments or aquarium systems and ranged from 2298 and 2350 μmol kg$^{-1}$ over the course of the study.

4.4.4 CCA calcification

There was no interactive effect of $pCO_2$ and turf algae ($F(1,53)= 0.08, P = 0.78$) and no significant main effects on calcification rates of CCA chips during the four-week growth period ($pCO_2: F(1,53) = 0.34, P = 0.57$; turf: $F(1,53) = 3.22, P = 0.08$). Calcification rates during this time were not significantly different from zero in all treatments ($t = -0.171, P = 0.865$).

4.4.5 Growth of filamentous turf algae

Percent cover of filamentous turfs increased over time in Ambient $CO_2 + turf$ and High $CO_2 + turf$ treatments. At the end of the four-week growth period, epiphyte cover was significantly higher on CCA chips in the high $CO_2$ aquaria, with 82±6% compared to 63±5% cover in the ambient $CO_2$ treatment aquaria ($t = 2.39, P = 0.03$).

4.5 Discussion

We have demonstrated a striking increase in the diel range of pH and $pO_2$ within the boundary layer above coralline algal crusts driven by an increase in epiphytic turf algal abundance under ocean acidification conditions. The large diel amplitudes in pH and $pO_2$ will have implications for coralline algae under future ocean acidification scenarios since overgrowth by epiphytic turf algae is likely to: (1) increase CCA resilience to ocean acidification through acclimation to a wider pH range than normally experienced and; (2) to modify CCA metabolic rates and thus their overall response to environmental change. Given the importance of coralline algae in many marine communities, understanding this interaction is essential for predicting the state of macroalgal habitats in the near future.

Coralline algae can often persist, or thrive after being overgrown by turf algae in the natural environment, depending on the particular habitat (Airoldi, 2000). Our findings suggest
that coralline algae may regularly encounter extensive diel pH fluctuations within their DBL. Indeed, in the ambient CO$_2$ treatment CCA experienced diel ranges of 0.63 pH units when turf algae were absent, whereas the diel pH range increased to 0.80 in the presence of turf algae. Metabolically-driven increases in local pH will reduce $p$CO$_2$ at the DBL in the light and this local interaction may counterbalance the detrimental effects of ocean scale high levels of CO$_2$ on light mediated calcification (Ries et al., 2009). As such, overgrowth by turf algae may increase coralline algal tolerance, and produce a microclimate that allows continued calcification in CCA under ocean acidification.

CCA crusts in the elevated CO$_2$ treatment supported denser epiphytic communities, suggesting that the amplified effect of turf algae on the diel range in seawater chemistry within the boundary layer was driven by an increase in turf algal biomass. Similarly, increased turf algal growth has been shown under acidified conditions both in the laboratory (Kuffner et al., 2008) and in the field (Porzio et al., 2011; Kroeker et al., 2012). Under an extreme ocean acidification scenario we observed major diel pH fluctuations within the DBL when CCA were overgrown by turf algae (1.4 pH units), and these fluctuations were less when CCA were grown alone (0.35 pH units). Indeed, when turf algae were present, DBL pH was increased to a mean of 8.6 in the light and reduced to 7.2 in the dark. The longer term effects of this on CCA calcification are likely to be two-fold, enhancing calcification during the day while facilitating dissolution at night. In the present study, there was no effect of turf algal overgrowth on CCA calcification rates, likely due to the relatively short growth period and variable turf algal densities. Temporal scale is extremely important when considering the effects of ocean acidification on coralline algae. Longer term studies report temporally variable effects of ocean acidification on coralline algal metabolic rates, due to the additional influence of seasonal changes in seawater properties, such as irradiance and nutrients levels or to shifts in the relative importance of species interactions (Martin et al., 2013; Short et al., 2014). As such, the effects of turf algal overgrowth on CCA calcification are complex and require further investigation for an extended period to determine the longer term impacts.
The presence of turf algae also affected diel O$_2$ fluctuations in the boundary layer (Fig. 4.1). In two out of three replicates, the boundary layer immediately above the CCA turned anoxic shortly after the light was switched off, whereas anoxia in the boundary layer never occurred in the absence of turf algae. To our knowledge, anoxia tolerance of CCA has never been studied and we can only speculate on the effect that anoxia exerts upon the red algae and their ability to continue metabolic processes in the dark, not to mention survive such events in the longer term. Moreover, in the light O$_2$ reached much higher levels in the boundary layer in the presence of turf algae, in particular under elevated CO$_2$ (Fig. 4.1). High pO$_2$ levels in the light may also adversely affect the CCA as the combination of high pH (and therefore low pCO$_2$) and high pO$_2$ would result in an increase in O$_2$ inhibition of photosynthetic O$_2$ consumption in CCA (Borowitzka, 1981b). We thus propose that effects of O$_2$ as derived from higher rates of photosynthesis are not overlooked when evaluating the combined effect of increasing CO$_2$ and colonisation of turf algae as reduced CCA performance measured at the level of photosynthesis could also translate into lower calcification rates.

CCA respiration (per unit Chl a) was significantly higher in the elevated CO$_2$ treatment, indicating a reduction in total Chl. a content under higher CO$_2$. The effects of ocean acidification on coralline algal respiration have rarely been investigated, and existing studies have not detected an effect of CO$_2$ dark respiration (Semesi et al., 2009; Martin et al., 2013). However, reductions in Chl a pigments have been observed for some species of calcifying and non-calcifying red algae under elevated pCO$_2$ (Andría et al., 2001; Gao and Zheng, 2010). In contrast, we found no effect of turf algal overgrowth on CCA photosynthesis. Coralline algal photosynthetic response to elevated CO$_2$ is variable, with some species exhibiting increased rates of photosynthesis under elevated CO$_2$ (Semesi et al., 2009) while others had reduced rates of photosynthesis (Anthony et al., 2008; Martin et al., 2013). Similarly, if the increase in respiratory oxygen demand measured in the present study was driven by a reduction in Chl a, reductions in photosynthetic rates would also be expected over time.
Increases in the abundance of turf algae and other fleshy macroalgal species, and declines in calcifying macroalgal species are expected with the increase of CO$_2$ in seawater (Nelson, 2009; Kroeker et al., 2010; Brodie et al., 2014). Correspondingly, shifts in benthic community structure from habitats dominated by calcareous to fleshy algal species have been predicted with ocean acidification (Kroeker et al., 2012). Here we have documented a significant effect of epiphytic overgrowth on seawater chemistry within the DBL above CCA crusts; a property which is known to affect rates of CCA metabolism. Consequently, overgrowth by turf algae is likely to affect coralline algal response to predicted near-future ocean acidification scenarios and further investigation is required to quantify this complex interaction. The effect of turf algae on seawater chemistry within the DBL above crustose coralline algae reported here highlights the importance of ecological interactions in predicting species vulnerability to ocean acidification.
Chapter 5 General Discussion

Crustose coralline algae overgrown by several species of fleshy and filamentous algae in Marmion Lagoon, Perth, Western Australia
This thesis investigated the impacts of ocean warming and acidification on the metabolism and ecology of Corallinaceae coralline algae as well as the diverse communities in which they play ecologically critical roles. This was achieved by way of three main aims (Fig. 5.1). First, I assessed coralline algal response to ocean warming in the natural environment by investigating the impacts of a marine heatwave on seasonal patterns in growth, calcification and mortality for three Corallinaceae sub-families (Porolithoideae, Lithophylloideae and Hydrolithoideae). From this study I concluded that coralline algae are sensitive to heating, as indicated by counter-seasonal growth and calcification rates in combination with high rates of mortality during anomalously warm summers. However, coralline algal response to heating was variable, and I hypothesized that some of this variability was driven by the influence of additional environmental and ecological factors.

Due to the apparent importance of ecological interactions in governing individual species response to environmental disturbance, I then examined how the common interaction between a Hydrolithoideae crustose coralline alga and an overgrowing assemblage of filamentous turf algae was affected by ocean acidification. This was explored in the laboratory by measuring the effects of turf algal overgrowth on Hydrolithoideae calcification and photosynthesis under ambient and elevated CO\textsubscript{2} for 10 weeks. During this experiment the interaction between the two algal taxa was highly variable. At times, turf algae appeared to compete with the corallines, exacerbating the effects of ocean acidification on the latter, while at other times the presence of turf algae enhanced coralline algal calcification under elevated CO\textsubscript{2}. I hypothesized that changes in seawater chemistry were driving alternate metabolic feedbacks in the two algal taxa, the nature of which were dependent on environment.

Finally, I investigated the mechanism behind the facilitative interaction between turf algae and coralline algae observed during the 10-week tank experiment. This was achieved by measuring the effect of turf algal overgrowth on seawater chemistry within the diffusive boundary layer (DBL) above Hydrolithoideae crusts under ambient and elevated CO\textsubscript{2}. I concluded that turf algae increased the diel amplitude of pH and O\textsubscript{2} within the DBL and this is likely to have implications for underlying coralline algae under ocean acidification.
As a whole, this thesis demonstrates that coralline algae are sensitive to ocean warming and acidification, but this response is largely influenced by ecological interactions, environmental conditions and the complex relationship between the two. In particular, interactions between turf and coralline algae can be both competitive and facilitative under varying environmental conditions. This has major implications for coralline algae under near-future climate change scenarios, highlighting the importance of investigating the effects of environmental change on a community scale.

Fig. 5.1 Conceptual model of this thesis, summarising how the chapters fit together to describe the role of a series of environmental and ecological drivers of coralline algal response to ocean warming and acidification.

5.1 *In situ* response of coralline algae to anomalously high temperatures

In order to investigate coralline algal response to a period of warming associated with a marine heatwave which occurred along the coast of Western Australia during summer 2010-11 (Feng et al., 2013), rates of coralline algal calcification, growth and mortality were measured for 9-24 months in this region immediately following the heatwave. This period of warming had negative impacts on the three most abundant Corallinaceae sub-families along the Western
Australian coast (Porolithoideae and Lithophylloideae rhodoliths and Hydrolithoideae CCA) from Coral Bay (~23°S) to Marmion Lagoon off Perth (~31°S). High coralline algal mortality rates when temperature anomalies were most pronounced and aseasonal growth patterns relative to those measured for Corallinaceae species in other marine habitats at similar latitudes across the globe were indicative of thermal stress. Prior work has shown that rates of coralline algal calcification generally increase with temperature in both the laboratory (Martin and Gattuso, 2009; Büdenbender et al., 2011; Martin et al., 2013) and in the field (Martin et al., 2006). However, rates of algal calcification have also been shown to decline at very high temperatures in the laboratory resulting in a Gaussian-like dependency of calcification on temperature (Agegian, 1985). The results of the field study are consistent with these observations, with calcification rates generally increasing with temperature up to some maximum or optimal rate and declining thereafter. However, much of the variability in the coralline algal growth rates reported was not explained by changes in seawater temperature alone.

In addition to temperature, a complex set of physical and chemical factors were likely influencing coralline algal growth and calcification (Adey and Macintyre, 1973; Steneck, 1985; Steneck, 1986; Steneck et al., 1991; Figueiredo et al., 2000; Andersson et al., 2008). At the local scale, biotic factors such as grazing pressure and competition for space on the substratum are known to influence coralline algal growth and distribution (Adey and Macintyre, 1973). Furthermore, shading by other species of macroalgae can have positive or negative effects on underlying coralline algal crusts (Airoldi, 2000; Figueiredo et al., 2000). Hydrolithoideae CCA at the southernmost location were shaded by kelps during the two year field study, suggesting that species interactions had important local effects. I observed distinct reductions in rhodolith calcification rates at the lower latitude reefs at Coral Bay and the Abrolhos Is. compared to the relatively constant rates of Hydrolithoideae CCA growth and calcification in the temperate fringing reef system at Marmion Lagoon. I hypothesized that shading by the kelp *Ecklonia radiata* may have been responsible for the change in seasonal pattern observed at Marmion Lagoon, relative to the other study locations, since shading by kelps can dampen seasonal patterns in growth rates of underlying coralline algal in the field by reducing the seasonal range.
in environmental parameters such as light and temperature (Melville and Connell, 2001; Irving et al., 2004). Thus, rates of coralline algal growth and calcification measured during the field study were affected by high seawater temperature anomalies, but some of the temporal and latitudinal variability observed was likely driven by ecological interactions, particularly by facilitative interactions with other species of macroalgae.

5.2 The effects of ocean acidification on the interaction between coralline and turf algae

There is a growing body of research which aims to untangle the relative influences of environment and ecology in the context of climate change, in order to understand the effects of environmental change on marine communities (Kroeker et al., 2012; Kroeker et al., 2013). Indeed, as observed during the field study, species interactions may play a crucial role in determining the overall effect of an environmental disturbance on a particular community. Furthermore, species interactions may be modified with environmental change, altering the impact of environmental stressors on individual organisms. For example, under ocean acidification conditions, Asnaghi et al (2014) demonstrated an increase in the detrimental effect of grazing by urchins on calcifying macroalgal species. Similarly, Ferrari et al (2011) described changes in damsel fish predator-prey dynamics under higher CO$_2$. In order to explore the relative roles of ecological interactions and environmental stressors in driving coralline algal metabolism, I investigated the effects of higher CO$_2$ on the interaction between Hydrolithoideae CCA and an overgrowing filamentous turf algal assemblage from the temperate reef system at Marmion Lagoon in an ocean acidification tank experiment. Although there was an overall negative effect of elevated CO$_2$ on CCA calcification rates, the effect of turf algal overgrowth was variable over the 10-week experimental period. The interaction between turf and coralline algae was at times competitive, with negative effects on the corallines. I hypothesized that this was associated with the effects of shading on CCA photosynthesis in a low-light environment, and the tight coupling between the processes of calcification and photosynthesis. Susceptibility to this was increased under acidified conditions wherein turf algal growth rates were higher.
Unexpectedly, turf algal overgrowth appeared to intermittently facilitate coralline algal calcification under elevated CO$_2$. I hypothesized that the positive correlation between rates of turf algal photosynthesis and the $p$CO$_2$ in seawater would result in enhanced CO$_2$ removal under ocean acidification, decreasing $p$CO$_2$ locally and allowing for underlying coralline algal crusts to calcify at normal rates. This study demonstrated that the effects of ocean acidification on a single organism can be modified by species interactions, and added to the growing body of research supporting the idea that the overall effect of environmental change on marine communities may be greater than the sum of its impacts on sensitive individuals.

The facilitative interaction between turf and coralline algae under ocean acidification observed during the 10-week tank experiment was further explored by investigating the effects of turf algal overgrowth on seawater pH and $p$O$_2$ within the DBL above coralline algal crusts under ambient and elevated CO$_2$. As hypothesized, turf algal overgrowth had significant effects on seawater chemistry within the DBL, increasing $p$CO$_2$ to present-day ambient levels in the light despite surrounding seawater with very high levels of CO$_2$, representing far-future ocean acidification conditions. Rather than an increase in rates of turf algal photosynthesis under higher CO$_2$, I determined that this was facilitated by an increase in turf algal biomass. Unexpectedly, coralline algal calcification rates were unaffected by turf algal overgrowth during this study, despite the observed effect of turf algae on local seawater chemistry. This may be related to the length of time over which CCA were cultured under the experimental conditions, and requires further exploration. Nonetheless, the effect of turf algal overgrowth on seawater chemistry within the DBL under ocean acidification conditions was striking, and has important implications for the resilience of coralline algae under ocean acidification.

Evidence for species interactions ameliorating the effects of ocean acidification exists but has rarely been investigated in a controlled environment. For example, the abundance of some crustaceans and fleshy algal species increases, while that of some carnivorous polychaetes decreases with reduced pH (Kroeker et al., 2011). Reduced rates of predation or increases in available habitat within macroalgal canopies may contribute to the observed increase in
crustacean abundance under decreased pH. Similarly, elevated CO₂ levels can increase rates of bioerosion for some marine calcifiers, due to the negative effects of ocean acidification on calcification (Stubler et al., 2014). Furthermore, manipulation of boundary layer thickness by macroalgal canopies can ameliorate the effects of ocean acidification on both canopy and understory algal species (Cornwall et al., 2013). Thus, species interactions will play a central role in overall community response to environmental change and this may be through simple mechanisms such as changes in species abundance leading to releases from or increases in the pressures of competition and predation or the provision of habitat. As observed herein, these mechanisms can also be more complex, such as via the manipulation of micro-scale seawater chemistry, which may facilitate the maintenance of normal metabolic rates for nearby organisms, despite regionally stressful environmental conditions.

5.3 Implications for macroalgal and reef habitats

This thesis found that coralline algae will be negatively impacted by seawater warming and acidification. However, I demonstrated that the precise nature of this response is dependent on additional factors such as the synergistic effects of additional environmental drivers and the influence of ecological interactions. During the 10-week tank experiment I observed contrasting effects of turf algae on Hydrolithoideae CCA, with rates of calcification both inhibited and enhanced during the experimental period. CCA photosynthetic parameters were reduced in the presence of turf algae and I hypothesized that this was due to the effects of shading under low-light conditions. If this was the case, an associated decrease in CCA calcification rates when turf algae were present would be expected. In contrast, the turf algal-driven increase in boundary layer pH relative to that in the surrounding seawater was likely responsible for the brief positive effect of turf algal overgrowth on CCA calcification. Under this model, coralline algae under ocean acidification conditions would benefit from turf-algal driven changes in seawater chemistry only in high-light environments wherein shading would not inhibit rates of coralline algal photosynthesis (Fig. 5.2A, B). Thus, in environments such as shallow tropical reefs where
irradiance levels are high, overgrowth by turf algae could allow for CCA to maintain their structural integrity under ocean acidification. Many organisms occurring in such habitats are highly dependent on reef structure (Idjadi and Edmunds, 2006), and on the coralline algae in particular to provide food and suitable substrata for settlement (Morse et al., 1988; Heyward and Negri, 1999). Thus, the interaction between turf and coralline algae may allow for the maintenance of higher reef diversity than would have otherwise been expected under ocean acidification conditions. Conversely, in lower light environments such as those dominated by kelp or macroalgae at greater depths or higher latitudes, shading by turf algae would be expected to have detrimental effects on CCA photosynthesis. Therefore, despite the potential for a facilitative interaction between turf and coralline algae in acidified seawater, the outcome of this interaction would ultimately be detrimental for underlying corallines in benthic habitats where light is limiting (Fig. 5.2C, D).

5.4 Implications for future research

There are multiple studies which have investigated the effects of ocean acidification alone as well as in combination with additional stressors such as higher temperature on coralline algae over a broad geographical range (Anthony et al., 2008; Martin and Gattuso, 2009; Johnson and Carpenter, 2012; Kamenos et al., 2013; Martin et al., 2013) and experimental duration is variable between studies. This was also the case in this thesis, with the laboratory studies ranging from 5-10 weeks in length, and the field study ranging from 9-24 months. I have shown that coralline algae are sensitive to the synergistic effects of multiple environmental factors and that their response to applied stressors can vary on both short and long-term scales. The results of the 10-week ocean acidification tank experiment revealed that while the overall effect of higher CO₂ remained constant over the experimental period, when considered together with turf algal overgrowth coralline algal metabolic response was temporally variable.
Fig. 5.2 Model of the effects of turf algal overgrowth on crustose coralline algae (CCA) in high (top panel) and low (bottom panel) irradiance conditions under ocean acidification. The combined effects of ocean acidification, irradiance level and turf algal overgrowth on CCA metabolism are indicated in the dot points in each sub panel and summarized by the large positive and negative symbols.

Therefore, it is a concern that some of the variability discovered in both this and prior work could be attributed to the length of the experimental period, particularly when investigating the effects of multiple stressors on coralline algal metabolism. Despite this, I have attributed much of the temporal variability in Hydrolithoideae metabolic rates observed during the ocean acidification tank experiment to the simultaneous contrasting effects of turf algal overgrowth on the processes of calcification and photosynthesis in a light-limited environment (Fig. 5.2).
Furthermore, I have demonstrated a potential mechanism behind the positive effect of turf algal overgrowth on coralline algal calcification under elevated CO$_2$. Future research should take note of the high temporal variability observed throughout this thesis, particularly during the 10-week ocean acidification tank experiment and should proceed with caution when interpreting coralline algal metabolic response to multiple applied stressors in experiments of limited duration.

In this work, I have investigated the effect of turf algal overgrowth on coralline algae in the context of ocean acidification and have demonstrated an effect of this interaction on coralline algal response to environmental change. In the natural environment, coralline algae interact with many other groups in several ways, which also have the potential to be modified by a changing environment. For example, reductions in coralline algal calcification rates can increase their susceptibility to grazing by urchins (Johnson & Carpenter 2012). In addition, due to the nature of the interaction between coralline and turf algae demonstrated herein, herbivory of fleshy algal species will indirectly affect the coralline algae. Further investigation of the many additional ecological interactions involving the Corallinaceae in the context of projected environmental change is required. Together with the information presented in this thesis, such knowledge will contribute towards a more complete understanding of the future fate of coralline algae in the various benthic habitats in which they occur.

5.5 Conclusions

The overall aim of this thesis was to assess the impact of climate change on the Corallinaceae coralline algae. The results of the three studies presented support the hypothesis that this group is negatively impacted by high seawater temperatures and ocean acidification. However, throughout these studies coralline algal response to warming and acidification was dependent on additional environmental factors such as light. Furthermore, competitive interactions with other macroalgal species played a critical role in driving coralline algal response to elevated temperature in the field, and modified their response to ocean acidification in the lab. As such, the effect of climate change on coralline algae within the Corallinaceae is
the result of a complex balance between its impacts on multiple metabolic processes which are closely integrated, as well as feedbacks between environmental change and the nature of ecological interactions, which may both facilitate and impede coralline algal metabolism under higher seawater temperature and CO$_2$, depending on the particular environment.
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Appendix A

First page of proof for publication arising from Chapter 3:

Interactions between filamentous turf algae and coralline algae are modified under ocean acidification

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1. Introduction
Crustose coralline algae (CCA) are a ubiquitous component of euphotic benthic communities from the tropics to the poles and from the intertidal to the deepest recorded depths for a photosynthetic organism. They also form biotic reefs over sediments (maerl) that occupy large areas of the continental shelf e.g. (Ryan et al., 2007). CCA require seawater conditions favouring the precipitation of calcium carbonate during the process of biogenic calcification for normal growth and production, and as the partial pressure of carbon dioxide (pCO2) in seawater increases, the saturation state of carbonate minerals decreases, adversely affecting the rate of calcification (Hoegh-Guldberg et al., 2007). Higher levels of pCO2 negatively affect coralline algal recruitment, growth, mortality, productivity and calcification (Anthony et al., 2008; Diaz-Pulido et al., 2012; Kuffner et al., 2008; Martin et al., 2013). Indeed, ocean acidification threatens the growth of these important organisms more so than other calcifying organisms such as aragonite-forming corals (Anthony et al., 2008; Jokiel et al., 2008; Ries et al., 2009), potentially due to the high solubility of their magnesium calcite skeletons (Feely et al., 2004; Morse et al., 2006) and possibly their biological response to changing seawater pH (McCulloch et al., 2012).

Ocean acidification is a decrease in seawater pH and carbonate ion concentration due to increased uptake of atmospheric carbon dioxide by the world’s oceans. This has major implications for many marine organisms, particularly the calcifiers. Crustose coralline algae (CCA) are among the most sensitive calcifying organisms to ocean acidification. In contrast, filamentous turf algae, which compete with CCA for space on the substratum, could potentially benefit from high pCO2 conditions, suggesting that the effects of filamentous turf on coralline algae may be amplified in a high pCO2 environment. The effect of ocean acidification on the growth of coralline algae, however, has rarely been investigated in combination with ecological interactions such as competition with filamentous turf algae. Here we tested the combined effects of ocean acidification and overgrowth by filamentous turf algae on CCA calcification, photosynthetic capacity and quantum yield of photosynthesis. We observed a positive effect of algal turfs on CCA calcification but a negative effect on photosynthesis in the high pCO2 treatments, however, these effects were variable over time. Our results have demonstrated the importance of investigating how inter-species interactions such as competition will complicate the impacts of ocean acidification.
Appendix B. Seawater properties observed just prior to midday in the large aquarium system (weeks 1-4, n = 3 tanks) and averaged between morning and evening in the small aquarium system (week 5, n = 2 measurements per day). pH \(_T\) (a), \(\rho CO_2\) (b) aragonite saturation state (\(\Omega_{Ar}\)) (c) and temperature (d) in high and ambient \(\rho CO_2\) treatment tanks. Data are means ± 1 s.d.