The ecophysiology of coralline algae in southern New Zealand

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Abstract

Coralline algae (Rhodophyta) are an important indicator of global environmental change in coastal ecosystem due to their global distribution and sensitivity to changing ocean climate. In temperate latitudes, coralline algae are a dominant component of macroalgae communities, are autogenic ecosystem engineers. They provide hard substrate for colonization of other marine algae and invertebrates, and food that helps sustain diverse communities of associated organisms. The metabolic processes, growth and reproduction of coralline algae heavily depend on environmental factors including light, temperature and ambient nutrients in seawater. These changes, in turn, directly affect physiological responses of coralline algae.

The primary aim of this study was to compare the nutrient and photosynthetic physiology between an important articulate coralline alga species (*Arthrocardia* sp.) and a group of indistinguishable crustose coralline species and their response to environmental fluctuations over seasonal cycles and depth gradients. Light, temperature and seawater nutrient concentration were sampled at 2 m and 10 m depth strata in a kelp forest where coralline algae were the dominant encrusting group over 21 months. The seasonal patterns in underwater light and temperature showed maximal values in summer and minimal values in winter, while the ambient nutrient concentration in seawater seasonally varied with higher concentration in winter, especially nitrate (7.68 ± 1.69 µM and 5.5 ± 0.77 µM at 2 and 10 m depths in winter, respectively). The highest temperatures at these depths were recorded in December 2017 (approximately 20 ºC at 2 m depth and 17 ºC at 10 m depth) when a marine heatwave occurred in New Zealand. Depth, season and the interaction between them clearly influenced to environmental conditions in seawater. These environmental parameters at the 2 m depth stratum were more seasonally variable, whereas these factors at 10 m depth were more stable. The nutrient and photosynthetic status of *Arthrocardia* sp. at 2 m depth, and crustose coralline algae at 2 m and 10 m depths also were investigated to evaluate effects of depth and season on nutrient and pigment concentration. Season influenced nutrient and photosynthetic pigment concentration of both *Arthrocardia* sp. and crustose coralline algae. Depth did not influence these concentrations of crustose coralline algae except for soluble tissue ammonium, C%, chlorophyll d and chlorophyll c concentration. Time-course nutrient
depletion experiments were conducted in winter and summer to determine the uptake rates and incubation time for nutrient uptake kinetics experiments for *Arthrocardia* sp. and crustose coralline algae at 2 m depth. An incubation of 120 minutes was suitable for multiple-flask experiments to determine the kinetics of nutrients of these coralline algae. The kinetics parameters (*V*<sub>max</sub> and *K*<sub>s</sub>) of ammonium, nitrate and phosphate of these coralline algae was determined. The ammonium uptake of both species exhibited a saturable kinetics with *V*<sub>max</sub> of *Arthrocardia* sp. (2.07 ± 0.32 µmol. gDW<sup>-1</sup>. h<sup>-1</sup>) was significantly higher than that of crustose coralline algae (0.58 ± 0.17 µmol. gDW<sup>-1</sup>. h<sup>-1</sup>). A linear relationship with concentration was observed in nitrate and phosphate uptake by both coralline algae. Higher surface area to volume ratio of *Arthrocardia* sp. was probably the primary factor of higher nitrogen and phosphorus uptake rates and differences in kinetic parameters than the group of crustose coralline algae.

Photosynthesis *versus* irradiance for *Arthrocardia* sp. and crustose coralline algae at 2 m and 10 m depths was determined to understand the effects of depth distribution on photosynthesis. The differences between the two coralline groups were the main factor driving the differences in maximal photosynthesis rates normalized to dry mass, wet weight and chlorophyll *a* between *Arthrocardia* sp. and crustose coralline algae at the same stratum. The maximum photosynthetic rate *P*<sub>max</sub> of *Arthrocardia* sp. (20.38 ± 2.38 µmol O<sub>2</sub>. gDW<sup>-1</sup>. h<sup>-1</sup>) was significantly higher than crustose coralline algae (3.72 ± 0.74 µmol O<sub>2</sub>. gDW<sup>-1</sup>. h<sup>-1</sup>) at the same 2 m stratum. Differences in depth distribution did not affect the photosynthetic parameters of crustose coralline algae measured. The photosynthetic characteristics of these coralline algae showed a shade adapted organism with low saturation irradiance (*E*<sub>k</sub> values are less than 100 µmol photons m<sup>-2</sup> s<sup>-1</sup>). This study provides the first information on nutrient and photosynthetic physiology of coralline algae on the subtidal rocky reef habitats under kelp forest communities in southern New Zealand. The findings from this study are broadly applicable to temperate rocky reef ecosystems and provide fundamental data for further studies related to coralline algae and to environmental conditions in coastal ecosystem.
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List of abbreviations

N: Northern
C: Central
NADPH: Nicotinamide adenine dinucleotide phosphate
ATP: Adenosine triphosphate
GTP: Guanosine triphosphate
OA: Ocean acidification
PE: Photosynthesis versus irradiance
PPFD: Photosynthetic photon flux density
PAR: Photosynthetically active radiation
PE: Phycoerythrins
PC: Phycocyanins
AP: Allophycocyanins
PBS: Phycobilisomes
PS I: Photosystem I
PS II: Photosystem II
PVC: Polyvinyl chloride
C:N ratio: Carbon: nitrogen ratio
CDOM: Chromophoric dissolved organic matter
PML: Portobello Marine Laboratory
SE: Standard error
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Table 5.1. Fitted parameters including $P_{\text{max}}$, $\alpha$, $E_k$, $E_c$, $R_d$ for photosynthesis versus irradiance curves (n=6) standardized to wet-weight, chlorophyll $a$ and surface area of coralline algae and the result of a Student’s t-Test between *Arthrocardia* sp. and crustose coralline algae at the same 2 m and between depths (2 m vs. 10 m).

Table A 2.1. The names of coralline algae in this study in GenBank.
Chapter 1
General introduction
1.1. Coralline algae

Coralline algae (Rhodophyta, Corallinales) are globally abundant red macroalgae (Steneck 1985) distributed in polar, temperate and tropical climates (Adey 1986, Nelson 2009, McCoy and Kamenos 2015). They grow in a variety of marine habitats such as rocky shores, seagrass meadows, tropical reefs, mollusk shells and artificial substrates or even unattached as rhodoliths (or maerl) (Steneck 1986, Nelson 2009). These species have wide depth ranges from intertidal zone to deep-waters and are known as the deepest growing benthic algae on the earth, being found at depths exceeding 200 m (Steneck 1986, Littler et al. 1991). One of the remarkable characteristics of coralline algae in comparison with other orders is calcification in the cell walls. This makes coralline algae one of the most important structural elements in marine ecosystem (Bosence 1991, Björk et al. 1995).

1.1.1. Taxonomy/diversity

The subclass Corallinophycidae was proposed by Le Gall and Saunders (2007) including the orders Corallinales, Rhodogorgonales (Fredericq and Norris 1995). Le Gall et al. (2010) separated Sporolithales from Corallinales to become one order in this subclass Corallinophycidae (see detail in Nelson et al (2015)). In 2015, Nelson et al. proposed one new order Hapalidiales originated form the family Hapalidiaceae of Corallinales. Table 1.1 shows the order, families and subfamilies of sub-class Corallinophycidae with the basic features to identify each order. However, modern methods of DNA sequence analysis are revolutionizing our understanding of coralline diversity worldwide, there are far more coralline algae species than ever described. For example, Gabrielson et al. (2018) indicated that the DNA sequences of encrusting Porolithon onkodes revealed the complexity and diversity of coralline algae genomes with the presence of abundant speciation of crustose coralline algae.

There are two major groups based on morphology of coralline algae, geniculate and non-geniculate. Geniculate coralline algae are referred to as articulated or turf coralline algae, while non-geniculate are known as crustose or encrusting coralline algae and rhodoliths (maerl) (Harvey et al. 2005, Farr et al. 2009). Geniculate coralline algae are branched, while the growth forms of non-geniculate coralline algae vary from parasitic to smooth crusts to free-living species, and correlate with environmental parameters especially
Table 1.1. The Sub-class Corallinophycidae and the basic reproductive feature characteristics of each family (Bosence 1991, Fredericq and Norris 1995, Harvey et al. 2003, Harvey et al. 2005, Farr et al. 2009, Nelson et al. 2015)

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Subfamily</th>
<th>Thallus</th>
<th>Male, female, carposporophyte conceptacles</th>
<th>Tetrasporangial conceptacles; tetrasporangia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corallinales</td>
<td>Corallinaceae</td>
<td>Corallinoideae</td>
<td>Geniculate</td>
<td>Uniporate</td>
<td>Uniporate; zonate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metagoniolithoideae</td>
<td>Geniculate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lithophylloideae</td>
<td>Non-geniculate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mastophoroideae</td>
<td>Non-geniculate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hapalidiales</td>
<td>Hapalidiaceae</td>
<td>Austrolithoideae</td>
<td>Non-geniculate</td>
<td>Uniporate</td>
<td>Multiporate; zonate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Choreonematoideae</td>
<td>Non-geniculate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Melobesioideae</td>
<td>Non-geniculate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporolithales</td>
<td>Sporolithaceae</td>
<td>No subfamilies</td>
<td>Non-geniculate</td>
<td>Uniporate</td>
<td>Calcified compartments; cruciate</td>
</tr>
<tr>
<td>Rhodogorgonales</td>
<td>Rhodogorgonaceae</td>
<td></td>
<td>Non-geniculate</td>
<td></td>
<td>None recorded</td>
</tr>
</tbody>
</table>
1.1.2. Ecological role

Coralline algae play a significant role in marine environment systems. The multiple functions of coralline algae in marine ecosystem are relatively well described (Nelson 2009, McCoy and Kamenos 2015).

Many aquatic organisms settle on the surfaces of coralline algae (Gherardi and Bosence 1999). The selective settlement on crustose coralline algae of barnacles *Balanus* is a typical example of this phenomenon (Steneck 1986). The snail *Turbo undulatum* settled on red turfing macroalgae *Corallina officinalis* in rocky shores (Worthington and Fairweather 1989). In addition, many marine invertebrate larvae such as starfish (*Acanthaster planci*), abalone (*Haliotis laevigata, Haliotis* spp.), sea urchin (*Heliocidaris erythrogramma*) preferentially settle on coralline algae (Johnson et al. 1991, Daume et al. 1999, Roberts 2001, Huggett et al. 2006).

Coralline algae are a food source for many organisms. Sea urchins such as *Strongylocentrotus droebachiensis* and *Evechinus chloroticus* eat branched coralline species (Himmelman and Steele 1971, Steneck 1983, Padilla 1984, Andrew 1988). Limpets (Patellacea) and chitons (Polyplacophora) graze hard substrates using unique dentition with a heavy silicate and iron mineral coating that allows them to graze on calcified corallines (Steneck 1983). Some fishes in tropical areas like parrot-fishes (*Scarus* spp.) also consume corallines (Steneck 1983, 1985, 1986). Although the branching coralline algae *Neogoniolithon strictum* provides refuge to the herbivorous crab *Mithrax sculptus*, the crab still consumes the coralline algae as well as the other macroalgae (*Halimeda* and *Dictyota*) (Stachowicz and Hay 1996).

Coralline algae are an important group to consider when seeking to understand ecosystem impacts of global environmental change because they can be dominant cover component of rocky reefs (Nelson 2009, Hepburn et al. 2011, McCoy and Kamenos 2015) and they are sensitive to changing seawater chemistry (e.g. Björk et al. 1985, Noisette et al. 2013, Cornwall et al. 2013b). Calcifying corallines algae in shallow subtidal reef communities can cover the majority (>52%) of rock surfaces (Shears and Babcock 2007, Hepburn et al. 2011).
The corallines, particularly crusts, have an extraordinary fossil record that is able to be used to analyze adaptive strategies over evolutionary time (Adey 1986, Steneck 1986). Coralline algae are a useful group for palaeoenvironment analysis because of their long life, ecological restrictions and flexibility of growth form in relation to environmental parameters (Bosence 1991). Free-living rhodoliths are sensitive indicators of turbulence in the shallow marine environment and their growth form is related to hydraulic energy. For example, open-branched forms of rhodoliths are found in the quieter water environments, while rhodoliths processing densely branched forms were observed in higher energy conditions (Bosence 1991).

1.1.3. Productivity

Growth rate of coralline algae are generally slower than other macroalgae species (see Fisher and Martone 2014). Crustose coralline algae belong to the slowest growing forms in the algal world (Steneck 1985). Lewis et al. (2017) summarized vertical and marginal growth and calcification rates of coralline algae worldwide. Among them, articulated coralline algae Calliarthron spp. has lowest vertical growth rate with 1-8 mm yr⁻¹, while encrusting coralline algae Porolithon pachydermum has lowest marginal growth rate with 3.6-18 mm yr⁻¹ (see Lewis et al. 2017). The annual carbonate production rates of coralline algae from 0.38-10.3 kg CaCO₃ m⁻² yr⁻¹ (Edyvean and Ford 1987, Freiwald and Henrich 1994, Chisholm 2000).

1.2. Physiology of coralline algae

1.2.1. Nitrogen and phosphorus physiology

Inorganic carbon, water, light and various mineral ions are vital elements for the photosynthesis and growth of macroalgae. C, H, O, N, P, Mg, Cu, Mn, Zn and Mo are required essential nutrients by all algae. Among these elements, nitrogen is a major part of metabolic compounds such as amino acids, purines, pyrimidines, amino sugars, amines (see Lobban and Harrison 1997, Hurd et al. 2014).

Nitrogen is the necessary element for macroalgae growth in the sea and the important forms for their growth are nitrate (NO₃⁻), ammonium (NH₄⁺) and urea ((NH₂)₂CO) (Hanisak 1983, Phillips and Hurd 2004). Although nitrogen gas N₂ is 20 times
more abundant than nitrate, macroalgae are unable use it directly (Lobban and Harrison 1997). The uptake rate for nitrate relates to its concentrations in the environment and the organism’s uptake abilities relative to shore position (Phillips and Hurd 2004). For example, the N uptake ability of Stictosiphonia arbuscula is highest because it grows at the highest intertidal position of the investigated macroalgae. The mid-intertidal Scytosiphon australis and low-intertidal Xiphophora gladiata follow in order of uptake ability at low concentrations (Phillips and Hurd 2003, Phillips and Hurd 2004). These authors showed that the uptake of nitrate is saturated in assay species in both winter and summer. Ammonium is an important N source in winter while urea is significant in summer for seaweed based on the relative importance of each N form to overall N nutrition (Phillips and Hurd 2003). These studies suggest that N sources are utilised in the order \( \text{NH}_4^+ > \text{NO}_3^- > \text{urea} \) in winter, whereas the order is \( \text{NH}_4^+ = \text{NO}_3^- > \text{urea} \) in summer.

Similarly, phosphorus is contained in important compounds such as ATP, GTP, and nucleic acids. The sources of phosphorus that are required by algae are orthophosphate ions (\( \text{PO}_4^{3-} \)), inorganic polyphosphates and organic-phosphorus compounds (Lobban and Harrison 1997). A study by Brown et al. (1977) indicated that enriched orthophosphate medium inhibits the growth of articulated coralline algae because phosphates interfere with calcification. These authors observed that Jania rubens died after 1-2 weeks culture with 30 \( \mu \text{MPO}_4^{3-} \) concentration, and Corallina officinalis grew slowly and stopped growth after a four or five-week experiment at the same concentration.

The parameters \( V_{\text{max}} \) – maximum uptake rate at saturating substrate concentration and \( K_s \) – half-saturation constant for substrate, obtained from hypothetical plots (Michaelis-Menten equation) are prevailing factors in nutrient absorption ability measurements of macroalgae (Lobban and Harrison 1997, Phillips and Hurd 2004). These parameters can be used to evaluate nitrogen and phosphorus uptake kinetics (Hurd and Dring 1990, Phillips and Hurd 2004).

### 1.2.2. Photosynthetic physiology

Photosynthesis is the transforming light energy into chemical energy by photoautotrophs (Falkowski and Raven 2007, Beer et al. 2014), thereby providing the
primary organic matter source for the growth and metabolic demands of other aquatic organisms in the marine ecosystem (Falkowski and Raven 2007). Photosynthesis in the marine environment is a challenge process as marine macrophytes live in special environment with large variation of light, UV-radiation, temperature, nutrient and salinity (Hanelt et al. 2003 Beer et al. 2014). There are two photosystems (I and II) of photosynthetic activity in the membrane that are connected by electron-transport chain. Each photosystem has its own light-harvesting complex (Cole and Sheath 1990, Lobban and Harrison 1997, Hurd et al. 2014). The reaction center pigment of PS I is P700 and contain many chlorophyll a molecules which are directly related in electron transfer (see detail in Hurd et al. 2014). Meanwhile, the reaction center pigment of PS II is P680 and contain a few chlorophyll a molecules. In red algae, phycobilisomes contribute the main light-harvesting in the PS II (see Cole and Sheath 1990). Photosystem II particularly oxidizes water to evolve oxygen and provides electrons and protons during light reaction (Barber 1987, Schulze and Caldwell 1995) (Figure 1.1). The transfer of electron and protons contribute to the driving force for NADPH and ATP synthesis (Schulze and Caldwell 1995, Falkowski and Raven 2007). Net photosynthesis of algae can be estimated by measurements of oxygen evolution or carbon dioxide consumption basing on a net exchange of CO₂ and O₂ during these process (Lobban and Harrison 1997, Falkowski and Raven 2007).

Photosynthesis is used as a main descriptor of the macroalgae responses variations to environmental conditions (Torres et al. 1991). Photosynthetic activity of algae are proved to be related to the temperature, pH, salinity, and other environmental factors in previous study (Martins et al. 2011, Comeau et al. 2014, Polo et al. 2014, Terada et al. 2016, Borlongan et al. 2018). For example, the photosynthesis of *Sargassum fusiforme* seedlings (photosynthetic electron transport rate) was significantly decreased by elevation of CO₂ under the low light conditions. A higher photosynthetic rates and compensation irradiance was observed on seedlings grown under the low light intensity and these seeding showing higher photosynthetic pigment contents and light absorption compared to seedlings grown under high light intensity (Chen et al. 2018). In addition, macroalgal morphology in combination with other factors can affect the photosynthesis. Stewart and Carpenter (2003) revealed a balance between maximizing photosynthetic ability and limiting susceptibility of the thallus to mechanical damage on five algal species.
(Sargassum palmeri, Zonaria farlowii, Dictyopteris undulata, Laurencia pacifica, Pterocladiella capillacea) representing three functional forms of marine macroalgae at Santa Catalina Island, California, USA. The result of this study also indicated that the rate of biomass- specific net photosynthesis increased with increasing surface area to volume ratio across functional groups. A trade-off between maximizing biomass specific net photosynthesis and reduction of the susceptibility of the thallus to mechanical damage may be a result of the interaction between plasticity of morphology and flow (Stewart and Carpenter 2003). Diverse photo-acclimation mechanisms and complications in regulation systems are challenges when determining the photosynthesis of macroalgae (Longstaff et al. 2002, MacIntyre et al. 2002), especially red macroalgae. The light harvesting system of red macroalgae contains phycobilisomes attached to the stromal surface of photosynthetic membranes and the absence of grana in thylakoid structure (Cole and Sheath 1990, Büchel and Wilhelm 1993). Phycobiliproteins in phycobilisomes of red macroalgae are considered to be major light harvesting pigments (Gantt et al. 1979, Gantt et al. 1986, Gantt 1990) which traps energy from sunlight to the photosynthetic reaction centres in the photosynthetic membrane (Gantt 1981, Gantt 1996). Three main classes of phycobiliproteins including phycoerythrins (PE), phycocyanins (PC), and allophycocyanins (AP) are primary components of phycobilisomes (Gantt 1981, Grossman et al. 1993) (Figure 1.2). Chlorophyll a still is the principal chlorophyll of red algae, and it also contains chlorophyll d (Manning and Strain, 1943).
Figure 1.1. Schematic diagram of light energy captured by phycobilisomes, attached to the stromal surface of thylakoids (Staehelin et al. 1978, see detail in Cole and Sheath 1990). The transfer of electrons occurs during the photosynthesis process to synthesise NADPH (Schulze and Caldwell 1995, Falkowski and Raven 2007).
Figure 1.2. Structure of phycobilisomes (see details in Cole and Sheath (1990))
1.3. Environmental threats to corallines and ecosystem services

1.3.1. Light/sedimentation

One of the most crucial factors that drive the distribution, morphology and physiological activities of macroalgae is light (Adey 1986, Gattuso et al. 2006). Many studies provide evidence for acclimation by macroalgae to significant periods of variable, low and undetectable light (Lüning and Dring 1979, Hepburn et al. 2011, Pritchard et al. 2013, Desmond et al. 2015). Macroalgal productivity and community structure can be significantly changed by variability in light (Fortes and Lüning 1980, Middelboe et al. 2006, Aumack et al. 2007, Desmond et al. 2015, Smale et al. 2016). For example, macroalgal biomass in kelp forests surrounded by forested catchments (low sediment run off and lower turbidity) were 2–5 times higher than kelp forests from coastlines modified by human activities (Desmond et al. 2015). The photo-acclimation of coralline algae differs among species and some of them have the ability to adapt to low light conditions, even overcome several months of darkness (polar winters) (Littler et al.1985, Freiwald and Henrich 1994). Various evolutionary physiological adaptations enhance the survival ability of coralline algae, such as their ability to utilise carbon during dark periods when photosynthesis is not possible (Freiwald and Henrich 1994). The authors indicated that coralline algae storage carbohydrates which is produced in photosynthesis process via production of starch grains in perithallial cell compartments. This assists coralline algae can survive during winter season when there was several darkness months and low light conditions within Arctic Circle. The photosynthetic pigment content of corallines also changes under different light intensities. The chlorophyll α concentration of Corallina officinalis increases in low light and decreases in high light (Kim et al. 2013).

1.3.2. Temperature

Temperature affects the geographical distribution and species composition of coralline algae (Wilson et al. 2004). Coralline algae are sensitive to seasonal variations in temperature including changes in their physiology so that they normally grow faster in warmer water (Steneck 1986). For example, seawater temperature increases from 10°C to 16°C causes a rise in primary production, calcification and respiration rates of Lithothamnion corallioides (Martin et al. 2006). Each macroalgal species has an ideal
temperature range for growth. When the temperature is over their optimal growth values, their growth rate is reduced (Hurd et al. 2014). A lack of normal seasonal growth patterns and high rates of mortality of crustose coralline algae Hydrolithoideae was observed when they were subjected to thermal stress after the 2010-2011 heatwave in Western Australia where temperature increased 3–5°C above average (Short et al. 2015). The increase of temperature (3°C higher than normal) negatively affects photosynthesis, calcification and survival of coralline algae (see Martin and Hall-Spencer 2017). Both sea surface temperature and the frequency of marine heatwaves are predicted to increase in the future (Law et al. 2017, Shears and Bowen 2017, Alexander et al. 2018, Oliver et al. 2018), which could result in a reduction of the cover of coralline algae and an increase of the cover of turf-forming algae (Wernberg et al. 2012).

1.3.3. Nutrient pollution

Eutrophication is one of the main factors degrading reefs due to its effects on the abundance and composition of reef macroalgae such as turfing and calcified algae which are important to the reefs’ ecological, environmental, aesthetic, social, and economic value (McCook, 1999). Habitat change, geographical and temporal expansion of some harmful algal blooms have been caused by eutrophication (Smayda, 1990, Anderson et al. 2002). Although there are many factors leading to increasing nutrients in the water column, the rate of nutrients sourcing from anthropogenic activity exported to coastal waters has noticeably increased in recent years (Glibert et al. 2005). The effects of terrestrial runoff on coralline algae associated with coral population were well summarized in previous work (see details in Fabricius 2005). The cover of coralline algae was observed to decrease when they were exposed to sewage water with high phosphate levels (Björk et al. 1995). The development of large fleshy algae and grazers under nutrient enrichment resulted in the decreasing abundance of crustose coralline algae (Chisholm 2003). In context of changing climate, the elevation of freshwater input in ocean from melting sea ice is predicted to lead to an increasing density gradient between surface and deeper waters (e.g. stratification) (Hetzinger et al. 2013). Reduced vertical mixing will, in turns, decrease the upward supply of nutrients to the surface from deeper waters (Boyd and Law 2011). Hetzinger et al. (2013) investigated the relationship between Ba/Ca ratios in algae and changes in surface ocean salinity and freshwater balance over
the past decades. The trace elemental composition (Mg/Ca, Ba/Ca, U/Ca, Sr/Ca) in skeletons of coralline algae is potential indicator for this phenomenon. These authors suggested that variation in Ba/Ca concentrations in the skeleton of crustose coralline algae *Clathromorphum compactum* is resulted from changes in the transport of cold, nutrient- and Ba-enriched deep waters (relative to shallow ice-diluted waters). The decrease in Ba/Ca ratios in the skeleton of *C. compactum* was observed in surface ocean freshwater variability conditions in northwestern North Atlantic shelf (Hetzinger et al. 2013).

### 1.3.4. Ocean acidification

Coralline algae are considered to be susceptible to changes in the seawater carbonate system associated with ocean acidification (OA), especially decrease in survival, calcification, growth, development and abundance (Kuffner et al. 2008, Cornwall et al. 2013b, Kroeker et al. 2013, Roleda et al. 2015, Law et al. 2018). Coralline algae are among the marine organisms most vulnerable to ocean acidification due to the solubility of their high magnesium-calcite skeletons (Martin and Hall-Spencer 2017). Most coralline algae show a decreased calcification rate at elevated pCO₂ (Harley et al. 2012) but this response is variable depending on species (see more details in Martin and Hall-Spencer 2017). The comparison of the lateral growth of crustose coralline algae under pH 7.60 (ocean acidification) and pH 8.05 in a six-week period indicates that the corallines are able to maintain their presence in the benthic rocky reef species assemblage, but the growth rates are lower at pH 7.60. This led to less capacity to recolonize and a reduction in coralline algae cover under OA conditions (James et al. 2014). *In situ* investigation of effects of the high carbon dioxide concentration on early life stages of crustose coralline algae communities indicated that crustose coralline algae lost 43% and 85% of cover when pH of seawater decreased from 8.0 to 7.8, respectively (Fabricius et al. 2015). In addition, ocean acidification induced the growth of other competitors such as noncalcifying algae, which contributed to the decline in coralline cover in the elevated CO₂ environment (Hepburn et al. 2011, Hofmann and Bischof 2014). Early successional benthic coral reef communities on PVC tiles deployed at two shallow-water volcanic CO₂ seeps and two adjacent control sites in Papua New Guinea were dominated by calcifying algae under ambient CO₂ but non-calcifying algae (predominantly green filamentous algae, cyanobacteria and macroalgae) increased from ~30% to ~80% cover when CO₂ increased
Recruitment, growth and abundance of coralline algae are usually negatively affected under OA (Kuffner et al. 2008, Martin and Hall-Spencer 2017).

1.4. Coralline algae in New Zealand

Coralline algae are common and clearly visible both in intertidal and subtidal habitats in New Zealand (Harvey et al. 2005). The taxonomy of common coralline algae species in the northern and central New Zealand (Figure 1.3) were described by Harvey et al. (2005) and Farr et al. (2009) in detail. 20 species of coralline algae have been confirmed in central New Zealand and more than 30 species in northern New Zealand (Table 1.2). Only recently has information become available on the taxonomy of coralline algae in southern New Zealand with identification of 77 species (Twist, 2019). This author reanalyzed the northern and central datasets of coralline algal species and updated the number of coralline algal species in these regions were 49 and 42, respectively. Much of the work to date on corallines in New Zealand has focused on ocean acidification (Cornwall et al. 2013, James et al. 2014, Roleda et al. 2015) and little is known about fundamental physiology of these key species in southern New Zealand.
Figure 1.3. Map of New Zealand, northern, central and southern New Zealand study area (after Harvey et al. (2005))
Table 1.2. The coralline algae species found in northern (N) and central (C) New Zealand (Harvey et al. 2005 and Farr et al. 2009)

<table>
<thead>
<tr>
<th>Family</th>
<th>Subfamily</th>
<th>Species name</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corallinaceae</td>
<td>Corallinaceae</td>
<td><em>Arthrocardia &amp; Corallina spp.</em></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Corallina officinallis</em></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Jania sagitata</em></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Jania rosea</em></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Jania verrucosa</em></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Jania sp. aff. unguilata</em></td>
<td>N</td>
</tr>
<tr>
<td>Lithophylloideae</td>
<td></td>
<td><em>Amphiroa anceps</em></td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Lithophyllum carpophylli</em></td>
<td>N &amp; C</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Lithophyllum corallinae</em></td>
<td>N &amp; C</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Lithophyllum johansenii</em></td>
<td>N &amp; C</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Lithophyllum pustulatum</em></td>
<td>N &amp; C</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Lithophyllum stictaeforme</em></td>
<td>N &amp; C</td>
</tr>
<tr>
<td>Mastophoroideae</td>
<td></td>
<td><em>Hydrolithon improcerum</em></td>
<td>N &amp; C</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Hydrolithon rupestris</em></td>
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</tr>
<tr>
<td></td>
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<td><em>Hydrolithon onkodes</em></td>
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</tr>
<tr>
<td></td>
<td></td>
<td><em>Neogoniolithon brassicaflorida</em></td>
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<td><em>Mastophorapacifica</em></td>
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</tr>
<tr>
<td>Genus</td>
<td>Species</td>
<td>Authorship</td>
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<tr>
<td><strong>Pneophyllum</strong></td>
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<td>N &amp; C</td>
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<td>fragile</td>
<td>N &amp; C</td>
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<tr>
<td></td>
<td>yendoi</td>
<td>N &amp; C</td>
<td></td>
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<tr>
<td><strong>Choreonema</strong></td>
<td>thuretii</td>
<td>N &amp; C</td>
<td></td>
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<tr>
<td><strong>Lithothamnion</strong></td>
<td>indicum</td>
<td>N</td>
<td></td>
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<tr>
<td></td>
<td>proliferum</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td><strong>Melobesia</strong></td>
<td>membranacea</td>
<td>N &amp; C</td>
<td></td>
</tr>
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<td><strong>Mesophyllum</strong></td>
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<td>N &amp; C</td>
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<td></td>
<td>erubescens</td>
<td>N &amp; C</td>
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<td></td>
<td>macroblastum</td>
<td>N &amp; C</td>
<td></td>
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<tr>
<td></td>
<td>printzianum</td>
<td>N &amp; C</td>
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<tr>
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1.5. Thesis outline

This research focuses on the fundamental physiology of coralline algae on subtidal rocky reefs in a *Macrocystis pyrifera* kelp forest in southern New Zealand (Shears and Babcock 2007, Hepburn et al. 2011, Desmond et al. 2015). Insights are provided on how differences between an articulated coralline algae species *Arthrocardia* sp. and a group of indistinguishable crustose coralline algae species, depths (2 m vs. 10 m) and season influences (i) nutrient and photosynthetic physiology, (ii) nutrient uptake kinetics, and (iii) photosynthesis *versus* irradiance relationship of coralline algae. Quantitative *in situ* measurements of environmental conditions at 2 m and 10 m depth strata, biological parameters of coralline algae, and controlled laboratory experiments were conducted to achieve this objective. The aim of this research is to answer the question:

1) How do environmental conditions in a shallow subtidal zone inhabited by coralline algae change seasonally?

2) How does the nutrient and photosynthetic physiology differ between the dominant articulate coralline algal species (*Arthrocardia* sp.) and crustose coralline species?

3) How does depth (2 m vs. 10 m) influence nutrient status and pigment concentrations of crustose coralline algae?

1.5.1. Chapter 2: Environmental conditions in a shallow subtidal zone inhabited by coralline algae in a kelp forest

The growth, reproduction and distribution of coralline algae are directly affected by abiotic factors such as light, temperature and available nutrients in marine ecosystem (Raven and Geider 1988, Hurd et al. 2014, Watanebe et al. 2014a, b, Smale et al. 2016). This chapter investigated seasonal variations in environmental conditions within a kelp forest. *In situ* light, temperature and available nutrients (nitrogen and phosphate) were measured at two depths (2 m and 10 m) in the shallow subtidal over 21 months. This environmental dataset was used as a baseline for evaluating seasonal and depths effects on nutrient and photosynthetic physiology of coralline algae. Additionally, these datasets were used to select suitable nutrient concentrations and irradiance for nutrient uptake kinetics and photosynthesis *versus* irradiance experiments of coralline algae.
1.5.2. Chapter 3: Spatial and temporal variability in nutrient status and pigment concentration of coralline algae in a kelp forest

Macroalgae need to be physiologically and morphologically flexible to obtain a balance between capacity to generate energy through photosynthesis and energetic requirement for growth and reproduction (Walters 2005, Gómez and Huovinen 2011). Nutrient status and pigment concentration in algae can be modified in response to environmental factors (Lapointe and Ryther 1979, Wheeler and Srivastava 1984, López-Figueroa 1992). Investigation of pigment and nutrient physiology of different coralline algal species will help to understand nutrient status and pigment concentration of coralline algae and to explain the ability of nutrient uptake and photosynthetic rate of each coralline algae species. The main focus of this chapter was to investigate the seasonality of nutrients and pigments between the articulated coralline alga *Arthrocardia* sp. belongs to the order Corallinales (geniculate form) and a group of crustose coralline algae species in the order Hapalidiales (non-geniculate form) at 2 m depth. A comparison between crustose coralline algae at 2 and 10 m depth strata was also made to compare the physiological traits of crustose coralline algae at different depths and light environments. Tissue nutrient concentrations (soluble tissue nitrogen and phosphorus, C:N ratio, nitrogen %, carbon %,) and pigment concentrations (phycobiliproteins and chlorophylls) were determined as indicators for nutrient and photosynthetic status of these coralline algae across season and depth. Investigation of pigment and nutrient physiology of different coralline algal species clarifies the relationship between species/group, depth and season on nutrient status and pigment concentration of coralline algae.

1.5.3. Chapter 4: Nitrogen and phosphorus uptake in articulated and crustose coralline algae in winter and summer

Nitrogen and phosphorus are limiting nutrients for macroalgal growth (Harrison and Hurd 2001, Hurd et al. 2014). Although the nutrient uptake characteristics of many macroalgae species are well known (Harrison et al. 1986, Thomas et al. 1987, Taylor et al. 1998, Martínez and Rico 2004, Phillips and Hurd 2004, Pritchard et al. 2015), the information on nutrient uptake and the uptake kinetics of coralline algae is lacking in the literature. The present study was the first study to compare nutrient uptake kinetics of a
geniculate/articulated species \textit{(Arthrocardia sp.)} and non-geniculate/crustose (order Hapalidiales) groups. Time-course nutrient depletion experiments were conducted to evaluate the changes in uptake of nitrate, ammonium and phosphate over time, in winter and summer, and to determine a suitable incubation for multiple-flask experiments to determine the kinetics of nutrients for \textit{Arthrocardia} sp. and the group of crustose coralline algae species. The nutrient uptake kinetics experiments were conducted to determine the kinetic parameters (\(V_{\text{max}}\) and \(K_s\)) for ammonium, nitrate and phosphate uptake for coralline algae (Harrison and Druehl 1982, Hurd and Dring 1990, Rees 2003, Harrison and Hurd 2001, Phillips and Hurd 2004, Hurd et al. 2014, Pritchard et al. 2015).

1.5.4. Chapter 5: Photosynthesis versus irradiance relationship for coralline algae

Macroalgae can acclimate to change in their photosynthesis activity to survive and growth in variable underwater light conditions (Ramus et al. 1976a, b, Hanelt et al. 2003). To evaluate the photosynthetic efficiency of photosynthetic organisms across different light levels, photosynthesis \textit{versus} irradiance (PE) curves are useful models to compare photosynthetic responses to light of different species (Coutinho and Zingmark 1987, Henley 1993, Watanabe et al. 2014a, Terada et al. 2016). In this chapter, photosynthesis over a range of irradiances was determined by measuring oxygen evolution of coralline algae under different irradiance levels. A comparison of photosynthesis parameters was made between \textit{Arthrocardia} sp. and the group of crustose coralline algae species at the same 2 m stratum. The effect of depth (2 m vs. 10 m) on photosynthesis was also incorporated by comparison of photosynthetic parameters delivered from the PE curves of crustose coralline algae species growing at different depths.

1.5.5. Chapter 6: General discussion

This chapter integrates findings from previous chapters and analyses the relationships between environmental conditions (daily quantum dose, temperature, ambient nutrients) and physiological parameters (endogenous nutrients and pigment contents) of coralline algae. This is followed by implications to relevant research, summary of findings and directions for future research.
Chapter 2

Environmental conditions in a shallow subtidal zone inhabited by coralline algae in a kelp forest
2.1. Introduction

Light, temperature and nutrients directly impact the growth, reproduction and distribution of macroalgae, key primary producers in shallow subtidal zones (Dring 1982, Hurd et al. 2014, Watanabe et al. 2014a, b, Smale et al. 2016). These effects are important as macroalgae are autogenic ecosystem engineers (Jones et al. 1997) providing habitat, food sources, and settlement substrata in marine ecosystem (Harvey et al. 2005, Nelson 2009).

Light plays a primary role in defining productivity in coastal ecosystems, providing energy for photosynthesis (Falkowski and Raven 2007). During the photosynthetic process, energy from sunlight is transformed into chemical energy which in turn is used to build carbohydrates (Larkum and Barrett 1983, Falkowski and Raven 2007). Light passes through the atmosphere before reaching the surface of the ocean and then enters the water column in natural marine environment (Kirk 1994). Differences in light availability are driven by attenuation by water itself and particles in water (Dring 1982). The presence of particles such as microorganisms (phytoplankton), biological detritus and sediments increases light attenuation (Dring 1982, Carr et al. 2010, Dickey et al. 2011) through the processes of absorption and scattering, which occur when the light interacts with these particles in the water column (Yentsch 1962, Lorenzen 1972, Stramski and Mobley 1997). Underwater irradiance decreases with depth in conformity with Beer’s Law (Gordon 1989, MacIntyre et al. 2000). The attenuation by the water itself reduces not only the amount of light but also, its spectral composition (Dring 1982). Hurd et al. (2014) indicated that vertical attenuation coefficient can be used to assess water turbidity which is a major factor affecting light availability at a certain depth. In the nine coastal water types classified by Dring (1982), curves representing spectral transmittance of the photosynthetically active radiation (400 to 700 nm, PAR) per meter in each water type were broad and similar at 1 m but these curves were transformed from broad into sharply peaked distributions as depth increased to ten meters depth. A compression of 70 % of the total visible quanta within a waveband of 100 nm bandwidth was particularly evident by a sharp peak spanning 100 nm in most coastal water types at 10 m depth. (see Dring 1982). According to Dring (1981), the peak of waveband fluctuated significantly with water type. The waveband ranges from a broad peak at 500-550 nm in clear coastal waters to a sharp
peak at 575 nm in the most turbid coastal waters. The light penetrates much deeper in the clearest oceanic waters. Therefore, benthic algae are able to grow at greater depths (Dring 1981, 1982).

Light availability can also be strongly affected by biogenic structures in the water column such as macroalgae (Reed and Foster 1984), seagrass (Duffy 2006), mangroves (Krauss et al. 2008) and corals (Fine et al. 2013). Reed and Foster (1984) demonstrated that shading by surface and subsurface canopies of the large kelps *Macrocystis pyrifera* and *Pterygophora californi* can reduce light at the benthos to < 3% of that above the canopy. Light attenuation through the water column can reduce growth and impact on competitive interactions of photosynthetic algae which in turn influences the structure of benthic macroalgae community and primary productivity (Dring 1982, Miller et al. 2006, Pritchard et al. 2013, Desmond et al. 2015). In cold temperate climate of southern New Zealand, the large brown macroalgae Ochrophyta (orders Laminariales, Durvillaeaales and Fucales) such as *Marginariella boryana*, *Carpophyllum flexuosum* and *Macrocystis pyrifera* predominantly distributed from 1 to 7 m depth, while the Rhodophyta such as filamentous turf forming rhodophytes *Anotrichium crinitum* and crustose coralline algae are dominant at greater depths from 9 to 12 m (Shears and Babcock 2007, Hepburn et al. 2011, Pritchard 2011, Desmond et al. 2015). The spatial distribution of macroalgal species across depths is typically regulated by the quality and intensity of light (Ramus 1981, Cole and Sheath 1990, Talarico and Maranzana 2000). The utilisation of light energy for photosynthesis at different depths is different among macroalgal species depending on the presence and the variability of light components (Talarico and Maranzana 2000, Johansson and Snoeijs 2002).

Macroalgae still require nutrients from seawater for growth and reproduction (Hurd et al. 2014). Among nutrients required for the growth of macroalgae, nitrogen and phosphorus are the two primary limiting macronutrients for metabolism, structure and energy transfer (Harrison and Hurd 2001). In seawater, the concentrations of these nutrients have been reported to be driven by many factors such as nutrient cycles (Herbert 1999), anthropogenic activity (Seitzinger et al. 2005), and season (Kain 1989). The seasonal fluctuation of nitrogen sources including nitrate, ammonium is well documented in previous studies. For example, the availability of nitrate (one of most abundant sources
of inorganic nitrogen in seawater) shows strong seasonal patterns in many temperate locations, with higher concentration in winter than summer (e.g. Dugdale and Goering 1967, Wheeler and Srivastava 1984, Brown et al. 1997, Phillips and Hurd 2003, Kregting et al. 2008, Stephens and Hepburn 2014, Pritchard et al. 2015). Ammonium is typically much more spatially and temporarily variable as it is regenerated from animal excretion (Bracken 2004, Hepburn and Hurd 2005, Fram et al. 2008, Aquilino et al. 2009). The presence of ammonium in marine environment is important nitrogen source during periods of low nitrate (Bracken and Nielsen 2004, Hepburn and Hurd 2005, Pritchard et al. 2015). Phosphate also plays an important role as a source for the growth of macroalgae (Hurd and Dring 1990, Glibert et al. 2005). In seawater, concentration of inorganic P (PO$_4^{3-}$) is generally lower than that of inorganic N (NO$_3^-$ + NO$_2^-$ + NH$_4^+$), for example, concentration of inorganic P is approximately 2 µM while inorganic N is 30-40 µM in the north east Pacific winter, and many macroalgae require more nitrogen compared to phosphorus nutrient to obtain a maximal growth (Harrison and Hurd 2001).

Temperature is an important abiotic factor that fundamentally affects geographic distribution, metabolic rates, enzymatic processes, and reproduction of macroalgae in marine ecosystems (Raven and Geider 1988, Lobban and Harrision 1997, Hurd et al. 2014). Macroalgae are able to adapt to changes in temperature through their biochemical and physiological evolution, for example, varying in the type and concentration of proteins and the properties of cell membranes (Eggert 2012). However, when temperature exceeds the tolerance range of a species, cellular and subcellular damage of can occur (Davison and Pearson 1996, Eggert 2012). According to Wernberg et al. (2012), the presence of heat waves in Jurien Bay, Australia caused a general reduction in cover of the dominant kelp (Ecklonia radiata), encrusting coralline algae and a significant decrease in total cover of macroalgal canopy. Meanwhile, an increase in cover of turf-forming algae was observed following the appearance of these heat waves (Wernberg et al. 2012). Similarly, rising surface seawater temperature (increase of 0.3°C/decade) is considered to be the main reason for the decline of temperate macroalgae species Sargassum spp. (Fucales) and gradual expansion of distribution of tropical macroalgae species, S. ilicifolium at Kochi Prefecture, southwestern Japan on the western North Pacific (Tanaka et al. 2012). The sea surface temperature is predicted to increase in future. For example, New Zealand sea surface temperature is forecasted to rise by 2.5°C in 2100 and this is predicted to
reduce in primary productivity by 4.5% and macronutrients at the ocean surface by 7.5-20% (Law et al. 2017). In addition, an extensive tropicalization in temperate regions will be due to warming patterns in western boundary current regions under future climate change (Shears and Bowen, 2017).

The south-eastern coast of New Zealand’s south island coast provides good examples of a cold temperate kelp forests (Shears and Babcock 2007, Hepburn et al. 2011, Desmond et al. 2015). The large brown macroalgae form canopies on the surface while subcanopies are formed by smaller primarily fleshy macroalgae (often Rhodophytes). Coralline algae species groups provide a foundation (Nelson 2009), with more than 50% of rock surfaces covered by crustose coralline algae in the shallow subtidal (Hepburn et al. 2011). To understand the influence of variability in environmental conditions on coralline algae growing within a kelp forest, light, temperature and available nutrients (nitrogen and phosphorus) were monitored over a 21 months at two depths in the shallow subtidal. These environmental data were collected as a baseline and provide context for evaluating physiological responses of coralline algae growth on the subtidal rocky habitats in the following chapters.
2.2. Methods

2.2.1. Study site

This study was conducted at wave-exposed coast Butterfly Bay (Te Awa Mokihi), Karitāne (45°38’26.98”S, 170°40’37.08”E), which is near the tip of Huriawa Peninsula and on the south-east coast of the South Island, New Zealand. The sampling site is 200 m from the edge of Karitāne village and is near the mouth of the Waikouaiti River (Figure 2.1). This sampling site was selected for this study because it was well known as an area possessing abundance of macroalgae communities (Hepburn et al. 2011, Prichard et al. 2013, Desmond et al. 2015). Seawater were collected at 2 and 10 m depths for analysing environmental parameters. The previous studies at the same area showed that these depth strata (2m vs. 10 m depth) were typical examples for shallow and depth water with different light concentration (see Desmond et al. 2015, Pritchar et al. 2013). In addition, the distinct macroalgae communities at these depth strata are described in detail by Hepburn et al. (2011). Articulated coralline algae are abundant in shallow waters and decrease in abundance with water depth, while crustose coralline algae are abundant coverage (from 52% to 81%) at all depths. Additionally, Xiphophora gladiata dominated in the 1-3 m depth stratum while Ecklonia radiata and Landsburgia quercifolia were most common in the 9-12 m depth stratum.
Figure 2.1. Butterfly Bay (Te Awa Mokihi), Karitāne, South Island, New Zealand
2.2.2. In situ irradiance - photosynthetically active radiation (PAR)

The photosynthetic photon flux density (PPFD) of photosynthetically active radiation (PAR) was recorded at surface, 2 and 10 m below mean low water of Butterfly Bay using the Odyssey® photosynthetic irradiance recording system (Odyssey, Dataflow Systems Limited, Christchurch, New Zealand) and HOBO® loggers (HOBO Pendant® Temperature/Light 64k Data Logger, Onset Computer Corporation, USA) (Long et al. 2012) from 05/04/2016 to 11/12/2017 (616 days). Each location set up one Odyssey logger and one HOBO logger, and each had one backup for changing. The Odyssey® loggers were attached to a 30 cm long aluminium stake using cable ties and stainless-steel hose clamps. Using SCUBA, the stake was driven into a crack between boulders and fixed in place using underwater epoxy (Expocrete UA, Fosroc, Petone, New Zealand). The sensors of the loggers were positioned parallel to the surface and protruding above the stake to prevent shading from the mooring system (see detail in Hepburn et al. 2011 and Pritchard et al. 2013). Similarly, HOBO® loggers were attached with 25 cm aluminium stand with the sensor parallel to the water surface by using cable ties (see detail in Desmond et al. 2015). The surface data loggers were installed in an unshaded location on the shore at Butterfly Bay. In order to prevent fouling of the sensor by algae and invertebrates and failure to operate correctly, the loggers were deployed for a maximum of three months (Pritchard et al. 2013). The loggers were cleaned, and batteries were replaced after deploying. The Odyssey® and HOBO® loggers were programmed to record irradiance every ten minutes.

The calibration of the Odyssey® loggers was carried out one day before deployment by using LI - 250A Light meter (LI-COR Inc, Lincoln, Nebraska, USA) connected to LI-COR light sensor. The loggers placed underwater were calibrated by both the LI - 192 underwater quantum sensor and the LI-190R quantum sensor in air while, the loggers positioned on the shore were only calibrated by the LI-190R quantum sensor in air. All logger sensors and LICOR quantum sensors were put in parallel in a specially designed box (20 x 40 x 10 cm) and placed in sunlight. The loggers were set up at one-minute recording intervals and these reading were compared with the referenced LI-COR meter that was measuring at the same time. The raw data over a range of light intensities from each calibration were pooled and natural log transformed from the Odyssey loggers
and LI-COR meters via linear regression (Figure A 1.1 and figure A 1.2). From this regression analysis, different calibration factors were required for logger data when they were deployed under water or in air. This was then applied to all data in underwater and in air to convert to µmol photons m$^{-2}$ s$^{-1}$.

Daily dose of light (mol photons m$^{-2}$ day$^{-1}$) was calculated by integrating the ten-minute light readings over 24 hours. Average daily dose (mol photons m$^{-2}$day$^{-1}$) for each month from April 2016 to December 2017 at surface, 2 m and 10 m depths was averaged from daily dose of light each month (n ≈ 30 days). Data from HOBO® were replaced when the Odyssey® data was lost due to the logger malfunction (from 2nd May to 18th July 2017 at 10 m depth stratum) and using the calibration formula from Desmond et al. (2015). The availability of photosynthetically active radiation (PAR), henceforth referred to as light. Daily dose of light (mol photons m$^{-2}$ day$^{-1}$), henceforth referred to as daily dose.

2.2.3. In situ temperature

The temperature at three different sites including surface, 2 m and 10 m depths at Butterfly Bay, Karitāne were recorded by HOBO® loggers (HOBO Pendant® Temperature/Light 64k Data Logger, Onset Computer Corporation, USA) that were described in 2.2.2 and were measured from 20/06/2016 to 11/12/2017 (540 days). These HOBO® loggers were programmed to record both light and temperature (˚C) every ten minutes. Daily mean temperature (˚C) was calculated by averaging the ten-minute temperature readings over 24h. The maximum and minimum values were also calculated from the daily mean temperature. The monthly temperature was averaged from daily mean temperature each month. The change of temperature per hour was calculated by averaging from the ten-minute temperature readings per hour for 540 days. Due to the logger malfunction from 2nd to the 29th May at 2 m depth and 14th September until the 19th October at 10 m depth, temperature data was provided from the same location from mooring data, SeaFET (http://satlantic.com/seafet (CARIM - Coastal acidification: Rate, Impacts and Management - project).
2.2.4. *In situ* nutrient seawater concentration

Seawater samples (n = 5) were collected at 2 m and 10 m depths depending on weather and turbidity conditions using 10% HCl washed 35 ml tubes from 05/04/2016 to 22/11/2017 (14 times). The tubes were filled rapidly by opening the caps underwater approximately 30 cm off the substratum by using SCUBA. The filled tubes were transferred to a boat and the seawater samples were immediately filtered (Whatman™ GF/C Glass Microfiber Filters, 1.2 µm) using an acid washed syringe (Terumo, Tokyo, Japan) and Swinnex filter holder (Millipore, Billerica, MA, USA). The syringes and holders were cleaned using ultra-high purity water (18.2 MΩ·cm Milli-Q®) and the filters were changed for each sample (see Pritchard et al. 2015). The seawater tubes were immediately transported to the Portobello Marine Laboratory (PML) and frozen for later analysis. Nitrate, ammonium and phosphate concentration contained in seawater were analysed by using a QuickChem® 8500 Series 2 Automated Ion Analyser (Lachat Instruments, a Hach Company Brand, USA). Nitrate concentration was calculated by quantitative reduction to nitrite by passage of the sample through a copperized cadmium column. The nitrite was then estimated by spectrophotometric analysis of the pink azo dye formed after addition of N-(1-naphthyl)-ethylenediamine dihydrochloride (NEDD), at 520 nm. Ammonium was determined by spectrophotometric analysis using the phenol-hypochlorite method, which measures the formation of indophenol-blue at 630 nm (see detail in Pritchard et al. 2015). Phosphate concentration was determined by absorbance of a blue complex, which indicate the reaction between PO₄³⁻ and ammonium molybdate and antimony potassium tartrate under acidic conditions, at 880nm (The Lachat Instruments 2008).

2.2.5. Data analysis

Percent surface irradiance at each depth were calculated from daily integrals of light at 2 m and 10 m depth. The downwelling attenuation coefficient $K_d$ (m⁻¹) is an important index which can be used to evaluate the quality or transparency of water (Dring 1982) in which a lower value indicates cleaner water (Dunton et al. 2009). The following formula (Kirk 1994) was used to calculate the attenuation coefficients between 2 m and 10 m depth:
\[
Kd = \frac{\ln \left( \frac{I_1}{I_2} \right)}{(d_2 - d_1)}
\]

where \( I_1 \) and \( I_2 \) are the daily doses calculated at depth 1 and depth 2, respectively; \( d_1 \) and \( d_2 \) are depths of two measurement (\( d_2 > d_1 \)) in meters.

The differences in daily dose, temperature and nutrient seawater concentration between times and depths were determined by using a two-way analysis of variance (Two-way ANOVA) followed by Tukey’s honest significant different (HSD) post hoc test with 95% family-wise confidence level. Data sets were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene’s median test) before statistical analysis. A Box-cox transformation was applied to improve normality and equal variance if the data failed to meet the assumptions of an ANOVA analysis.

All statistical analyses were performed using R Statistical Software Package (R Development Core Team, 2010).
2.3. Results

2.3.1. In situ irradiance

2.3.1.1. Daily dose of light

Light availability (daily dose) in the shallow subtidal zone was significantly affected by depth and month over the 616-day sampling period (Figure 2.2 and Table 2.1) and there was a significant interaction between those factors (Two-way ANOVA, $F_{40,1785} = 15.97, P<0.001$, Table 2.1). The effect of depth on average daily dose interacted with the months of year. The average daily dose in winter months was considerably lower than that in summer months and between 10 m and 2 m depth. The average daily dose decreased from 2 m to 10 m depth where the value at 10 m depth ($0.80 \pm 0.13$ mol photons $m^{-2} day^{-1}$) was 7 times lower than that at 2 m depth ($5.79 \pm 0.74$ mol photons $m^{-2} day^{-1}$) (Figure 2.3). The terrestrial daily dose was $18.65 \pm 1.54$ mol photons $m^{-2} day^{-1}$. During the survey period, there were 22 days in total of 616 days of deployment when light was undetectable at 10 m depth during daylight hours, which were usually recorded during late autumn and in winter from May to July. There was a significant influence of season on light availability at all depths investigated. The highest daily quantum dose was recorded in summer (December to February), and it then significantly declined in autumn before reaching a minimum dose in winter (May to July).

2.3.1.2. Percentage of surface irradiance at different depth

The percentage of surface irradiance at 2 m depth was approximately on average 28%. However, it varied highly, ranging from 0.15% to 99% (Figure 2.4). Meanwhile, at 10 m depth, the percentage of surface irradiance ranged from 0% to 23% and its mean was 4%.

2.3.1.3. Attenuation coefficients $K_d (m^{-1})$

Light attenuation was derived from the in situ underwater PAR measurement at 2 m and 10 m depths. The attenuation coefficient $K_d (m^{-1})$ varied from $0.00 – 1.13$ (m$^{-1}$) between 2 m and 10 m depth (Figure 2.5).
2.3.1.4. The photosynthetic photon flux density of PAR

The photosynthetic photon flux density (PPFD) of PAR varied depending on the depth (Figure 2.6). The PPFD averaged 211 \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \) terrestrially, whereas 64 and 9 \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \) were recorded at 2 m depth and 10 m depth, respectively. The highest PPFD recorded terrestrially was 2258 \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \). The maximal PPFD at 2 m depth was approximately three times higher than that of 10 m depth with 1353 \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \) and 390 \( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \), respectively.
Figure 2.2. Daily dose (mol photons m\(^{-2}\) day\(^{-1}\)) at the surface (top), 2 m (middle) and 10 m (bottom) depths from April 2016 to December 2017. Logging interval for each logger was 10 minutes. Lines represent a weighted least-squares regression smoother (loess). Shades area represents 1.96- times the standard error (approximate 95% C.I) of the loess smoother.
Figure 2.3. Average daily dose (mol photons m$^{-2}$day$^{-1}$) for each month from April 2016 to December 2017 at surface, 2 m and 10 m depths. Error bars represent mean ± standard errors (SE) for n ≈ 30 days.
Table 2.1. Two-way ANOVA statistics testing for the differences in the means across both depth and time (months) for daily dose, temperature, seawater nutrients.

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<td>13</td>
<td>35.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Depth x Month</td>
<td>12</td>
<td>3.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure 2.4. Percent of surface irradiance at 2 m (top) and 10 m (bottom) depths from April 2016 to December 2017. Logging interval for each logger was 10 minutes. Lines represent a weighted least-squares regression smoother (loess). Shades area represents 1.96- times the standard error (approximate 95% C.I) of the loess smoother.
Figure 2.5. The attenuation coefficient ($K_d$) calculated from daily integrals of PAR between 2 m and 10 m depth from April 2016 to December 2017. Note that this figure excludes 22 days of readings where $K_d$ could not be calculated because daily dose at 10 m depth was zero.
Figure 2.6. Histogram of PAR – Photosynthetically active radiation at surface, 2 m and 10 m depths from April 2016 to December 2017 (616 days).
2.3.2. *In situ* temperature

The ambient temperature considerably varied depending on the interaction between depth and month (Figure 2.7 and Table 2.1, Two-way ANOVA, $F_{36,1563} = 22.30$, $P < 0.001$). Temperature in winter months (June to August) was significantly lower than in summer months (December to February). The terrestrial temperatures are higher than the 2 m and 10 m readings in summer, but in winter they are lower than in deeper water. It is noted that the temperature in December 2017 was considerably higher than in December 2016 and this was the beginning of a heatwave in the summer of 2017/2018 in New Zealand. The variability between maximal and minimum temperature were gradually reduced in concomitance with the increasing depth from 2 m to 10 m (Figure 2.8). The gap between minimum and maximal temperature at 2 m and 10 m depths ranged from 0.00 - 3.82°C and 0.00 – 3.25°C, respectively. The thermal variation in seawater was high in summer and low in winter at all depths. The mean of water temperature at 2 m ($12.24 \pm 0.03°C$) was significantly 0.3°C higher than at 10 m depth ($11.92 \pm 0.01°C$) (Table 2.1, $P<0.001$). The temperature ranged from 7.58 to 19.66°C at 2 m depth and from 8.18 to 17.09°C at 10 m depth over ten-minute temperature readings of HOBO® loggers. The maximal temperatures at these depths were recorded in December 2017.

The changes in temperature during 24 hours at surface, 2 m and 10 m depths are illustrated in Figure 2.9. The terrestrial environment exhibited the greatest fluctuations of temperature for 24 hours while greater temperature stability was observed at 10 m depth. At 2 m depth stratum, the temperature changed slightly between day time and night time and reached a peak at 15 h ($12.54 \pm 0.03°C$). Meanwhile, the highest daily mean temperature recorded at 12 h ($21.99 \pm 0.15°C$) in terrestrial and 17 h ($12.33 \pm 0.04°C$) at 10 m depth.
Figure 2.7. The mean monthly of temperature (°C) in the 19-month study from June 2016 to December 2017 at surface, 2 m and 10 m depths. Error bars represent mean ± SE for n ≈ 30 days.
Figure 2.8. The variability of daily temperature (°C) at 2 m and 10 m depths from June 2016 to December 2017 (540 days) (these lines showed max, mean and min values, respectively)
Figure 2.9. The change of temperature over 24 hours at surface, 2 m and 10 m depths. Error bars represent mean ± SE for n = 540 days.
2.3.3. Ambient nutrient seawater concentration

Generally, there was significant variation in concentration of nitrate, ammonium in seawater depending depths and months (Figure 2.10, Table 2.1). Significant differences in nitrate concentration were found between winter and summer (Figure 2.10, $F_{13,103} = 51.49$, $P < 0.001$, Table 2.1). High concentrations of nitrate were detected from May to August and reaching a peak in June 2016 with 7.68 µM ± 1.19 µM and 5.5 µM ± 0.77 µM at 2 m and 10 m depths, respectively. During summer from December to March, the nitrate concentration remained at a low concentration with ranging from 0.43 µM ± 0.05 µM to 0.90 µM ± 0.07 µM. Similarly, the ammonium concentration varied depending on months ($F_{13, 103} = 10.71$, $P < 0.001$, Table 2.1) but in situ ammonium concentration did not show a clearly seasonal pattern (Figure 2.10). The concentration of ammonium in seawater ranged from 0.60 µM to 2.19 µM over 21 months. Ammonium concentration at 10 m depth was generally higher than at 2 m depth ($F_{1, 103} = 23.91$, $P < 0.001$, Table 2.1). The concentration of phosphate in seawater ranged from 0.2 µM to 0.4 µM. The phosphate concentration varied significantly between months but there was no significant difference in phosphate concentration between depths (Two-way ANOVA, $F_{1, 103} = 2.24$, $P > 0.05$, Table 2.1).
Figure 2.10. Seawater nutrient concentration at 2 m and 10 m depths. Error bars represent mean ± SE for n = 5.
2.3.4. Correlations between environmental parameters

Daily dose, temperature and ambient nutrient in Karitāne, southern New Zealand showed a clear seasonal pattern, which is typical of environmental parameters on temperate oceans. The correlations between these factors are described in Table 2.2. There was a positive correlation between daily dose and average temperature in the sampled area at 2 m depth \((r = 0.807, P = 0.002)\) and 10 m depth \((r = 0.629, P = 0.03)\). The daily dose and temperature were negatively correlated with nitrate and phosphate concentration in seawater at 2 m depth. At 10 m depth, negative correlations were found between daily dose or temperature and phosphate concentration \((r = -0.779, P = 0.003\) and \(r = -0.788, P = 0.002\), respectively) and temperature and nitrate concentration \((r = -0.703, P = 0.011)\).
Table 2.2. Pearson’s correlation based on mean values for environmental factors from 2 m and 10 m depths. Significant correlations are in bold.

<table>
<thead>
<tr>
<th>Depth</th>
<th>Correlation</th>
<th>df</th>
<th>r</th>
<th>P</th>
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</thead>
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<td>Daily dose and temperature</td>
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<td>0.807</td>
<td><strong>0.001</strong></td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Daily dose and nitrate</td>
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<td>-0.628</td>
<td><strong>0.029</strong></td>
</tr>
<tr>
<td></td>
<td>Daily dose and phosphate</td>
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<td>-0.863</td>
<td><strong>0.0003</strong></td>
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<tr>
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<td><strong>0.02</strong></td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>Temperature and phosphate</td>
<td>10</td>
<td>-0.788</td>
<td><strong>0.002</strong></td>
</tr>
</tbody>
</table>
2.4. Discussion

Light, temperature, and nutrients strongly influence physiological processes including growth, distribution, reproduction of macroalgae (Wernberg et al. 2012, Tanaka et al. 2012, Hurd et al. 2014, Watanabe et al. 2014a, b). Investigations focused on variability of these factors across environmental gradients provide a baseline and help understanding of physiological and biological responses of macroalgae to a changing marine environment (Guenther and Martone 2014, McCoy and Kamenos 2015, Smale et al. 2016). In order to obtain a baseline of environmental condition for subsequent chapters, this study provided temporal and spatial context for light, temperature and ambient nutrients in the subtidal temperate rocky reefs at Butterfly Bay, Karitāne, New Zealand.

Data gathered here, over a 616-day period demonstrated that in-situ irradiance at the Huriawa Peninsula in present study was comparable with those of the previous studies in the same area by Hepburn et al. (2011), Pritchard et al. (2013) and Desmond et al. (2015). In particular, surface and underwater PAR measured continuously from April 2016 to December 2017 showed a clear influence of depth and season on the daily dose reaching macroalga communities. In the present study, the daily dose at 10 m depth ($0.80 \pm 0.13 \text{ mol photons m}^{-2} \text{ day}^{-1}$) was on average seven times lower compared to that at 2 m depth ($5.79 \pm 0.74 \text{ mol photons m}^{-2} \text{ day}^{-1}$). A similar finding was report by Hepburn et al. (2011) who indicated that the average light dose was ten times higher at the 2 m depth than 10 m depth during summer with 9.85 and 0.84 mol photons m$^{-2}$ day$^{-1}$, respectively. In addition, the seasonal change of underwater light was also reported at Otago Harbour, South Island, New Zealand (Hepburn and Hurd 2005) with low values in winter and high values in summer (Brown et al. 1997). Rodgers and Shears (2016) also showed that depth and season clearly influenced to light distribution in northern New Zealand. In that location, the average daily photon flux density at 14 m was 2.5 lower than 6 m depth and the winter received less light ($\sim 50\%$) than in summer all depths. The seasonal variation of daily dose of light at different depths including 2 m, 4 m, 8 m, 10 m and 15 m was observed in the sublittoral region near Helgoland - North Sea (Lüning and Dring 1979). Instantaneous PAR measurement indicated that the light was attenuated from surface to 10 m depth. The maximal photon flux density measured at 10 m depth (390 µmol photons m$^{-2}$ s$^{-1}$) was 3.5 times lower than that of 2 m depth (1353 µmol photons m$^{-2}$ s$^{-1}$). The difference in photon flux density between the depths
affects the diversity and distribution of benthic photosynthetic organism (Dennison 1987, Gattuso et al. 2006). Distinct depth gradients of macroalgae communities along in the temperate region have been described in many previous studies (Wernberg et al. 2003, Shears and Babcock 2007, Wernberg et al. 2011, Hepburn et al. 2011). Particularly, the dominance of the Phaeophyceae – large brown macroalgae was observed at shallower water, whereas understory species such as non-calcareous algae and coralline algae (Rhodophyceae) were distributed at deeper area. Gattuso et al. (2006) indicated that the daily dose ranging from 0.4 to 5.1 mol photons m\(^{-2}\) d\(^{-1}\) was minimum daily dose required for growth of benthic communities. However, crustose coralline algae can survive at a 268 m depth at which the irradiance was only 0.015 to 0.025 µmol photons m\(^{-2}\) s\(^{-1}\) corresponding 0.0005% surface irradiance (Littler et al. 1985). The light availability at each depth also affects the biomass of macroalgae. The biomass of macroalgae at 2 m depth is considerably more abundant than those at 10 m depth (Harrer et al. 2013, Desmond et al. 2015).

Notably, no light was detected at 10 m depth stratum for 22 days (3.6% of total study period) in winter from May to July. Similarly, no light was detectable within 10 m depth during 14 days at the same site in winter 2013 (Desmond et al. 2015) and for 20 days in 2006 (Hepburn et al. 2011). Lüning and Dring (1979) demonstrated no light detected on some days during late autumn and in winter in the sublittoral region near Helgoland (North Sea). There are many reasons which can cause no light at 10 m depth stratum for 22 days of this study period such as low surface irradiance due to weather conditions, sediment load or even fouling of light sensors. In this case, it is possible to rule out the fouling of light sensors. These loggers were cleaned periodically and deployed for a maximum of 3 months in order to prevent fouling of the sensor by algae and invertebrate as described by Pritchard et al. (2013). In addition, based on observation from previous studies (Hepburn et al. 2011, Desmond et al. 2015) and personal observation at the same sampling site, it is high possibility that floods and wave action may be the major factors for no light at 10 m depth recorded in the present study. Some macroalgae inhabited in cold, deep waters at high latitudes can survive for long periods under low light or complete darkness (Dunton and Schell 1986, Weykam et al. 1997). The rhodophyte macroalgae at the high Arctic or Antarctic was proved to maintain biomass during 6 months of complete darkness through utilizing stored carbohydrate reserves under low respiration rate R\(_d\) enforced by very cold ambient temperatures and equally long periods of high light during summer (ice-free)
periods (Gomez et al. 2009). At present, knowledge about the survival of coralline algae without light is very limited. Recently, the minimum light requirements of *Anotrichium crinitum*, which dominates near the maximum depth limit for macroalgae throughout New Zealand and southern Australia was examined by Pritchard et al. (2013). The result of this study showed that *A. crinitum* can maintain biomass under sub-compensation (critical) light levels for at least 5 days. Therefore, the survival and growth of coralline algae under a long window of darkness time (*in situ* condition) should be done in future.

The attenuation coefficients ($K_d$) show the decline of irradiance by absorption and scattering in water column, and these were calculated based on the daily dose measurement at different depths. The attenuation coefficient $K_d$ (m$^{-1}$) varied from 0.00 – 1.13 (m$^{-1}$) between 2 m and 10 m depth and the season of attenuation of light through the water column was not observed in this study. The study site, Butterfly Bay is near the mouth of the Waikouaiti River which receives sediment runoff from the surrounding catchment particularly during floods (Hepburn et al. 2011, Pritchard et al. 2013). Evidence for relatively high turbidity at the study site is also provided by Desmond et al. (2015) who showed that the turbidity at coastline modified by human activity was two times higher than that of forested catchments. More fouling of sensor in loggers positioned at 2 m depth was observed in comparison with loggers at 10 m depth over two-month logger deployment (personal observation, Nguyen, H. T. T). Apart from anthropogenic factors, the diversity and distribution of algae also affect the light attenuation. Evidently, the over shadowing presence of the kelp forest on temperate rocky reefs directly affects the light capture of understory species through canopy shading (Wing and Patterson 1993, Graham et al. 2007). Krause-Jensen and Sand-Jensen (1998) reported that the light attenuation in macrophyte community habitats was lower than that of phytoplankton and benthic microalgal communities due to higher chlorophyll concentration of microalgae in water. Moreover, light attenuation in oceanic water is different with estuarine water because of the different light absorption in water column. The abundance of chromophoric dissolved organic matter - CDOM, phytoplankton and detritus in estuarine water column lead to light more quickly attenuated and blue light absorbed than in oceanic water which dominantly attenuated in red light (MacIntyre et al. 2000, Lawrenz et al. 2010).
In the present study, temperature varied depending on season and depth. The mean temperature at 2 m depth was 0.3°C higher than that at 10 m depth. Similarly, the long-term in situ water temperature recorded from 1988 to 1995 at different water depths (8, 12, 15, 18, and 21 m) in the Point Loma kelp forest community off San Diego, California, USA indicated that in situ water temperature considerably decreased with depth (Dayton et al. 1992, 1999). Additionally, the calculation of seawater temperature based on Mg/Ca ratio of benthic foraminifera in Belek, East Antalya, Turkey revealed that the water temperature declined depending on depth (Parlar and Görmüs 2011). Data from the present study indicated that the variability of daily temperature in seawater at 2 m was higher than 10 m. The deeper the water, the more stable temperature. At all investigated depths, temperature changed seasonally during the study period (from June 2016 to December 2017). The maximal temperature was recorded in summer, and the minimal temperature was observed in winter. This is consistent with data of Dayton et al. (1992, 1999), who found that the biomass of macroalgae is affected by the seasonal change of temperature. For instance, Sargassum canopy biomass in a coral reef ecosystem was higher in summer (Fulton et al. 2014). According to Desmond et al (2015), the peak biomass of benthic macroalgae community at Karitāne was observed in summer. Similarly, during 15 consecutive months, maximum biomass of estuarine intertidal macrophytes was recorded in summer (136 to 168 g dry weight 0.1 m−2) while minimum biomass (34–48 g dry weight 0.1 m−2) was found in winter at Cedar Point, Little Bay, New Hampshire, USA (Chock and Mathieson, 1983). Changes of temperature directly impact the photosynthesis, growth and geographic distribution of seaweed (Harley et al. 2012, Eggert 2012, Lideman et al. 2013, Tereda et al. 2013). For example, both of the macroscopic sporophyte and microscopic gametophyte stages of Costaria costata (Laminariales, Phaeophyceae) had maximal net photosynthetic rate and maximal quantum yield (Fv/Fm) at optimal temperature ranging from 14°C to 22°C. Although photosynthetic activity of C. costata decreased when the temperature increased above 24°C, low temperature did not inhibit the photosynthesis of this brown seaweed. This is likely a reason explained the distribution limitation of C. costata sporophytes which disappears in summer in Japan (Borlongan et al. 2018). In Karitāne, the temperature measurements in December 2017 were part of an unprecedented coupled ocean-atmosphere summer in New Zealand where sea surface temperature anomaly increased +3.7°C (Salinger et al. 2019)
The ambient nitrate concentration in seawater varied seasonally with higher concentration in winter. This result is consistent with previous studies in temperate regions where high nitrate concentration are generally observed seawater in winter (Chopin et al. 1989, Fujita et al. 1989, Harrison and Hurd 2001, Phillips and Hurd 2003, Hepburn et al. 2007, Kregting et al. 2008, Pritchard et al. 2015). The concentration of nitrate reached a peak in June 2016 with 7.68 µM ± 1.19 µM and 5.5 µM ± 0.77 µM at 2 m and 10 m depths, respectively. The ambient ammonium concentration varied from 0.60 to 2.19 µM in this study. The major factor causing this phenomenon may be due to the occurrence of mixing with deeper water masses and nitrate delivery from deep water where nitrate is very high and is produced by bacteria (Hepburn, 2003). Ammonium sources are most likely animal excretion (Hepburn and Hurd 2005, Pritchard et al. 2015). The concentration of phosphate was low in both depths ranging from 0.19 ± 0.009 µM to 0.49 ± 0.01 µM and did not show a clear seasonal change. This phosphate concentration was similar to phosphate concentration recorded in Antarctic coastal waters (0.11 – 0.39 µM) (Dunton et al. 2009).

Daily dose, temperature and nutrients interact strongly in coastal environments (Hurd et al. 2014). It is difficult to analyse separately the influence of these environmental factors (Wernberg and Goldberg 2008). The results of the present study are also in agreement with Kain (1989) indicating that the seasonal change of light obviously impacts on temperature with positive correlation between these factors, while ambient nitrogen had a significantly negative correlation with temperature. Additionally, this study indicated daily dose and temperature showed negative correlations with ambient nitrate and phosphate concentration at 2 m depth. At 10 m depth phosphate concentration was negatively correlated with daily dose or temperature, while nitrate concentration only showed significantly negative correlation with temperature. In the summer, the water column is thermally stratified (a thermocline forms), therefore deep-water nitrate cannot reach areas where algae grow (Manasrah et al. 2006). Moreover, the increase of light in the summer means that large take up most of the nitrate for high primary productivity and this nutrient will not replenished until the waters and the thermocline breaks up (Hepburn 2003, Manasrah et al. 2006). Taken together, the negative correlation between nitrogen and temperature found in the present study is reasonable. The interaction between these factors can have significant effects on the growth rate and reproduction of macroalgae (Kain 1989, Riebesell et al. 2009). For example, Gao et al. (2017) indicated that temperature and nutrient availability interacted to affect the
relative growth rate of small sporophytes of *Saccharina japonica* (Laminariales, Phaeophyceae) especially at 5 to 10°C. However, the growth rate of larger size sporophytes decreased at 10 and 15°C due to the reduction of available nutrients when temperature increased.

In conclusion, the daily dose, temperature and nutrient concentration at Karitāne significantly varied depending on depth and season. Daily dose, temperature and nutrient concentration was more variable at 2 m compared to at 10 m depth stratum. The seasonal patterns of underwater light and temperature showed low values in winter and high values in summer. The maximal PPDF at 2 m and 10 m depths were 1353 µmol photons m\(^{-2}\) s\(^{-1}\) and 390 µmol photons m\(^{-2}\) s\(^{-1}\), respectively. The temperature ranged from 7.58 to 19.66 °C at 2 m depth and from 8.18 to 17.09 °C at 10 m depth over ten-minute temperature readings of loggers. The highest temperatures at these depths were recorded in December 2017 when a marine heatwave occurred in New Zealand. The ambient nitrate concentration in seawater varied seasonally with higher concentration in winter, while ammonium and phosphate concentration did not show a clearly seasonal change. Seasonal change of light showed positive correlation with temperature, whereas light and temperature negatively correlated with ambient nutrient concentration except for temperature and ammonium relationship. Understanding temporal and spatial patterns in these parameters at different depths is crucial in understanding seasonal and depth effects on photosynthetic process and nutrient physiology of coralline algae which dominate on the subtidal rocky reef habitats. Furthermore, the ambient environmental dataset provided here is key in selecting appropriate nutrient concentrations and irradiance levels for experiments evaluating the nutrient (nitrate, ammonium, phosphate) uptake ability and photosynthetic physiology of coralline algae.
Chapter 3

Spatial and temporal variability in nutrient status and pigment concentration of coralline algae in a kelp forest
3.1. Introduction

In marine ecosystems, environmental conditions are highly variable (Chopin et al. 1989, Brown et al. 1997, Dayton et al. 1999, Dunton et al. 2009, Rodgers and Shears 2016). For example, light availability in coastal water varies depending on a large variety of factors including water motion, tidal cycles, turbidity, clouds, season and anthropogenic activities (Dring and Lüning 1994, Kirk 1994, Anthony et al. 2004, Desmond et al. 2015). The availability of nutrients also varies, often strongly correlated with season, especially nitrogen which is a limiting nutrient for macroalgal growth (Phillips and Hurd 2003, Hurd et al. 2014, Pritchard et al. 2015, Chapter 2. Section 2.3.3). To adapt to such variability, it is necessary for macroalgae to be physiologically and morphologically flexible in order to obtain a balance between the capacity to generate energy through photosynthesis and energetic requirements for growth and reproduction (Walters 2005, Gómez and Huovinen 2011).

Red algae have short- and long-term acclimation strategies at different levels (individual, cellular and molecular level) to survive under a constantly changing light field (see in Talarico and Maranzana 2000). For example, amounts of the phycobillisomes (PBSs, a light-harvesting complex of red algae) per cell and number of PBSs per square micrometer of red algae at low light areas are higher in comparison with species growing under high light (Gantt 1981, Wehrmeyer 1990). The differences in size, organization and number of chloroplasts between macroalgae growing in low and high light are well documented. Either larger chloroplasts with more thylakoids per chloroplast or higher number of chloroplasts are observed in macroalgae growing in lower light (Talarico and Maranzana 2000). Pigment concentration in algae can also be modified in response to other environmental conditions such as nutrient availability, light regime and water depth (Ramus et al. 1976 a, b, Lapointe and Ryther 1979, Wheeler et al. 1984, López-Figueroa 1992). Lapointe and Ryther (1979) observed nitrogen-related pigment changes in Gracilaria foliifera with an inverse relationship between absolute levels of both phycoerythrin and chlorophyll and C:N ratio.

Depending on the availability of ambient nutrient concentration, macroalgae have different strategies for nutrient uptake, assimilation and internal nutrient stores (Martínez et al. 2012, Hurd et al. 2014). Nutrients can be stored intracellularly to be utilized in case of nutrient deficiency (Hanisak 1979, Thomas and Harrison 1985, Chopin et al. 1989). Nitrate
is stored in the vacuole and cytoplasm or reduced to nitrite, then nitrite is transported to the chloroplasts where it is reduced to ammonium. Ammonium formed from nitrate or urea is quickly assimilated to amino acids (Raven and Smith 1976, Harrison and Hurd 2001). Storage of internal nitrate (NO$_3^-$) in tissues of sugar kelp *Saccharina latissima* occurred when levels of NO$_3^-$ in enriched medium exceed the satisfactory levels required for growth (Chapman et al. 1978). This excess nutrient is possibly utilized when external nutrient is limited (Hanisak 1979, Phillips and Hurd 2003). The importance of phosphate storage is still unclear because polyphosphate, a form of accumulated phosphate, was found in different cells depending on species. For example, polyphosphate was found in the medullary cells of *Chondrus crispus* (Chopin et al. 1997), whereas *Ulva lactuca* stored polyphosphate in the vacuole (Lundberg et al. 1989).

In cold temperate climates, coralline algae, a dominant component of benthic communities with often over 50% coverage on rocky reefs (Hepburn et al. 2011), play an important role in ecological marine systems (Nelson 2009, McCoy and Kamenos 2015). The calcification in cell walls of coralline algae significantly contribute to the stability of coral reef ecosystem, and calcium carbonate deposition by coralline algae is part of the global carbon cycle (Goreau 1963, Farr et al 2009, Nelson 2009). In addition, marine organisms such as starfish (Johnson et al. 1991), abalone (Daume et al. 1999), sea urchins (Huggett et al. 2006), fleshy macroalgae and diatoms (Figueiredo et al. 1997, James et al. 2014) use the surface of coralline algae for settlement. Geniculate and non-geniculate are two groups of coralline algae commonly observed in both intertidal and subtidal habitats (Harvey et al. 2005, Shears and Babcock 2007, Farr et al. 2009, Hepburn et al. 2011, Noisette et al. 2013, Guenther and Martone 2014, McCoy and Kamenos 2015). Geniculate corallines have alternating calcified and uncalcified segments on upright branches, whereas non-geniculate species either lack branches or have entirely calcified branches but no uncalcified joints or nodes (Harvey et al. 2005).

There is a limitation of study related to the physiological response of coralline algae to variation of environmental condition. Noisette et al. (2013) initially evaluated the physiological response between geniculate alga *Corallina elongata* and two non-geniculate coralline alga species *Lithophyllum incrustans* (crustose) and *Lithothamnion corallioides* (rhodolist or maerl beds) to near future ocean acidification in the north-western coast of
Brittany, Atlantic Ocean. The physiological response of coralline algae to near-future ocean acidification is species-specific and that the elevated CO₂ could indirectly impact this coralline species physiology making them more sensitive to other stresses (Noisette et al. 2013). Pigment composition including chlorophyll a and phycobiliproteins (phycoerythrin, phycocyanin, allophycocyanin) between two species of geniculate group including *Calliarthron tuberculosum* and *Corallina vancouveriensis* living in tidepool and non-tidepool, respectively, was also investigated during a simulated tidal cycle in North Pacific Ocean (Guenther and Martone 2014). The authors indicated that *C. vancouveriensis* was more photosynthetically active than *C. tuberculosum* in both high tide (light level increased) and low tide. However, pigment composition was not significantly different between these species, suggesting that light harvesting pigment does not related to this difference. In southwestern Pacific Ocean, both groups geniculate and non-geniculate recently received much more attention, evaluating their response to ocean acidification in condition of elevated CO₂ concentrations and altered pH (Cornwall et al. 2013a, b, Cornwall et al. 2014, James et al. 2014, Roleda et al. 2015). For example, flow conditions were found to promote the formation of thick boundary layer, and this enhanced the subsistence of *Arthrocardia corymbosa* through creating localised hydrodynamic conditions where metabolic activity ameliorates the negative impacts of ocean acidification (Cornwall et al. 2014). Despite their global importance and broad depth distribution, surprisingly little is known about internal nutrient and pigment concentration of coralline algae over seasonal cycles, over depth gradients and between morphological groups (articulated vs. crustose coralline algae). Therefore, the main focus of this study was to investigate the seasonality of nutrient and pigments contents of two different groups of coralline algae, *Arthrocardia* sp., a species of articulated coralline algae in the order Corallinales (geniculate form) and a group of morphologically indistinguishable crustose coralline algae species in the order Hapalidiales (non-geniculate form) at 2 m depth. A group of morphologically indistinguishable crustose coralline algae species in the order Hapalidiales at 10 m depth stratum were also added to compare the physiological traits of crustose coralline algae at different depths in a kelp forest in southern New Zealand. Tissue nutrient concentrations (soluble tissue nitrogen and phosphorus, C:N ratio, nitrogen %, carbon %,) and pigment concentrations (phycobiliproteins and chlorophylls) were determined every month or two months, depending on weather conditions, over 18 months. These factors were used as indicators for
nutrient and photosynthetic status of these macroalgae across season and depth. Investigation of pigment and nutrient physiology of different coralline algal species aimed to clarify effects between species/group, depth and seasons on nutrient status and pigment concentrations of coralline algae. These data were then used to support the design of experiments that relate to nutrient uptake kinetics and photosynthesis versus irradiance for coralline algae in the following chapters.
3.2. Methods

3.2.1. Collection and pre-treatment of coralline algae

Articulated coralline algae were collected at 2 m depth, while crustose coralline algae were collected at 2 m and 10 m depth strata using SCUBA at Butterfly Bay, Karitāne, southern New Zealand (Figure 2.1) from June 2016 to November 2017 (12 times). Entire thalli of articulated coralline algae or sections of crustose coralline algae (~ 3 cm diameter per section) were used to measure nutrient and pigment concentration of coralline algae. The number of coralline algae samples were collected depending on divers and turbidity condition due to the difficulty of removing coralline algae from substrate. However, the minimal number of samples for each collection at each depth were greater than 10 individuals of Arthrocardia sp. or section of coralline algae. The coralline algae samples collected at each stratum were kept in separate zip-lock plastic bags and immediately transported to the Portobello Marine Laboratory (PML) at University of Otago in an insulated cool box containing seawater from the sampling site. The specimens were held for less than three days at a 12: 12 light: dark photoperiod in two fibreglass tanks (70 cm x 60 cm x 30 cm) with flow through natural seawater until they were processed. The process of keeping samples in tanks up to less than 3 days prior to analysing was acceptable because nitrate and phosphate concentration was proven to be stable over short periods in seawater from harbor (Hepburn, 2003). These tanks were shaded by mesh to ensure that light conditions were similar to in situ irradiance at the two depth strata. All visible epiphytes on coralline algae were gently removed using fine forceps and tissues that were cleaned using filtered (Whatman™ GF/C Glass Microfiber Filters, 1.2 µm) seawater.

Entire thalli of articulated coralline algae and section of crustose coralline algae (3 cm diameter per section) from each collection were placed in a nappy liner in a zip-lock plastic bag with silica gel (Farr et al. 2009) and sent to the National Institute of Water and Atmospheric Research, NIWA in Wellington for species identification based on DNA analysis. At NIWA, these samples were ground into a powder and DNA extracted using a Qiagen DNeasy Tissue DNA extraction kits following protocols by Broom et al. (2008). Subsequent PCR reactions were performed using primer combinations psbA-F1 and psbA-F2 (Yoon et al. 2002) and sequences commercially by Macrogen Inc. (Seoul, Korea). DNA
sequences were compared using phylogenetic tree building techniques to a library of coralline algae collected around the New Zealand coast, many of these species undescribed (B A Twist, unpublished data). These DNA sequences were uploaded in GenBank (see detail in Twist, 2019) (Table A 2.1). Based on this phylogenetic tree, the articulated coralline algae belong to the order Corallinales and was Arthrocardia sp. which was found to be the only southern New Zealand distributed species of this genus. Crustose coralline algae taxa were a group of morphologically indistinguishable crustose coralline algae or crustose coralline community from the order Hapalidiales (hereafter, crustose coralline algae). At present, it is extremely challenging to identify morphology of crustose coralline algae to species level due to the convergent growth forms and the lack of available morphological markers to identify them (Richards et al. 2018) (see detail in Twist, 2019). These results of DNA analysis have brought surprisingly not only for phycologist but also for taxonomists by the diversity of species of crustose coralline algae in this area. The diversity of crustose coralline algae is still controversial of many taxonomists worldwide. This uncertainty and inability to identify crustose coralline algae species using other methods (i.e. crustose coralline algae were morphologically identical) than genetic approaches resulted in a comparison between one species of articulated coralline algae – Arthrocardia sp. and a group of indistinguishable crustose coralline algae (that was originally assumed to be one species). This causes issues when extrapolating findings out to make generalized comparisons between articulated and crustose species along with replication at the level of morphology (e.g. replicate articulate and crustose species that can be identified non-lethal manner) is required to make generalization regarding differences in the physiology of articulated and crustose corallines. Regarding the collection of a group of crustose coralline algae at 2 m depth and a group of crustose coralline algae at 10 m depth, the groups were chose, which have the same functional role at each depth.

3.2.2. Soluble tissue nitrate, ammonium, and phosphate

The concentration of soluble tissue nitrate (NO$_3^-$), ammonium (NH$_4^+$), and phosphate (PO$_4^{3-}$) were measured as described by Hurd et al. (1996). Apical Arthrocardia sp. and crustose coralline algae sections (0.5 g ± 0.1 g) were placed into boiling tubes containing 40 ml of ultra-high purity water (18.2 MΩ·cm Milli-Q®) with five replicates. These coralline algae were collected from June 2016 to November 2017 (12 times). The tubes were covered
by aluminium foil and placed into a 50 ml boiling water bath. Subsequently, the tubes were removed from the water bath after 20 minutes of boiling. The water samples were decanted, cooled, filtered (Whatman™ GF/C Glass Microfiber Filters, 1.2 µm) and stored in 35 ml acid-washed polyethylene tubes. The water samples were then refilled with a further 40 ml of ultra-high purity water (18.2 MΩ·cm Milli-Q®) and boiled for another 20 minutes. The third boil was conducted to completely extract nitrate, ammonium, and phosphate in soluble tissue. All of the extracts were frozen for later analysis by a QuickChem® 8500 Series 2 Automated Ion Analyser (Lachat Instruments, a Hach Company Brand, USA) and the coralline algae specimens were bottled dry, weighted (wet weight) and frozen.

3.2.3. C:N ratio, % nitrogen, % carbon

Arthrocardia sp. and sections of crustose coralline algae with three replicates were kept in 1M HCl solution for ~ 2 hours to remove all inorganic tissue. These coralline algae were collected from September 2016 to November 2017 (10 times). These coralline algae were then washed with ultra-high purity water (18.2 MΩ·cm Milli-Q®) before being dried at 60 °C for 48 hours in an oven to reach a constant weight. The samples were then ground to a fine powder in a mortar and pestle and stored in 1.7 ml micro-centrifuge tubes. Tissue organic carbon and nitrogen were determined by isotope ratio mass spectrometer CF-IRMS at Iso-trace Research – Department of Chemistry – University of Otago. The amount of carbon or nitrogen for analysis must be at least 2 micromoles. Samples were weighted out and put into tin capsules, then encapsulated and sample weights were recorded. Results were written to computer file by the instrument control software after finishing analysis processes of samples.

3.2.4. Photosynthetic pigment concentration

Pigment concentration was measured following the method described by Pritchard et al. (2013) with slight modification. Coralline algae (0.03 ± 0.01 g) with five replicates for each species/group were cleaned with filtered seawater (Whatman™ GF/C Glass Microfiber Filters, 1.2 µm) and ground using a tissue grinder under dim light in 1.5 ml of ice-cold 0.1 M phosphate buffer (pH 6.8). These coralline algae were collected from June 2016 to November 2017 (12 times). In order to totally decant the pellets in the tissue grinder, 0.2 ml of ice-cold 0.1 M phosphate buffer was added after pouring these mixes in 1.7 ml micro-
centrifuge tubes. Water soluble phycobiliproteins were extracted after 24 hours in the dark at 4°C. The tubes were then centrifuged at 13000 x g for 20 minutes. The supernatant decanted and absorbance between 400 nm and 800 nm were determined using the scanning spectrophotometer (Jenway 7315, Bibby Scientific, UK) setting a 1 nm scan interval zeroed with 0.1M phosphate buffer. The remaining pellets were re-suspended in 1.5 ml ice-cold 96% ethanol. Chlorophylls were extracted at 4°C for three hours after successive re-centrifugation and measurement of absorbance by a scanning spectrophotometer (Jenway 7315, Bibby Scientific, UK) setting a 1 nm scan interval between 400 nm and 800 nm zeroed with 96% ethanol. The resulting pellets lost colors after this extraction. Phycobiliproteins (R – phycocyanin and R - phycoerythrin) and chlorophylls (chlorophyll a, chlorophyll d, chlorophyll c) concentrations were calculated using formulas and coefficients of Sampath-Wiley and Neefus (2007) and Ritchie (2008), respectively.

3.2.5. Data analyses

Data were tested for normality and equal variances using Shapiro-Wilk and Levene’s test, respectively. Data which did not meet these assumptions were Box-Cox family transformed to obtain homogeneity of variance. Data were analyzed with two-way ANOVAs followed by Tukey’s post-hoc test comparing Arthrocardia sp. and crustose coralline algae at the same 2 m depth stratum and months as factors to investigate effects of species/group and season on soluble tissue nitrogen and phosphorus, C:N ratio, nitrogen % content, carbon % content as well as photosynthetic pigment concentration. Meanwhile, depths (2 m vs. 10 m) and months were used as factors to evaluate effects of depth and months on nutrient and pigment concentration of crustose coralline algae.

All statistical analyses were performed using the R Software (Version 1.0.153 - R Development Core Team) and significance was determined at an α of 0.05.
3.3. Results

3.3.1. Soluble tissue nitrate, ammonium, and phosphate of coralline algae

The average intracellular NO$_3^-$ concentration (67.42 ± 8.86 µmol gWW$^{-1}$) in soluble tissue of coralline algae was higher than the average intracellular NH$_4^+$ and PO$_4^{3-}$ concentration (40.74 ± 1.18 µmol gWW$^{-1}$ and 3.87 ± 0.24 µmol gWW$^{-1}$, respectively) (Figure 3.1). There was a significant interaction between effects of species/group and season on NO$_3^-$ (two-way ANOVA, $F_{11,92} = 7.804, P < 0.001$) and PO$_4^{3-}$ concentration ($F_{11,92} = 4.376, P < 0.001$) but not with NH$_4^+$ ($F_{11,92} = 1.590, P > 0.05$). Simple main effect analysis showed that seasonal changes significantly affected soluble tissue ammonium, nitrate and phosphate (two-way ANOVA, all $P<0.05$) of coralline algae. However, the intracellular NO$_3^-$ concentration was more variable in comparison with the intracellular NH$_4^+$ and PO$_4^{3-}$ concentration during the survey period from June 2016 to November 2017 (Figure 3.1). In particular, the average of NO$_3^-$ concentration of Arthrocardia sp. in winter was 62.87 ± 29.94 µmol gWW$^{-1}$ and it was approximately 1.5 higher than that of summer (45.05 ± 11.44 µmol gWW$^{-1}$). The differences between Arthrocardia sp. and crustose coralline algae at the same depth stratum considerably affected soluble tissue nitrate ($F_{1,92} = 9.242, P = 0.003$) and phosphate ($F_{1,92} = 35.16, P < 0.001$), whereas there was no significant difference in the soluble tissue ammonium between these species/group (Table 3.1). The average NO$_3^-$ concentration in Arthrocardia sp., was significantly lower than that of crustose coralline algae with 55.63 ± 11.73 µmol gWW$^{-1}$ and 80.13 ± 18.35 µmol gWW$^{-1}$, respectively. The interaction between season and depth (crustose coralline algae at 2 m vs. 10 m depth) had significant effects on the three nutrients investigated (NH$_4^+$: $F_{11,92} = 2.577, P < 0.05$; NO$_3^-$: $F_{11,92} = 9.796, P < 0.001$; PO$_4^{3-}$: $F_{11,92} = 4.455, P < 0.001$). In relation to simple main effect analysis, the concentration of soluble tissues in crustose coralline algae at both 2 m and 10 m depth seasonally varied (NH$_4^+$: $F_{11,92} = 2.213, P < 0.05$; NO$_3^-$: $F_{11,92} = 6.730, P < 0.001$; PO$_4^{3-}$: $F_{11,92} = 3.667, P < 0.001$). Meanwhile, significant effects of depth on soluble tissue concentration was only detected in NH$_4^+$ concentration ($F_{1,92} = 5.317, P < 0.05$). Average NH$_4^+$ content of crustose coralline algae at 10 m depth (37.60 ± 2.67 µmol gWW$^{-1}$) was significantly lower than that of 2 m depth (42.65 ± 1.91 µmol gWW$^{-1}$).
Figure 3.1. Seasonal change in soluble tissue ammonium, nitrate and phosphate concentration in *Arthrocardia* sp. (a), crustose coralline algae Hapalidiales 2 m depth (b) and Hapalidiales 10 m depth (c). Points are means ± SE (n=5).
Table 3.1. Two-way ANOVA testing for the differences in the means across both species/group and months and depth and months for soluble tissue ammonium, soluble tissue nitrate, soluble tissue phosphate of coralline algae.

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3.3.2. Atomic C:N ratio, percent nitrogen and carbon of coralline algae

Interaction between species/group and season (months) had a statistically significant effect on atomic C:N ratio (two-way ANOVA, \(F_{9,40} = 3.781, P < 0.05\)), % nitrogen (N) content (two-way ANOVA, \(F_{9,40} = 6.149, P < 0.001\)) and % carbon (C) content (two-way ANOVA, \(F_{9,40} = 5.912, P < 0.001\)) of coralline algae (Table 3.2, Figure 3.2). In addition, statistically significant differences in C:N ratio (two-way ANOVA, \(F_{1,40} = 55.36, P < 0.001\)), total tissue nitrogen (two-way ANOVA, \(F_{1,40} = 400.4, P < 0.001\)) and carbon (two-way ANOVA, \(F_{1,40} = 469.1, P < 0.001\)) were detected between *Arthrocardia* sp. and crustose coralline algae at 2 m depth (Table 3.2). In particular, the average C:N ratio of *Arthrocardia* sp. was 10.14 ± 0.57 and it was significantly lower than that of crustose coralline algae (14.79 ± 1.48) (Figure 3.2 a). In contrast, percent N (4.25 ± 0.31 %) and C (35.57 ± 1.62 %) of *Arthrocardia* sp. were significantly higher those of crustose coralline algae at 2 m depth (N: 1.97 ± 0.31 % and C: 21 ± 1.42 %) (Figure 3.2 b and c). The ratio C:N, % tissue C and N varied significantly over the sampling period (all \(P< 0.001\)).

Significant interactions between effects of season and depth on atomic C:N ratio (two-way ANOVA, \(F_{9,40} = 2.705, P < 0.05\)), percent N of crustose were detected (two-way ANOVA, \(F_{9,40} = 4.195, P < 0.001\)). However, there was no interaction between depth and season influencing on percent C of crustose coralline algae \(F_{9,40} = 1.599, P >0.05\) (Table 3.2). Simple main effect showed that depth had significant effects on total tissue carbon \(F_{1,40} = 8.003, P < 0.05\) but not on the ratio C:N and percent N (Table 3.2). Percent carbon content of crustose coralline algae at 2 m (21 ± 1.42 %) was significantly higher than those at 10 m (18.46 ± 1.02 %). Although the atomic C:N ratio, total tissue nitrogen and carbon of crustose coralline algae at both 2 m and 10 m depths varied seasonally (C:N ratio: \(F_{9,40} = 6.096, P< 0.001\); % N: \(F_{9,40} = 13.16, P< 0.001\); % C: \(F_{9,40} = 5.942, P<0.05\)), there was no clear seasonal pattern in these variables.
Figure 3.2. Seasonal change in the C:N ratio (a), nitrogen % (b) and carbon % (c) of *Arthrocardia* sp., crustose coralline algae Hapalidiales at 2 m depth and 10 m depth. Points are mean ± SE (n=3). The %C and %N are expressed as a percentage of dry weight and C:N ratio determined on an atomic weight.
Table 3.2. Two-way ANOVA testing for the differences in the means across both species/group and time (months) and depth and time (months) for C:N ratio, nitrogen % and carbon % of coralline algae.

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3.3.3. Phycobiliprotein concentration of coralline algae

There was a significant interaction between species/group and season for both phycoerythrin and phycocyanin concentration of coralline algae at 2 m depth (Two-way ANOVA, F_{11,92} = 7.139, F_{11,92} = 5.166, both P < 0.001). Similarly, there was a significant interaction between depth (2 vs. 10 m depth) and season on concentration of phycobiliproteins of crustose coralline algae (phycoerythrin: F_{11,92} = 3.041, P < 0.05; phycocyanin: F_{11,92} = 2.564, P < 0.05). Differences in species/group between *Arthrocardia* sp. and crustose coralline algae at 2 m depth showed significant effects on phycoerythrin (F_{1,92} = 469.1, P < 0.001) and phycocyanin contents (F_{1,92} = 520.7, P < 0.001). Phycoerythrin concentration of *Arthrocardia* sp. (0.003± 0.0002 mg. gWW⁻¹) was significantly higher than that of crustose coralline algae at 2 m depth (0.001± 0.0001 mg. gWW⁻¹) (Figure 3.3). The same pattern was observed in phycocyanin concentration between these species. *Arthrocardia* sp. had significantly higher concentration of phycocyanin (0.0008 ± 0.00006 mg. gWW⁻¹) in comparison to crustose coralline algae, at 2 m depth (0.0002 ± 0.00002 mg. gWW⁻¹) (Figure 3.4). Concentration of phycobiliproteins of crustose coralline algae at 2 m and 10 m depths were not significantly affected by depth, whereas they varied seasonally (phycoerythrin: F_{11,92} = 4.437, P < 0.001; phycocyanin: F_{11,92} = 6.504, P < 0.001). The pigment concentration of all species showed similar seasonal trends with an increase during winter – early spring months (July and September) and decrease during summer – early autumn months (December and March) (Figure 3.3 and Figure 3.4).
Figure 3.3. Seasonal change in phycoerythrin concentration (mg. gWW⁻¹) of coralline algae. Means ± SE (n=5).
Figure 3.4. Seasonal change in phycocyanin concentration (mg. gWW\(^{-1}\)) of coralline algae. Means ± SE (n=5).
Table 3.3 Two-way ANOVA testing for the differences in the means across both species/group and time (months) and depth and time (months) for phycobiliproteins of coralline algae.

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3.3.4. Chlorophylls concentrations of coralline algae

Simple main effect analysis showed that the differences in species/group of coralline algae at 2 m depth had significant effects on the concentration of chlorophyll $a$ and $c$ ($F_{1,92} = 265.5$, $F_{1,92} = 16.04$, respectively both $P < 0.001$) but not with chlorophyll $d$ (Table 3.4). Chlorophyll $a$ concentration (0.252 ± 0.018 mg. gWW$^{-1}$) and chlorophyll $c$ concentration (0.023 ± 0.002 mg. gWW$^{-1}$) of *Arthrocardia* sp. were significantly higher than that of crustose coralline algae (0.099 ± 0.022 mg. gWW$^{-1}$ and 0.016 ± 0.003 mg. gWW$^{-1}$, respectively) at the same stratum (Figure 3.5 and Figure 3.7). Chlorophyll $a$, $d$ and $c$ concentrations of crustose coralline algae at 2 m depth varied seasonally over the sampling period ($F_{11,92} = 17.73$, $F_{11,92} = 3.352$, $F_{11,92} = 9.657$, all $P < 0.001$). The interaction between species/group and season significantly affected chlorophyll $a$ concentration (Two-way ANOVA, $F_{11,92} = 1.898$, $P = 0.049$) (Table 3.4). A clear seasonal pattern was only observed in chlorophyll $a$ concentration with an increase during winter and early spring months (June, July and September) and a decrease during summer and early autumn months (December and March) (Figure 3.5).

There was a significant interaction affect between depth and season for all chlorophyll concentrations of crustose coralline algae at 2 m and 10 m depths (Table 3.4). Chlorophyll $a$ concentration of these coralline algae was not significantly affected by depth. In contrast, the chlorophyll $d$ concentration of crustose coralline algae at 10 m depth (0.011 ± 0.002 mg. gWW$^{-1}$) was significantly higher than that of crustose coralline algae at 2 m depth (0.008 ± 0.001 mg. gWW$^{-1}$) (Figure 3.6, $F_{1,92} = 11.84$, $P < 0.001$). The chlorophyll $c$ concentration of crustose coralline algae at 10 m depth was 0.023 ± 0.004 mg. gWW$^{-1}$ and they were also significantly higher than that of crustose coralline algae at 2 m depth (0.016 ± 0.003 mg. gWW$^{-1}$) ($F_{1,92} = 20.62$, $P < 0.001$). All chlorophyll concentrations of crustose coralline algae at different strata were affected by season (Table 3.4, all $P < 0.001$).
Figure 3.5. Seasonal change in chlorophyll $a$ concentration (mg. gWW$^{-1}$) of coralline algae. Means ± SE (n=5).
Figure 3.6. Seasonal change in chlorophyll d concentration (mg. gWW\(^{-1}\)) of coralline algae. Means ± SE (n=5).
Figure 3.7. Seasonal change in chlorophyll c concentration (mg. gWW$^{-1}$) of coralline algae. Means ± SE (n=5).
Table 3.4. Two-way ANOVA testing for the differences in the means across both species/group and time (months) and depth and time (months) for chlorophylls of coralline algae.

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3.3.5. Ratios of accessory pigments to chlorophyll a of coralline algae

Simple main effect analysis showed that the differences in species/group of coralline algae at 2 m depth had significant effects on the ratio of chlorophyll d or chlorophyll c to chlorophyll a (F_{1,92} = 59.09, F_{1,92} = 43.60, respectively, both P < 0.001) but not with the ratio of phycobiliproteins to chlorophyll a (Figure 3.8, Table 3.5, P > 0.05). The ratios of chlorophyll d to chlorophyll a (0.10 ± 0.01) and chlorophyll c to chlorophyll a (0.20 ± 0.04) of crustose coralline algae were significantly higher than those of Arthrocardia sp. (0.03 ± 0.004 and 0.10 ± 0.01, respectively) (Figure 3.8). Similarly, there was a significant effect between depth (2 m vs. 10 m depth) on the ratio chlorophyll d or chlorophyll c to chlorophyll a (F_{1,92} = 5.504, P < 0.05; F_{1,92} = 14.23, P < 0.001, respectively) but not with the ratio phycobiliproteins to chlorophyll a (P > 0.05). The interaction between species/group and season significantly affected the ratio phycobiliproteins to chlorophyll a (Two-way ANOVA, F_{11,92} = 5.757, P < 0.001) and the ratio chlorophyll c to chlorophyll a (Two-way ANOVA, F_{11,92} = 3.653, P < 0.001). There was also a significant interaction effect between depth and season for the ratio phycobiliproteins to chlorophyll a of crustose coralline algae at 2 m and 10 m depth (Table 3.5, Two-way ANOVA, F_{11,92} = 5.350, P < 0.001). Meanwhile, the interaction effects between depth and season to the ratio chlorophyll d or chlorophyll c to chlorophyll a of crustose coralline algae at 2 m and 10 m depths was not significant (Two-way ANOVA, both P > 0.05).
Figure 3.8. The ratio of phycobiliproteins: chlorophyll $a$, chlorophyll $d$: chlorophyll $a$ and chlorophyll $c$: chlorophyll $a$ in coralline algae (mean ± SE, n=5).
Table 3.5. Two-way ANOVA statistics testing for the differences in the means across both species/group and time (months) and depth and time (months) for the ratio phycobiliproteins: chlorophyll \( a \), chlorophyll \( d \): chlorophyll \( a \), chlorophyll \( c \): chlorophyll \( a \).

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</tr>
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<td></td>
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</tr>
<tr>
<td>Species/group</td>
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<td>Chlorophyll ( a )</td>
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<td></td>
</tr>
<tr>
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</tr>
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<td>14.95</td>
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3.3.6. Correlations between environmental and physiological parameters of coralline algae

The statistical analysis of the correlation between environmental parameters (Chapter 2) and the physiological parameters of *Arthrocardia* sp. and crustose coralline algae at 2 m depth and crustose coralline algae at 10 m depth (Chapter 3) is shown in table 3.6, 3.7 and 3.8, respectively. At 2 m depth, the correlation between environmental and physiological parameters of coralline algae is different depending on species. For the *Arthrocardia* sp., there was a significant negative correlation between temperature and chlorophyll *a* of *Arthrocardia* sp. \((r = -0.605, P = 0.037)\), while the ratio phycobiliprotein:chlorophyll *a* was positively correlated with daily quantum dose and temperature \((r = 0.654, P = 0.021\) and \(0.774, P = 0.003\), respectively). Ammonium concentration in seawater indicated a positive correlation with the C:N ratio of *Arthrocardia* sp. \((r = 0.635, P = 0.049)\). For crustose coralline algae, there was no significant correlations between the daily quantum dose or temperature and the physiological parameters (Table 3.7). There was a negative correlation between ammonium concentration in seawater and soluble tissue nitrate \((r = -0.607, P = 0.036)\). Meanwhile, a positive correlation between nitrate concentration in seawater and the C: N ratio \((r = 0.641, P = 0.046)\) was detected in this species. At 10 m depth, the correlation between daily quantum dose and phycoerythrin of crustose coralline algae was negative \((r = -0.646, P = 0.023)\). Ammonium and phosphate concentration in seawater showed positive correlations with soluble tissue nitrate and phycoerythrin concentration of crustose coralline algae, respectively \((r = 0.683, P = 0.014\) and \(r = 0.582, P = 0.047\) (Table 3.8).
Table 3.6. Pearson’s correlation coefficients (top) and p-values for the correlations (below) based on mean values for tissue of *Arthrocardia* sp. or water samples collected from 2 m depth (n=8-10, depending on paired data). Significant correlations are in bold. Dash indicates an unjustified/meaningless comparison.

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<tr>
<th>Daily quantum dose</th>
<th>Temperature</th>
<th>Seawater ammonium</th>
<th>Seawater nitrate</th>
<th>Seawater phosphate</th>
<th>Soluble tissue ammonium</th>
<th>Soluble tissue nitrate</th>
<th>Soluble tissue phosphate</th>
<th>Phycoerythrin</th>
<th>Phycocyanin</th>
<th>Chlorophyll a</th>
<th>Phycobiliproteins: Chlorophyll a</th>
<th>Chlorophyll d: Chlorophyll a</th>
<th>Chlorophyll c: Chlorophyll a</th>
<th>% Nitrogen</th>
<th>Carbon</th>
</tr>
</thead>
<tbody>
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<td>-0.069</td>
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### Table 3.7. Pearson’s correlation coefficients (top) and p-values for the correlations (below) based on mean values for tissue of crustose coralline algae or water samples collected from 2 m depth (n=8-10, depending on paired data). Significant correlations are in bold. Dash indicates an unjustified/meaningless comparison.

<table>
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<th>Daily quantum dose</th>
<th>Temperature</th>
<th>Seawater ammonium</th>
<th>Seawater nitrate</th>
<th>Seawater phosphate</th>
<th>Soluble tissue ammonium</th>
<th>Soluble tissue nitrate</th>
<th>Soluble tissue phosphate</th>
<th>Phycoerythrin</th>
<th>Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>Chlorophyll c</th>
<th>Phycochlorophylls: Chlorophyll a</th>
<th>Chlorophyll d: Chlorophyll a</th>
<th>Chlorophyll d: Chlorophyll b</th>
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**Note:** CN ratio is calculated as the ratio of carbon to nitrogen, typically using a value of 6.67 for the molar ratio of carbon to nitrogen in biological systems.
Table 3.8. Pearson’s correlation coefficients (top) and p-values for the correlations (below) based on mean values for tissue of crustose coralline algae or water samples collected from 10 m depth (n=8-10, depending on paired data). Significant correlations are in bold. Dash indicates an unjustified/meaningless comparison.

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<th>Daily quantum dose</th>
<th>Temperate Seawater</th>
<th>Temperate Seawater</th>
<th>Soluble tissue ammonium</th>
<th>Soluble tissue nitrate</th>
<th>Soluble tissue phosphate</th>
<th>Phycoerythrin</th>
<th>Phycoerythrin</th>
<th>Phycobiliproteins: Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>Chlorophyll c</th>
<th>Chlorophyll d</th>
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<th>% Carbon</th>
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3.4. Discussion


The difficulty in identifying exactly crustose coralline algae species and ensuring the consistence of crustose coralline algae species collected for each sampling trip is one of the challenges of this study. To overcome this challenges, crustose coralline algae samples were pooled from mixture species and the sampling process was well replicated (12 times) in order to ensure objectivity in comparing nutrient and pigment concentration between a group of crustose coralline algae species at 2 m depth and a group at 10 m. Therefore, if there are differences in the community composition it is not relevant, the question is does the physiology of crustose coralline algae’s differ between depths a comparison of replicate samples was made. Therefore, the pooling of various species of crustose coralline algae is a valid approach. The result of this study indicated that different species/group coralline algae, depth distribution, season and the interaction between these factors significantly affect nutrient and pigments concentrations of coralline algae. These results are consistent with findings from previous studies for non-calcareous macroalgae and coralline algae as well. For example, chlorophyll $a$ concentration in Arthrocardia sp. and crustose coralline algae at 2 m depth increased in the winter and decreased in the summer. A similar pattern was observed for kelp (Nereocystis luetkeana) with high levels of chlorophyll $a$ in the winter and low levels in the summer (Wheeler et al. 1984). Chlorophyll $a$, carotenoids and phycobiliproteins levels of three coralline algae species including Corallina elongata,
*Corallina officinalis*, *Jania rubens* showed seasonal fluctuation with high concentration during winter and decreased in summer (Ismail and Osman 2016). Data from previous chapter (chapter 2) indicated that nitrogen concentration in the winter was significantly higher than that of summer. The availability of nitrogen can alter macroalgal pigment concentration (Shivji 1985, Mcglathery 1992, Mcglathery and Pedersen 1999, Gordillo et al. 2006). Higher chlorophyll *a* in winter is likely a result of lower light (shade acclimation) and higher seawater nitrogen which is required for synthesis of chlorophyll *a* (it requires lots on nitrogen). In addition, this study showed that pigment concentrations of crustose coralline algae including chlorophyll *d* and chlorophyll *c* increased with depth. According to Ramus et al. (1976), greater pigment concentrations with depth was observed when these authors investigated the relationship between changes in photosynthetic pigment concentration and water depth on green algae (*Ulva lactuca* and *Codium fragile*) and red algae (*Porphyra umbilicalis* and *Chondrus crispus*). The change pigment ratio, or simply increase the total amount of pigment, or both are strategies of macroalgae to adapt intensively and/or chromatically to limiting light conditions (Ramus et al. 1976). Hence, the increase in chlorophyll *d* and chlorophyll *c* of crustose coralline algae with depth detected in the present study are reasonable.

Intracellular NO$_3^-$ concentration was generally higher than the intracellular NH$_4^+$ concentration, while the intracellular PO$_4^{3-}$ concentration was the lowest in soluble tissues compared to NO$_3^-$ and NH$_4^+$ concentration of all coralline algae investigated. Ambient nutrient concentrations (NO$_3^-$, NH$_4^+$ and PO$_4^{3-}$) in seawater at the sampling site showed a similar pattern to intracellular nutrients (see more detail in Chapter 2). Average internal nitrate storage of these coralline algae in winter was significantly higher than in summer. This suggests storage of nitrate internally by coralline algae may positively correlate with ambient nutrient concentration in seawater. Storage of NO$_3^-$ is important during high nitrate periods for future growth of giant kelp *Macrocystis pyrifera* (Wheeler and Srivastava 1984, Brown et al. 1997, Hepburn et al. 2007, Fram et al. 2008, Stephens and Hepburn 2014), *Saccharina longicuris* (Gagné et al. 1982), and red macroalgae *Adamsiella chauvinii* (Kregting et al. 2008) and *Anotrichium crinitum* (Pritchard et al. 2015). The timing of decline in internal nitrogen concentration of these macroalgae in summer coincided with the decrease in nitrate concentration in seawater (e.g. Wheeler and Srivastava 1984, Brown et al. 1997, Kregting et al. 2008).
Nitrate and ammonium are important nitrogen sources for macroalgae (Hurd et al. 2014). However, nitrate was considered to be a main nitrogen storage pool in *Chaetomorpha linum* (Chlorophyta). During the nutrient enrichment period, intracellular concentrations of NO$_3^-$ of *C. linum* increased 7-15% of the total nitrogen tissue compared to ammonium content increase (less than 2% of total tissue nitrogen content) (McGlathery et al. 1996). In this study, the average of intracellular NH$_4^+$ pools of coralline algae were relatively stable around 40 µmol. gWW$^{-1}$, whereas intracellular NO$_3^-$ pools were on average 67.42 ± 8.86 µmol. gWW$^{-1}$ with frequent variation during sampling period. This suggests that coralline algae accumulate greater internal nitrate pools in tissue than ammonium. Some macroalgae have different strategies to store nitrogen. For example, four intertidal seaweeds (*Stictosiphonia arbuscula, Apophlaea lyallii, Scytothamnus australis* and *Xiphophora gladiata*) and a light-limited red alga *Anotrichium crinitum* distributed at 10 m depth showed larger internal ammonium pools than nitrate pool (Phillips and Hurd 2003, Pritchard et al. 2015). Notably, the average NO$_3^-$ and PO$_4^{3-}$ concentration capacity in different species/group at the same 2 m depth strata was significantly different. It is assumed that the erect branches and three - dimension form of *Arthrocardia* sp. enhanced absorption of dissolved nutrients from seawater. The different morphologies between two species *Ulva intestinalis* and *Ulva expansa* (Chlorophyta) also showed considerable differences in their NO$_3^-$ pools. In particular, NO$_3^-$ concentration in soluble tissue of *U. intestinalis* having a tube shape was five times lower than that of *U. expansa* having a broader thallus (Kennison et al. 2011). The phosphate soluble tissues of coralline algae had a low concentration in comparison to other nutrients. The PO$_4^{3-}$ soluble tissue in coralline algae is perhaps related to the low PO$_4^{3-}$ concentration in seawater (see detail in Chapter 2).

It is noticed that tissue nutrient concentrations and nutrient ratio are required to determine the severity of nutrient limitation of macroalgae and these parameters varied depending on species (Harrison and Hurd 2001). C:N ratio can be used as one of the indicators showing the nitrogen status of macroalgae in which a higher CN: ratio implies greater nitrogen limitation (Thomas and Harrison 1987). In red algae, the ratio higher than 10, generally suggests that nitrogen starved macroalgae (D’Elia and DeBoer 1978). In kelp, the values of the C: N ratio ranged from 10 to 15, which indicates storage of nitrogen, and higher values indicate nitrogen limited growth (see detail in Sjøtun et al. 1996). Peckol and Ramus (1988) also indicated that low tissue N levels and relatively high atomic C:N ratio
suggested more nutrient limitation. The C:N ratios of *Arthrocardia* sp. and crustose coralline algae in the present study were around or higher than 10 in all species/group during the study period. The C:N ratios of crustose coralline algae was significantly higher than that of *Arthrocardia* sp. in this research, but the tissue %N and tissue %C contents of *Arthrocardia* sp. were higher than of crustose coralline algae at 2 m and 10 m depths. Crustose coralline algae at 2 m depth contained lower tissue N (< 2%) and higher atomic C:N ratio (14.79 ± 1.48) in comparison with the *Arthrocardia* sp. (10.14 ± 0.57) at the same depth. A study on deep water macroalgae from Carolina outer continental shelf showed a similar result. In particular, the leathery thallus forms of *Zonaria tournefortii* had lower tissue N content and higher C:N ratios than the finely branched thallus of *Solieria tenera* and the flat species *Dictyopteris hoytii* (Peckol and Ramus 1988). Taken together, it is possible that coralline algae could be nitrogen limited species and the differences in nutrient status of these coralline algae was possibly caused by the difference in morphology between *Arthrocardia* sp. and crustose coralline algae. However, this assumption needs to be clarified in further studies by investigating the growth rate of coralline algae in a range of nitrogen concentrations (e.g Hanisak 1979).

Phycobiliproteins and chlorophylls are light harvesting pigments responsible for light capturing and transferring energy along the photosystem of red algae (Gantt et al. 1986, Gantt 1996). Changes in pigment concentration driven by different light regimes have been observed in many macroalgal groups (Ramus et al. 1976 a, b, López-Figueroa 1992, Flores-Moya et al. 1995, Stengel and Dring 1998, Pritchard et al. 2013). In the present study, photosynthetic pigments were significantly different between coralline algae examined, depth and season during the survey period. This result agrees with previous studies for a variety of macroalga species. For example, discoloration in macroalgae was observed in summer and this was thought to be related to loss of pigments due to environment stress (Ramus et al. 1976, Falkowski and LaRoche 1991, Raven and Geider 2003). The present data showed that phycobiliproteins (phycoerythrin and phycocyanin) and chlorophyll a increased during winter months and decreased during summer months. Again, the differences in ambient conditions, especially light intensity between winter and summer months (chapter 2) could be the main factors driving the increase of phycobiliproteins in the winter when light intensity was reduced. According to Lüning (1990), a greater amount of pigment is required to enhance the chance of photons being absorbed by the antenna
molecules of the photosystems. Therefore, the increase of phycobiliproteins in macroalgae implied more light harvested and improvement of photosynthesis capacities of macroalgae (Ramus et al. 1976). Phycoerythrin and chlorophyll a of Anotrichium crinitum distributed at the same 10 m depth stratum site also showed a clear seasonal pattern of lower concentration during summer months and high concentration during winter months (Pritchard et al. 2013). These results are consistent with findings by Ismail and Osman (2016) investigated seasonal fluctuation of photosynthesis pigments of five Rhodophyta species. However, phycobiliprotein and chlorophyll a concentration of crustose coralline algae were not significant different between depth. A study of Desmond et al. (2019) at the same area with present study found that total pigment concentration and pigment ratios of some brown macroalgae species (Phaeophyceae) at 10 m depth was also not considerably higher than at 2 m depth and low light at 10 m depth was suggested to limit the synthesis of pigments in macroalgae at this depth. Hence, no significant differences in phycobiliprotein and chlorophyll a concentration in crustose coralline algae between 2 and 10 m depth in this study are reasonable.

Phycoerythrin, phycocyanin and chlorophyll a concentration of Arthrocardia sp. were significantly higher than that of crustose coralline algae at the same stratum. Noisette et al. (2013) also showed that articulated coralline alga Corallina elongata had higher chlorophyll a concentration than crustose coralline alga Lithophyllum incrustans and maerl beds Lithothamnion corallioides. Peckol and Ramus (1988) demonstrated that a thinner thallus morphology species had higher pigment concentration and photosynthetic capacity than calcified, coarsely branched or leathery thallus forms species. These authors indicated high surface area: volume (SA: V) ratio makes thin flat thalli species exposing highest photosynthetic performance at all levels of ambient irradiance. In this study, the greater SA: V ratio of Arthrocardia sp. than crustose coralline algae (personal observation) probably led the higher photosynthetic pigment concentration of Arthrocardia sp. compared to crustose coralline algae. Chlorophyll c was detected in pigment extraction of coralline algae during the survey period. Chlorophyll c is traditionally found in diatoms, dinoflagellates, and brown algae (Strain and Manning 1942, Manning and Strain 1943). In brown algae, chlorophyll c is an accessory pigment which aid in the harvesting of light across the PAR spectrum and enable macroalgae to live in variable light environments (Caron et al. 2001). Interesting, a relatively small quantities of chlorophyll c had been reported in red algae (Sorby 1873) and
analyzing the light harvesting complex gene sequence of red algae revealed chlorophyll c in existence (Green and Durnford 1996, Tan et al. 1997, Bachvaroff et al. 2005). Bhattacharya et al (2013) indicated that red algal plastids and red algal nuclear genes are considered as chlorophyll c-containing lineages as seen in diatoms, haptophytes and cryptophytes through analyzing genome of unicellular red alga, *Porphyridium purpureum*. These authors suggested that ancestral red algae lineages acted as mediators of horizontal gene transfer between prokaryotes and photosynthetic eukaryotes. Thus, the finding of chlorophyll c in these coralline algae seems reasonable.

Alteration of the ratio between chlorophyll $a$ and accessory pigments allows a macroalga to use a wider range of light wavelengths (Fairhead and Cheshire, 2004). Quantifying pigment concentrations and the ratio of accessory pigments to chlorophyll $a$ will help to understand the response of algae species to irradiance changes in the subtidal (Fairhead and Cheshire, 2004)). For example, the phycoerythrin: chlorophyll $a$ ratio of red alga *Gracilaria folifera* was higher under low light intensity in comparison with high light (Lapointe 1981). In the present study, the ratio of phycobiliproteins to chlorophyll $a$ was similar between *Arthrocardia* sp. and crustose coralline algae at the same depth, and no significant differences was found in this ratio between crustose coralline algae at different depths. Meanwhile, the ratio chlorophyll $d$ and chlorophyll c to chlorophyll $a$ of crustose coralline algae was significantly higher than those of *Arthrocardia* sp. and these ratios increased with depth. An increase in the concentration of chlorophylls is a common acclimation response of macroalgae to lowering irradiance (Ramus et al. 1976, Wheeler et al. 1984, Henley and Ramus 1989, Iglesias Prieto and Trench 1994, Gomez et al. 1997, Stengel and Dring 1998). This response either increases the effective absorption cross-section of PSII and/or increases the number of photosynthetic units (PSUs), thus improving the capacity for capture of energy available for photochemistry. Wheeler (1980) and Falkowski and LaRoche (1991) suggested that an alteration in the ratio chlorophyll c to chlorophyll $a$ indicates change in photosystem II. Based on the increase of chlorophyll d and chlorophyll c concentration and the ratio chlorophyll d to chlorophyll $a$ and chlorophyll c to chlorophyll $a$ with depth stratum in crustose coralline algae (section 3.3.4 and section 3.3.5), I assumed that chlorophyll d and/or chlorophyll c may allow these algae to harvest more light for their photosynthesis to adapt low light condition in deeper water column or these chlorophyll confer other unknown physiological advantages. This is rising a question for
future studies. In fact, little is known about the light-harvesting complex of coralline algae and they can be found at depths exceeding 200 m on the earth (Steneck 1986, Littler et al. 1991). Therefore, the future research on pigment concentration and light harvesting complex of coralline algae is required.

Species/group, depth distribution (2 m vs. 10 m), season and the interaction between these factors significantly affected nutrient and pigment concentration of coralline algae. This finding is well supported by results from correlation analysis between these factors presented above. The nutrient pools of coralline algae were more closely correlated with changes in nutrient concentration of seawater, whereas the photosynthetic pigments were more correlated with changes in the daily quantum dose and temperature. A similar correlation between these factors was found by Pritchard (2011) who analyzed correlations between environmental parameters at 10 m depth at Karitāne and physiological parameters of Anotrichium crinitum. This author found that there was a strong negative correlation between daily quantum dose and phycoerythrin of A. crinitum ($r = -0.824, P = 0.012$). Ramus et al. (1976) indicated that high irradiance results in reduced pigment concentration and increase of pigment accumulation in tissue of macroalgae was observed at low irradiance. A positive correlation between ambient nitrogen and phosphorus concentration, and photosynthetic pigment contents of red algae was detected (Ismail and Osman, 2016). Seawater phosphate concentration was significantly positively correlated with phycoerythrin concentration of crustose coralline algae at 10 m depth in this study. The photosynthetic pigment concentrations of Hypnea musciformis (phycocyanin - PC and allophycocyanin – APC) are also positively correlated with the increase of phosphate in the medium (Martins et al. 2011). According to Ismail and Osman (2016), the increase in all photosynthetic pigment may be correlated with nitrogen and phosphorus of seawater due to the vital role of these nutrients in algal metabolic process. In particular, nitrate and phosphate are two of the most important elements required for algal growth and metabolism (DeBoer 1981, Lapointe 1987). The relationship between ambient nitrogen and phycoerythrin in macroalgae has been discovered in many previous studies (Lapointe 1981, Bird et al. 1982, Lapointe 1985). Lapointe (1981, 1985) revealed that a high nitrogen environment results in an increase of phycoerythrin and chlorophyll $a$ in Gracilaria tikvahiae. Similarly, phycoerythrin content of Pyropia yezoensis was increased five times by nitrogen enrichment (Amano and Noda 1988). In contrast, the interaction between ambient phosphate and photosynthetic pigment,
especially phycoerythrin content still unclear. Phosphorus involves in energy transfer mediated by ATP and other high energy compounds in photosynthesis and respiration (Lobban and Harrison 1997). Lapointe (1986) detected that phosphorus enrichment can stimulate the growth and the photosynthetic rates of some algae. Taken together, the significant positive correlation between phycoerythrin concentration of crustose coralline algae (10 m depth) and seawater phosphate concentration found in the present study may relate to photosynthetic response of this species to low light condition. However, this assumption needs to be clarified in future.

In conclusion, there were considerable differences in nutrient and photosynthetic pigment between Arthrocardia sp. and crustose coralline algae. The differences might be due to difference in growth form between these species/group coralline algae. This suggest that a different life strategy between these coralline algae indicate the difference in nutrient and pigment concentration. Arthrocardia sp. with upright branch thallus had higher accessory pigment concentration including phycobiliproteins, chlorophyll a than flattened thallus, crustose coralline algae, that probably forecast a higher potential photosynthetic capacity of Arthrocardia sp. However, more data is needed to investigate this suggestion because only one species of articulated coralline algae was compared to a group of crustose coralline algae in this study. The concentrations of nutrient and photosynthetic pigment of crustose coralline algae at 2 m and 10 m depth except for soluble tissue ammonium, C%, chlorophyll d and chlorophyll c concentration were not significant different. There was an interaction between species/group and season, between depth and season on nutrient and photosynthetic physiology of coralline algae. It is noteworthy that this is the first time, chlorophyll c has been quantified in a coralline algal. Therefore, further investigation into chlorophyll c in a range of coralline algae species is suggested.
Chapter 4

Nitrogen and phosphorus uptake in articulated and crustose coralline algae in winter and summer
4.1. Introduction

Macrolegae must take up and assimilate nitrogen and phosphorus, limiting nutrients for macroagal growth, from seawater to support their primary productivity (Harrison and Hurd 2001). In seawater, nitrate (NO$_3^-$) and ammonium (NH$_4^+$) are the primary forms of nitrogen (Dugdale and Goering 1967, Jackson and Williams 1985, Phillips and Hurd 2004, Hurd et al. 2014) available for uptake, while phosphorus is found as phosphate (PO$_4^{3-}$) (Jackson and Williams 1985, Iheagwara et al. 2013, White and Dyhrman 2013). These nutrients are introduced into the marine environment by several processes. Nitrogen comes into the euphotic zone by vertical mixing and upwelling in the ocean, atmospheric and anthropogenic inputs, nitrogen fixation by bacteria or cyanobacteria (Dugdale and Goering 1967, Banse 1974, Jackson and Williams 1985, Herbert 1999, Boyd and Hurd 2009, Hurd et al. 2014). In particular, an important source of nitrate is nitrification in nitrogen cycle or submarine discharge of ground water in coastal and terrestrial runoff (Herbert 1999, Boyd and Hurd 2009, Hurd et al. 2014, Den Haan et al. 2016), whereas ammonium originates from excretion of both sessile and mobile animals or is produced by bacterial decomposition of organic matter in sediments (Banse 1974, Herbert 1999, Hepburn and Hurd 2005, Glibert et al. 2005, Seitzinger et al. 2005, Boyd and Hurd 2009). Phosphate exists in the marine water column due to biological activity and coastal upwelling and probably from anthropogenic inputs into coastal waters such as fertilizers and the weathering of P rich sediments (Cembella et al. 1984, Rees 2003, White and Dyhrman 2013, Valiela 2015).

Macroleagal nutrient uptake rates are influenced by physical, chemical and biological processes (Fujita and Goldman 1985, Larned and Atkinson 1997, Hurd 2000, Hurd et al. 2014). Physical factors (irradiance, temperature and water velocity) have impacts on nutrient uptake rate of macroalgae (Haines and Wheeler 1978, Hurd 2000, Hurd et al. 2014). For instance, nutrient uptake rates of bladed macroalgae have been found to increase with increasing water velocity (Wheeler 1980, Gerard 1982, Hurd et al. 1996) due to the decrease of the thickness of diffusion boundary layers and increase rates of nutrient delivery to the cell (Hurd and Pilditch 2011). Chemical factors (molecular form of an ion and/or ion concentration, ambient nutrient concentration) affect nutrient uptake of macroalgae (Hurd et al. 2014). Tyler et al. (2005) suggested that ammonium was considered to be more desirable nitrogen source for Ulva lactuca to uptake in comparison with urea because the affinity of
*U. lactuca* fore urea was lower than that for ammonium at low concentration ($V_{\text{max}}/K_m = 4.5$ and $\sim 12$, respectively). Biological factors such as type and age of tissue, nutritional history, genetic factors and growth rate strategy may influence the nutrient demand thus influencing uptake rates (Wallentinus 1984, Fujita 1985, Druehl et al. 1989). For example, ecotypic and ontogenetic differentiation and physiological plasticity were the major reasons causing a lower uptake rate observed in the kelp *Saccharina groenlandica* exposed to relatively high ambient nitrogen conditions in comparison with those maintained in low nitrogen levels (Druehl et al. 1989). Transient uptake of $\text{NH}_4^+$ by all algae *Enteromorpha* spp., *Ulva lactuca* and *Gracilaria tikvahiae* growing at high nitrogen flux was slower in comparison with algae growing at low nitrogen flux or starved of nitrogen (Fujita 1985).

Strategies of nutrient uptake differ among algal species and even within functional groups and species (Arrigo 2005, Den Haan et al. 2016). The competitive ability of macroalgae to acquire nutrients at ecologically-realistic concentrations can be examined by the kinetic constants of the uptake system (Philips and Hurd 2004, Rees et al. 2007, Hurd et al. 2014) (Figure 4.1). The two kinetic parameters ($V_{\text{max}}$ and $K_s$) derived from hyperbolic plots of uptake rate at a range of concentrations have been used as indicators of an alga’s ability to take up nutrients at high and low concentrations (Harrison and Druehl 1982, Rees 2003, Hurd et al. 2014). $V_{\text{max}}$ is the maximum uptake rate achieved at saturating concentrations of a nutrient and $K_s$, the half saturation constant, is nutrient concentration at which uptake is 50% of $V_{\text{max}}$ (Rees et al. 2007, Hurd et al. 2014). A species with a high $V_{\text{max}}$ is considered to have a competitive advantage when a particular nutrient is available at high concentrations. In addition, nutrient status of a macroalgal species can be predicted based on $V_{\text{max}}$ in which nutrient-depleted cells generally show a higher $V_{\text{max}}$ than nutrient-replete cells (Boyd and Hurd 2009). $K_s$ is a useful indicator of the affinity for nutrients at low concentrations. A lower $K_s$ value indicates efficient nutrient uptake of species at low external concentrations (D'Elia and DeBoer 1978, Boyd and Hurd 2009).

Saturation of nutrient uptake is thought to be an energy-related process (Phillips and Hurd 2004, Hurd et al. 2014). $\text{NO}_3^-$ uptake typically exhibits saturable kinetics (Philips and Hurd 2004) although several authors report a linear increase in the uptake rate with $\text{NO}_3^-$ concentration (Harrison et al. 1986, Thomas et al. 1987, Lavery and McComb 1991, Pritchard et al. 2015). $\text{NO}_3^-$ is usually actively transported across the membrane against an
electrochemical potential gradient using ATP (Mulholland and Lomas 2008). \( \text{NH}_4^+ \) can be either passively diffused or actively transported depending on alga species; for instance, \( \text{NH}_4^+ \) uptake of \textit{Codium fragile} and \textit{Anotrichium crinitum} were suggested to be actively transported (Hanisak and Harlin 1978, Pritchard et al. 2015), whereas passive diffusion of \( \text{NH}_4^+ \) was found in some other macroalgae such as \textit{Gracilaria pacifica} (Thomas et al. 1987), \textit{Xiphophora chondrophylla} and \textit{Ulva} sp. (Taylor et al. 1998), \textit{Stictosiphonia arbuscular} (Phillips and Hurd 2004). The difference in energy requirements for uptake of nitrate versus ammonium is difficult to determine (Raven et al. 2008) but it is generally considered that nitrate uptake generally requires more energy in comparison to ammonium uptake (Raven 1984, Solomonson and Barber 1990, Falkowski and Raven 2007). Particularly, \( \text{NO}_3^- \) assimilation reduces \( \text{NO}_3^- \) to \( \text{NH}_4^+ \) in a two-step process, whereas ammonium is directly incorporated into amino acids (Hurd et al. 2014). Ammonium is probably therefore energetically favorable to macroalgae, particularly in winter when the decrease of light delivery may impose energy limitation (D'Elia and DeBoer 1978, Phillips and Hurd 2003, Pritchard et al. 2015). \textit{Anotrichium crinitum}, a light-limited red alga with low-energy input via photosynthesis, has a high affinity for \( \text{NH}_4^+ \) to be able to sustain growth under lowlight conditions in the field (Pritchard et al. 2015). Regarding phosphate uptake, some macroalgae show a biphasic kinetics of phosphate uptake with saturable kinetics at low concentrations and a linear uptake response at high external phosphate concentrations (Friedlander and Dawes 1985, Gordillo et al. 2002, Silkin and Chubchikova 2007). For \textit{Palmaria palmata} (Rhodophyta), phosphate uptake kinetics were observed to be biphasic and did not saturate (Martínez and Rico 2004). Meanwhile, five species of fucoid algae including \textit{Pelvetia canaliculata}, \textit{Fucus spiralis}, \textit{Ascophyllum nodosum}, \textit{Fucus vesiculosus}, \textit{Fucus serratus} exhibited saturable kinetics of phosphate in both winter and summer (Hurd and Dring 1990).

A significant literature is available on the kinetic characteristics of nutrient uptake for fleshy macroalgae while very little is known for diverse and ecological important coralline algae. The present study is the first to determine on the nutrient uptake kinetics of two different coralline algae at 2 m depth stratum. It was hypothesized that \textit{Arthrocardia sp.} would have higher nutrient uptake rates than crustose coralline algae based on higher surface area to volume ratio (SA:V) (Rosenberg and Ramus 1984), and tissue nutrient concentrations reported in Chapter 3. In addition, it was predicted that ammonium would be the preferred nitrogen source for uptake of these macroalgae species compared to nitrate, which has been
observed in a light-limited red alga (Pritchard et al. 2015). In order to examine these hypotheses, kinetics of nitrate, ammonium and phosphate uptake of *Arthrocardia* sp. and crustose coralline algae were investigated based on the results of time-course nutrient depletion experiments.
Figure 4.1. Hypothetical plots of nutrient uptake rate (V) and concentrations of the limiting nutrients (S) for: (a) Active transport plus passive diffusion (solid line), active transport (dashed line) and the passive diffusion component (dot–and–dash line) subtracted; (b) facilitated diffusion or active transport, where $V_{\text{max}2} = \frac{1}{2} V_{\text{max}1}$ and consequently $K_{s1} < K_{s2}$.

c) Hypothetical time series showing nutrient disappearance from the medium using the perturbation method, where saturated uptake is non-linear with time. (1) the initial surge uptake ($V_1$) which represents filling of internal pool; (2) ($V_i$) represents an assimilation rate under internal control; (3) ($V_e$) is the depletion of the nutrients when concentrations are limiting (i.e external control) (after Hurd et al. 2014).
4.2. Methods

4.2.1. Collection and pre-treatment of coralline algae

_Arthrocardia_ sp. and crustose coralline algae in the order Hapalidiales were collected at 2 m depth stratum using SCUBA at the wave-exposed coast of Butterfly Bay, Karitāne, southern New Zealand (45°38’26.98”S, 170°40’37.08”E) (Figure 2.1) in June 2016, February and March 2017. Species identification was based on DNA analysis described in the previous chapter (see detail in Chapter 3, section 3.2.1. Collection and pre-treatment of coralline algae). Entire thalli of _Arthrocardia_ sp. or sections of crustose coralline algae (3 cm diameter per section) were collected by chiselling these from the reef and were kept in separate zip-lock plastic bags. They were immediately transported to Portobello Marine Laboratory, University of Otago, New Zealand in an insulated cool box containing seawater from the sampling site. After arriving at the laboratory, the specimens were maintained at a 12: 12 light: dark photoperiod in a recirculating tank (70 cm x 60 cm x 30 cm) with natural seawater until they were processed. This tank was shaded by mesh to ensure that light condition was similar to _in situ_ irradiance levels at 2 m depth stratum (see detail in Chapter 2, section 2.3.1 _In situ_ irradiance). The day before experimental commencement, coralline algae tissues (1.0 ± 0.3 g) were cleaned using filtered (Whatman™ GF/C Glass Microfiber Filters, 1.2 µm pore size) seawater and all visible epiphytes were gently removed using fine forceps.

4.2.2. Time-course nutrient depletion experiments

In order to evaluate the changes in uptake of nitrate (NO₃⁻), ammonium (NH₄⁺) and phosphate (PO₄³⁻) of coralline algae over time, the time-course nutrient depletion experiments were conducted in June 2016 (winter) and February 2017 (summer) in accordance to methods described by Phillips and Hurd (2003) and Pritchard et al. (2015) with slight modification. Prior to the experiments, _Arthrocardia_ sp. (30-50 cm in length) and sections of crustose coralline algae (4-8 cm diameter per section) were prepared as mentioned above (see section 4.2.1). They were then stored in 10 L filtered (Whatman™ GF/C Glass Microfiber Filters, 1.2 µm pore size) seawater plastic tank at 12 °C in control temperature room (CT room) in the dark for 16 hours in order to acclimate with the experimental temperature incubation and to facilitate wound recovery (Hepburn et al. 2006).
In order to deplete nutrients in natural seawater, blades of *Ulva* sp. (1.5 g L\(^{-1}\)) was added to seawater used in these experiments for 48 hours before it was filtered by glass microfiber filters (Whatman™ GF/C, 1.2 µm pore size) (Pritchard et al. 2015). Subsequently, this filtered seawater was enriched with 15 µM of nitrate (as KNO\(_3\)) or ammonium (as NH\(_4\)Cl) or phosphate (as Na\(_2\)HPO\(_4\)). These enriched seawaters were used for the time-course nutrient depletion experiments. All experiments were conducted under conditions of water motion (110 rpm) using two orbital shakers (N-Biotek, GyeongGi-Do, Korea) to prevent boundary layer formation. The experiments used different levels of irradiance (20 µmol photons m\(^{-2}\) s\(^{-1}\) and 110 µmol photons m\(^{-2}\) s\(^{-1}\) for winter and summer, respectively) supplied by Viribright 18 PAR 38 LED light bulbs. Temperature was constantly maintained at 12°C (the same temperature was used in both seasons). Nutrient treatments were replicated four times in winter and five times in summer. Two jars (in winter) and six jars (in summer) contained the filtered seawater without coralline algae for each nutrient treatment were included as controls, in total, twenty-four controls were used in the experiments. All jars were randomly placed on the orbital shaker tables to minimise any effects of variability in irradiance.

After adding the coralline algae, ten ml of seawater sample from each jar was collected at 0, 30, 60, 120, 180, 240 and 300 minutes by using a separate acid washed syringe. The syringes and holders were rinsed with ultra-high purity water (18.2 MΩ· cm Milli-Q®) and glass microfiber filters (Whatman™ GF/C, 1.2 µm pore size) were changed after each sampling at different time points to prevent contamination for each replicate. These seawater samples were then stored in polyethylene vials and frozen at –20°C for later nutrient analysis by using Quickchem® 8500 Automated Ion Analyser (Lachat Instruments, a Hach Company Brand, USA). At the end of the experiment, the coralline algae were removed from the jars, blotted dry and weighed (wet weight) before being dried in an oven at 60°C for 48 hours to determine dry weight.

### 4.2.3. Nutrient uptake kinetics experiments

Nutrient uptake kinetics experiments were carried out to determine the maximal uptake rate (V\(_{max}\)) and half saturation parameters (K\(_m\)) for nitrate or ammonium or phosphate uptake of coralline algae in March 2017. Similar to the time-course nutrient depletion
experiments, the coralline algae specimens were maintained in a 10 L filtered (Whatman™ GF/C Glass Microfiber Filters, 1.2 µm pore size) seawater plastic tank at 12°C in a control temperature room in the dark for 16 hours in order to acclimate to the experimental conditions. The natural seawater was filtered by glass microfiber filters (Whatman™ GF/C, 1.2 µm pore size) after adding blades of Ulva sp. (1.5 g L⁻¹) for 48 hours as mentioned above (see section 4.2.3). This seawater was then enriched with single nitrate (KNO₃) or ammonium (NH₄Cl) or orthophosphate (Na₂HPO₄) at different concentrations including 0, 2, 4, 8, 16, 32, 48 or 64 µM. Each concentration of each nutrient treatment (NO₃⁻, NH₄⁺ and PO₄³⁻) was triplicated. Three additional seawater jars without adding coralline algae at 8 µM for each nutrient treatment were used as controls. All jars were randomly placed on the orbital shakers (N-Biotek, GyeongGi-Do, Korea) with 110 rpm under 110 µmol photons m⁻² s⁻¹ supplied by the Viribright 18 PAR 38 LED light bulbs in the CT room.

After the addition of coralline algae in each enriched nutrient concentration, the initial 10 ml of seawater was immediately sampled by a separate acid washed syringe to determine the initial nutrient concentrations (NO₃⁻, NH₄⁺ and PO₄³⁻) in each experiment (time zero). After 120 mins of incubation, the final 10 ml of seawater was sampled and stored in polyethylene tubes. The incubation time in these nutrient uptake kinetics experiments were based on the results of the preliminary time-course nutrient depletion experiments (see section 4.2.2). The data of the time-course depletion experiments indicated that 120 minutes was a suitable time for incubation and to compare the uptake rate between the different nutrient treatments (NO₃⁻, NH₄⁺ and PO₄³⁻) of these coralline algae. The syringes and holders used to take water samples were always rinsed with ultra-high purity water (18.2 MΩ·cm Milli-Q®) and glass microfiber filters (Whatman™ GF/C, 1.2 µm pore size) were changed after each sampling at different time points. This process was always done in order from lowest to highest concentration to prevent contamination for each replicate. At the completion of the experiment, coralline algae in each jar were removed, blotted dry and weighed (wet weight) in the same order as they were sampled at time zero and final time. They were then dried in an oven at 60°C for 48 hours to measure dry weight. All seawater samples were stored at -20°C for later analysis of nitrate, ammonium, and phosphate concentrations using Quickchem® 8500 Automated Ion Analyser (Lachat Instruments, a Hach Company Brand, USA).
4.2.4. Data analyses

The uptake rates in the time-course nutrient depletion and the nutrient uptake kinetics experiments were calculated using equation (Phillips and Hurd 2004):

\[
V = \frac{(S_i - S_f) \times vol}{(T_i - T_f) \times dw}
\]

Where \( V (\mu\text{mol. gDW}^{-1}. \text{h}^{-1}) \) is the uptake rate, \( S_i (\mu\text{mol}) \) is the concentration at the initial time point (\( T_i \) in hours), \( S_f (\mu\text{mol}) \) is the concentration at the final time point (\( T_f \) in hours), \( \text{vol} (\text{L}) \) is the volume, \( T (\text{h}) \) is the time and \( \text{DW} (\text{g}) \) is the dry weight.

The kinetics of the nutrient uptake in the nutrient uptake kinetics experiments were fitted to the Michaelis-Menten equation (Harrison and Druehl 1982):

\[
V = \frac{V_{\text{max}} \times S}{K_s + S}
\]

Where \( V \) is nutrient uptake rate, \( V_{\text{max}} (\mu\text{mol. gDW}^{-1}. \text{h}^{-1}) \) is maximal uptake rate, \( K_s (\mu\text{M}) \) is the substrate concentration at which the reaction rate is half of \( V_{\text{max}} \). \( S (\mu\text{M}) \) is substrate concentration. Data that showed saturation kinetics were fitted to the Michaelis-Menten model using nonlinear least square regression built in R software package (version 1.0.143) computing values of \( V_{\text{max}} \) and \( K_s \) parameters. A linear regression was replaced if the uptake rates could not follow Michaelis-Menten kinetics (Hurd and Dring 1990). Any negative uptake rates were excluded from analyses (Phillips and Hurd 2004, Martínez et al. 2011).

The differences in uptake rate of each nutrient treatment delivered from the time-course nutrient depletion data between two coralline algae in each season were analysed using a two-way analysis of variance (ANOVA). Similarly, the differences in maximal nutrient uptake rates delivered from the time-course nutrient depletion data in each season were analysed using a two-way analysis of variance. Where ANOVAs showed significant differences between means, post-hoc multiple comparisons were performed using Tukey’s HSD tests with 95% family-wise confidence level. Data sets were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene’s test) before statistical analysis.
A Box-cox transformation was applied to improve normality and equal variance if the data failed to meet with the assumption of an ANOVA comparison. A Student’s t-Test was used to examine for differences in kinetic parameters and slopes of the linear regression equations between species and for each nutrient, at $P < 0.05$ in the nutrient uptake kinetics experiments. All statistical analyses were conducted using the R statistical software package (version 1.0.143).
4.3. Results

4.3.1. Time-course nutrient depletion experiments

The result of time-course nutrient depletion experiments showed that ammonium and phosphate were predominately taken up during the first two hours of experiment by coralline algae during both winter and summer. This period of rapid uptake was followed by a decrease in uptake rate during the remaining time of experiment (Figure 4.2 and 4.4). However, a significant decrease in nutrient uptake rate over time was only detected for ammonium uptake on samples collected in summer (Two-way ANOVA, F$_{5,48}$ = 24.25, Table 4.1). For ammonium uptake, a surge in uptake was observed in both coralline algae during the first 30 minutes of the experiment in summer (Figure 4.2 b) and in crustose coralline algae in winter (Figure 4.2 a). The ammonium uptake rates at 30 minutes were 2.41 ± 0.27 µmol. g$_{DW}^{-1}$ and 1.48 ± 0.31 µmol. g$_{DW}^{-1}$ in summer for *Arthrocardia* sp. and crustose coralline algae, respectively. In winter, the maximal ammonium uptake rate of crustose coralline algae was 0.69 ± 0.36 µmol. g$_{DW}^{-1}$ at 30 minutes, whereas this value of *Arthrocardia* sp. was 1.55 ± 0.97 µmol. g$_{DW}^{-1}$ at 60 minutes.

For phosphate uptake, the time point to reach the highest uptake varied depending on species and season of samples collected (Figure 4.4). *Arthrocardia* sp. reached a maximal phosphate uptake rate after 2 hours of incubation (1.94 ± 1.25 µmol. g$_{DW}^{-1}$) for the winter samples, while the maximal rate was recorded after one hour for the summer samples (0.68 ± 0.29 µmol. g$_{DW}^{-1}$). In contrast, crustose coralline algae reached the highest uptake after 60 minutes of incubation in both seasons (1.82 ± 0.98 µmol. g$_{DW}^{-1}$ and 0.29 ± 0.15 µmol. g$_{DW}^{-1}$ for winter and summer, respectively). Unlike ammonium and phosphate, nitrate was not taken up by both coralline algae collected in winter (data not shown). However, a nitrate uptake was observed in both coralline algae collected in summer. There was a difference in time point at which nitrate uptake was detected between these coralline algae (Figure 4.3). The crustose coralline algae reached the maximal nitrate uptake (0.53 ± 0.17 µmol. g$_{DW}^{-1}$) during the first 30 minutes, whereas *Arthrocardia* sp. did not take up nitrate during the first hour of experiment. Nitrate uptake was only observed after 120 minutes of incubation with a particularly low uptake rate (0.1 ± 0.05 µmol. g$_{DW}^{-1}$) and a peak (0.21 ± 0.09 µmol. g$_{DW}^{-1}$) at 180 minutes.
In relation to comparison of nutrient uptake between two coralline algae, the ammonium uptake rates of *Arthrocardia* sp. during the time-course depletion experiment were significantly higher than that of crustose coralline algae regardless of season (winter: Two-way ANOVA, $F_{1,36} = 12.08, P < 0.001$; summer: Two-way ANOVA, $F_{1,48} = 118.2, P < 0.001$). Similarly, the phosphate uptake of *Arthrocardia* sp. was generally higher than that of crustose coralline algae in both seasons. However, the significant differences in phosphate uptake between these coralline algae were only found in summer samples (Two-way ANOVA, $F_{1,48} = 8.09, P < 0.05$). The nitrate uptake rate of crustose coralline algae was considerably higher than that of *Arthrocardia* sp. in summer (Two-way ANOVA, $F_{1,48} = 22.18, P < 0.001$).

For maximal nutrient uptake rate, there were no significant differences in maximal uptake rate among the three investigated nutrients and between the two coralline algae species collected in winter. In summer, for *Arthrocardia* sp., there were no significant differences in maximal uptake between nitrate and phosphate. The maximal uptake rate of these nutrients was significantly lower than that of ammonium (Two-way ANOVA, $F_{2,24} = 29.71, P < 0.001$). A similar pattern was observed for crustose coralline algae in summer. However, the significant difference was only found between ammonium and phosphate (Turkey HSD, $P < 0.05$). There were no significant differences in maximal uptake rate of each investigated nutrient between these species/group, but the interaction between species/group and nutrient significantly affected the maximal nutrient uptake rates of these coralline algae (Two-way ANOVA, $F_{2,24} = 3.81, P < 0.05$, Table 4.2 and Figure 4.5).
Figure 4.2. Uptake rates of ammonium derived from time-course depletion experiments in winter (a) and summer (b). Bars represent means ± SE (n = 4 in winter and n = 5 in summer).

Figure 4.3. Uptake rates of nitrate derived from the time-course depletion experiments in summer (no uptake rate data was observed in winter). Bars represent means ± SE (n = 4 in winter and n = 5 in summer). Negative values not shown.
Figure 4.4. Uptake rates of phosphate derived from the time-course depletion experiments in winter (a) and summer (b). Bars represent means ± SE (n = 4 in winter and n = 5 in summer).
Table 4.1. Two-way ANOVA testing for differences in the means across both species/group and time points for uptake rates derived from time-course depletion experiments in winter (n = 4) and summer (n = 5).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Season</th>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium</td>
<td>Winter</td>
<td>Species/group</td>
<td>1</td>
<td>12.08</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time points</td>
<td>5</td>
<td>0.515</td>
<td>0.763</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Species/group x time points</td>
<td>5</td>
<td>0.623</td>
<td>0.683</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>Species/group</td>
<td>1</td>
<td>118.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time points</td>
<td>5</td>
<td>24.25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Species/group x time points</td>
<td>5</td>
<td>1.58</td>
<td>0.184</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Summer</td>
<td>Species/group</td>
<td>1</td>
<td>22.19</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time points</td>
<td>5</td>
<td>0.359</td>
<td>0.874</td>
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<td></td>
<td></td>
<td>Species/group x time points</td>
<td>5</td>
<td>4.473</td>
<td>0.002</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Winter</td>
<td>Species/group</td>
<td>1</td>
<td>1.773</td>
<td>0.191</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time points</td>
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<td>1.757</td>
<td>0.147</td>
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<tr>
<td></td>
<td></td>
<td>Species/group x time points</td>
<td>5</td>
<td>0.337</td>
<td>0.887</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>Species/group</td>
<td>1</td>
<td>8.089</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time points</td>
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<td>0.166</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Species/group x time points</td>
<td>5</td>
<td>0.708</td>
<td>0.620</td>
</tr>
</tbody>
</table>
Figure 4.5. Maximal ammonium, nitrate, and phosphate uptake rates derived from the time-course nutrient depletion experiments in winter (a) and summer (b). Bars represent means ± SE (n = 4 in winter and n = 5 in summer). Letters denote significant differences between bars (Tukey’s HSD, P < 0.05 at each season (uppercase letter for winter and lowercase letters for summer).
Table 4.2. Two-way ANOVA testing for differences in the means across both species/group and nutrients for maximal uptake rates derived from time-course depletion experiments in winter (n = 4) and summer (n = 5).

<table>
<thead>
<tr>
<th>Season</th>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>Species/group</td>
<td>1</td>
<td>0.642</td>
<td>0.611</td>
</tr>
<tr>
<td></td>
<td>Nutrients</td>
<td>2</td>
<td>7.165</td>
<td>0.766</td>
</tr>
<tr>
<td></td>
<td>Species/group x nutrients</td>
<td>2</td>
<td>0.429</td>
<td>0.838</td>
</tr>
<tr>
<td>Summer</td>
<td>Species/group</td>
<td>1</td>
<td>3.219</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td>Nutrients</td>
<td>2</td>
<td>29.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Species/group x nutrients</td>
<td>2</td>
<td>3.811</td>
<td>0.037</td>
</tr>
</tbody>
</table>
4.3.2. Nutrient uptake kinetics

The rate of ammonium uptake by two coralline algae was followed saturating Michaelis-Menten uptake kinetics (Figure 4.6 a and b). The ammonium $V_{\text{max}}$ (2.07 ± 0.32 µmol. gDW$^{-1}$. h$^{-1}$) of Arthrocardia sp. was significantly higher than that of crustose coralline algae (0.58 ± 0.17 µmol. gDW$^{-1}$. h$^{-1}$) by 1.49 µmol. gDW$^{-1}$. h$^{-1}$ (Table 4.3, $P < 0.001$). Meanwhile, the ammonium $K_s$ of Arthrocardia sp. (3.43 µM) was lower than that of crustose coralline algae (4.60 µM) but no significant difference was found between two coralline algae species/group (Table 4.3, $P > 0.05$). The uptake rate of nitrate and phosphate for both coralline algae displayed linear relationships as concentrations increased (Figure 4.6 c, d, e and f; all $P < 0.001$). The slopes of linear regression for nitrate and phosphate kinetics of Arthrocardia sp. were significantly higher than those of crustose coralline algae (Table 4.3, all $P < 0.001$). Changes in N (NO$_3^-$ and NH$_4^+$) and P (PO$_4^{3-}$) concentration in control jars were minimal (< 5% for all nutrients and two coralline algae). Therefore, the changes in nutrient concentration in treated jars were attributed to uptake by macroalgae.
Figure 4.6. The ammonium, nitrate, and phosphate uptake kinetics of *Arthrocardia* sp. and crustose coralline algae. Nonlinear (Michaelis-Menten functions) and linear regression are fitted to pooled data from replicates (n = 3). Negative values not shown. a, c, e show ammonium, nitrate, phosphate uptake kinetics of *Arthrocardia* sp.. b, d, f show ammonium, nitrate, phosphate uptake kinetics of crustose coralline algae in the order Hapalidiales at 2 m depth.
Table 4.3. Kinetic parameters for ammonium, nitrate, and phosphate. Parameters were derived from a nonlinear regression of raw data using the Michaelis-Menten function. For data not displaying saturation kinetic, linear regression equation are given (all $P < 0.001$). Letters denote significant differences between two coralline algae at each nutrient treatment (Tukey’s HSD, $P < 0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Ammonium</th>
<th>Nitrate</th>
<th>Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_s$ (µM)</td>
<td>$V_{\text{max}}$ (µmol. gDW$^{-1} \cdot$ h$^{-1}$)</td>
<td>$K_s$ (µM)</td>
</tr>
<tr>
<td>Arthrocardia sp.</td>
<td>3.43±2.46$^a$</td>
<td>2.07± 0.32$^a$</td>
<td>-</td>
</tr>
<tr>
<td>Hapalidiales-2 m depth</td>
<td>4.60±5.58$^a$</td>
<td>0.577±0.17$^b$</td>
<td>-</td>
</tr>
</tbody>
</table>
4.4. Discussion

Nutrient uptake by algae plays a crucial role in driving primary productivity and the biological pump in the marine environment (Geider et al. 2001, Boyd and Hurd 2009). Macroalgae are important primary producers in temperate environments and supply physical structure to the ecosystem and modify many abiotic coastal processes (Gaylord et al. 2007). Therefore, the ability to efficiently take up nutrients is one of the factors enabling marine macroalgae to constantly maintain high levels of productivity (Rees et al. 2007). This study provides the first information on nitrate, ammonium and phosphate uptake of coralline algae and compares nutrient uptake kinetic parameters between *Arthrocardia* sp. and crustose coralline algae in the order Hapalidiales at the same 2 m depth stratum which are an important component of rocky reefs in subtidal zone (Harvey et al. 2005, Farr et al. 2009, Nelson 2009, Hepburn et al. 2011, Nelson 2013).

In order to determine an appropriate incubation time for uptake kinetics of nutrients, preliminary time-course nutrient depletion experiments were conducted using samples collected in both winter and summer. The results of these experiments revealed that there was a variation in the time for coralline algae to reach maximal uptake rates which was dependent on the growth form of coralline algae, season of samples collected and nutrient forms and sources. The maximum uptake rates of all nutrients occurred within the first two hours of the experiments for both coralline algae and across seasons. Hence, for this reason, an incubation of 120 minutes was considered appropriate for multiple-flask experiments to determine the kinetics of nutrients in these two coralline algae. This is also the time point (120 minutes) used by Pritchard et al. (2015) to investigate nitrogen ecophysiology of a light-limited red alga *Anotrichium crinitum*, is an important macroalgal species of the lower the subtidal zone of many temperate reefs in New Zealand (Nelson 2013, Pritchard et al. 2013).

In the time-course nutrient depletion experiments, all nutrient treatments were used at 15 µM to satisfy the demand of coralline algae in a short period as well as to compare the nutrient uptake ability between the two coralline algae. Although these concentrations were nontoxic for macroalgae, the concentration of ammonium higher 10 µM is outside the typical range of ammonium concentration in seawater (< 3 µM) (Harrison and Druehl 1982, Phillips and Hurd 2003, Pritchard et al. 2015). However, these coralline algae used in the present
study took up ammonium at 15 µM. Rapid uptake at 15 µM of ammonium occurred at the beginning of the experiment in both coralline algae regardless of season. Similar results have been observed for many other macroalga species such as *Enteromorpha intestinalis*, *Fucus gardneri*, *Mastocarpus papillatus*, *Gracilaria pacifica* (Thomas and Harrison 1987). The high intertidal macroalgae *Stictosiphonia arbuscula* and *Scytosiphon australis* also display a surge phase of NH$_4^+$ uptake at 30 µM concentrations (Phillips and Hurd 2003). The maximal ammonium uptake rate of both coralline algae was approximately threefold higher than that of nitrate in both seasons. Wallentinus (1984) also indicated that most macroalgae in the Baltic Sea, for example *Fucus vesiculosus*, *Furcellaria lumbricalis* and *Phyllophora truncata*, *Cladophora glomerata*, *Enteromorpha ahnneriana*, *Scytosiphon lomentaria*, *Dictyosiphon foeniculaceus* and *Ceramium tenuicorne*, exhibit higher NH$_4^+$ uptake rates than NO$_3^-$ and that nitrogen uptake rates were always higher than those of phosphorus at the same concentrations. The lower energetic cost of NH$_4^+$ uptake and assimilation compared to NO$_3^-$, makes NH$_4^+$ the primary source of nitrogen for many macroalgae, particularly slow growing algae with low nitrogen demands (Raven 1984 and Raven et al. 2008). These coralline algae in this present study displayed higher affinities for ammonium compared to nitrate uptake in both seasons. The nitrate and ammonium uptake of the other New Zealand intertidal macroalgae *Apophlaea lyallii* and *Xiphophora gladiata* (Phillips and Hurd 2004) and subtidal macroalga *Anotrichium crinitum* (Pritchard et al. 2015) is seasonally altered with a preference for taking up ammonium in winter (Phillips and Hurd 2003, Pritchard et al. 2015). Alternatively, the reduction in nitrate reductase activity under N limitation to conserve energy as seen in temperate kelps (Wheeler and Weidner 1983, Davison et al. 1984) is another explanation for a cease in nitrate uptake of two coralline algae in the winter. Coralline algae exhibited maximal phosphate uptake rates after 60 minutes and 120 minutes of incubation for summer and winter, respectively. The rapid responses of the two coralline algae in the present study are comparable with four intertidal fucoid algae *Pelvetia canaliculata*, *Fucus spiralis*, *F. vesiculosus*, *F. serratus* (Hurd and Dring 1990). A transient phase of high affinity of the phosphate uptake during the first 15 minutes of experiment was observed for macroalgae *Bifurcaria bifurcata* and *Nemalion helminthoides* (Martínez et al. 2011).

Both coralline algae collected in winter did not take up nitrate and nitrate uptake by *Arthrocardia* sp. sampled in summer was only detected after 120 minutes of incubation.
Seasonal change of background nutrient levels in seawater at the sampling area is typical of temperate seas with high concentrations of nitrate in winter and a low concentration in summer (see detail in Chapter 2). In addition, the average NO$_3^-$ concentration in tissue of coralline algae in winter was approximately 1.5 higher than that of summer. It is assumed that the nitrate accumulation in tissue of coralline algae in winter probably satisfies the requirement of nitrate, which leads to no nitrate uptake of coralline algae in winter. As the Chapter 3 mentioned, analysing soluble tissue of coralline algae indicated that coralline algae contained nutrients in tissue. Possibly, these nutrients were released into environment due to different gradients. In summer, the demand of nutrients due to low NO$_3^-$ concentration and high light favouring growth results in uptake of all available nitrogen forms and phosphorus for coralline algae. In relation to comparisons of nutrient uptake between two coralline algae in the time-course nutrient depletion experiments, the ammonium uptake rates of *Arthrocardia* sp. were significantly higher than that of crustose coralline algae regardless of season. Similarly, the phosphate uptake of *Arthrocardia* sp. was generally higher than that of crustose coralline algae in both seasons but the significant differences in phosphate uptake between these coralline algae were only found in summer samples. This suggests the growth form of *Arthrocardia* sp. with upright branch thallus and three -dimension form (personal observation, Nguyen Hang TT) could be more efficient at taking up ammonium and phosphate than the flattened thallus and two – dimension form of crustose coralline algae. A higher surface area: volume ratio of *Arthrocardia* sp. could be an advantage for *Arthrocardia* sp. uptake more nutrient in comparison with crustose coralline algae. Phillips and Hurd (2004) indicated that intertidal macroalgae with a higher surface area to volume ratio have more efficient uptake a low nitrogen concentration and using surface area to volume can explain some of the difference in nitrogen uptake rates among the intertidal macroalgae. *Ulva prolifera* (filament) and *Ulva linza* (blade) have disparate morphology exhibited a similar in nutrient uptake capacity to *Arthrocardia* sp. and crustose coralline algae, even though they are in the same clade (*Ulva linza–procer–prolifera complex*) (see detail in Luo et al. 2012). NO$_3^-$ and NH$_4^+$ uptake rates of *U. prolifera* were significantly higher than that of *U. linza* (Luo et al. 2012). Macroalgae with short-lived, opportunistic, filamentous or with numerous hairs such as *Cladophora glomerata*, *Enteromorpha ahlneriana*, *Scytosiphon lomentaria*, *Dictyosiphon foeniculaceus* and *Ceramium tenuicorne* also showed higher nutrient uptake rates than macroalgae with late-
successional, long-lived, coarse thalli with low surface: volume ratios such as *Fucus vesiculosus*, *Furcellaria lumbricalis* and *Phyllophora truncata* (Wallentinus 1984). The effects of morphological structure on the ability to take up nutrients is reported for many macroalgae species. For *Pyropia* species, species with thin thalli are more efficient to take up nutrient than thick globular or cylindrical-shaped species (see Pedersen et al. 2004).

Among nutrient forms and sources investigated, only the uptake of ammonium exhibited the saturable kinetics in both coralline algae, whereas the nitrate and phosphate uptake displayed increased linear uptake. There have been numerous studies indicating that many macroalgae exhibit saturable or biphasic uptake for ammonium (Phillips and Hurd 2004, Pritchard et al. 2015, Den Haan et al. 2016). Hence, saturable ammonium uptake which was observed on two coralline algae in the present study is reasonable. The linear increases in the rate of nitrate uptake were observed in two coralline algae. This result is not surprising because similar patterns for nitrate uptake were reported in several intertidal and subtidal algae species such as *Saccharina greelandica*, *Gracilaria pacifica*, *Chaetomorpha linum*, *Gracilaria vermiculophylla*, *Anotrichium crinitum* (Harrison et al. 1986, Thomas et al. 1987, Lavery and McComb 1991, Abreu et al. 2011, Pritchard et al. 2015). However, but the mechanisms behind these phenomena are unknown (Harrison and Hurd 2001). Similarly, phosphate uptake exhibited a linear relationship with concentration in both coralline algae. The opportunistic *Ulva intestinalis*, and the summer-annual *Nemalion helminthoides* from Asturian coasts (Spain) also showed a linear regression for phosphate uptake kinetics (Martínez et al. 2011). *Palmaria palmata* (Rhodophyta) displayed non-saturation for inorganic P uptake and biphasic uptake kinetics (Martínez and Rico 2004). Turf algae also exhibited strong linear increases of their PO$_4^{3-}$ uptake rates with increasing ambient PO$_4^{3-}$ concentrations (Den Haan et al. 2016). The transport system involving in such non-saturable response remains unknown in algae (Borchardt et al. 1994). Based on the low membrane permeability to orthophosphate anions, this response cannot be explained by passive diffusion that has been associated with linear responses (Reed 1990, Lobban and Harrison 1997). Hence, whether nitrate and phosphate levels could reach toxic levels inside the cells of algae showing a linear uptake of these nutrients is still unknown. According to Martínez and Rico (2002), the non-saturable nature of the uptake response may be considered as an adaptation to exploit such P sources by primary producers. Notably, the differences between *in situ* and laboratory estimates may be occurred due to differences in nutritional histories

In comparison, the kinetic parameters between two coralline algae, the \( V_{\text{max}} \) of ammonium uptake kinetics of *Arthrocardia* sp. \((2.07 \pm 0.32 \ \mu\text{mol. gDW}^{-1}. \text{h}^{-1})\) was significantly higher than that of crustose coralline algae \((0.58 \pm 0.17 \ \mu\text{mol. gDW}^{-1}. \text{h}^{-1})\). Clearly, *Arthrocardia* sp. have a greater efficiency of ammonium uptake than crustose coralline algae at low and high concentrations. The \( K_s \) of *Arthrocardia* sp. and crustose coralline algae was 3.43 and 4.60 \( \mu\text{M} \), respectively but there was no significant difference in ammonium \( K_s \) between these coralline algae. These \( K_s \) values are comparable to \( K_s \) of *Anotrichium crinitum* (3.23 \( \mu\text{M} \)) which has a relatively high affinity for ammonium. This supposed that *Arthrocardia* sp. and crustose coralline algae have high affinity for ammonium as they likely use ammonium as a sole source of nitrogen to sustain growth. Johnson and Carpenter (2018) exposed crustose coralline alga *Porolithon onkodes* to nitrogen-enriched solutions, including nitrate + nitrite \(< 1 \ \mu\text{M}\) and high ammonium concentration \(> 6 \ \mu\text{M}\), in ambient and high pCO\(_2\) treatments, and resulted in calcification increases by 90–130\%, respectively. These authors demonstrated that nitrogen enrichment, especially in the form of ammonium, can reduce negative effects of ocean acidification to coralline algae. However, a considerably negative effect of nutrient enriched plots on crustose coralline algae cover has also been observed (Belliveau and Paul 2002), especially high phosphate concentrations that inhibited the growth rate and the calcification of encrusting coralline algae *Lithophyllum kotschyanum* (Björk et al. 1995). Therefore, it is necessary to investigate the growth ability of these coralline algae with the different nutrient forms in further studies.

Regarding the kinetics of nitrate and phosphate uptake, both coralline algae followed linear relationship that resulted in the failure of kinetic parameters comparison between them. Hurd and Dring (1990) indicated that although the Michaelis - Menten model was the most effective tool to compare the ability of nutrient uptake between macroalgae in many cases, data often did not meet the requirements for applying the Michaelis - Menten equation. As a result, a linear regression was applied to compare the nitrate and phosphate uptake kinetics between two coralline algae. The statistical analysis showed that *Arthrocardia* sp. has significant higher slope of both nitrate and phosphate uptake in comparison with that of
crustose coralline algae. This indicated a faster increase in the rate of nutrient uptake with rising nitrate and phosphate substrate concentration of *Arthrocardia* sp. in comparison with crustose coralline algae.

This study demonstrates that ammonium is the preferred nitrogen source for both coralline algal groups studied when compared to nitrate in both summer and winter, whereas phosphate can be taken up regardless of season. Ammonium is considered as a good source for slow growing algae without high nitrogen demands (Pritchard et al. 2015), but also many invertebrates live within coralline habitats so NH$_4^+$ is likely to be available for uptake (Taylor and Rees 1998, Hepburn and Hurd 2005). There was a greater efficiency in nitrogen and phosphorus uptake rates and uptake kinetics of *Arthrocardia* sp. compared to crustose coralline algae. The advantage in morphological structure of *Arthrocardia* sp. with stiff and erect fronds (Nelson 2013) and three-dimensional form compared to the crustose which lies prostrate to the substrate (Harvey et al. 2005) and two-dimensional form can be a potential factor resulting in the difference in nitrogen and phosphorus uptake rates and uptake kinetics between these macroalgae through the surface area to volume ratio (lower value in crustose coralline algae, personal observation). However, this assumption needs to investigate in further study by calculating and comparing the ratio of SA: V between these coralline algae.
Chapter 5

Photosynthesis *versus* irradiance relationship for coralline algae
5.1. Introduction

Macroalgal productivity and the structural complexity of macroalgal communities are directly related to natural variation in light delivery in coastal waters (Larkum and Barrett 1983, Johansson and Snoeijjs 2002, Schwarz et al. 2003, Middelboe et al. 2006, Falkowski and Raven 2007, Raven and Hurd 2012, Desmond et al. 2017). A balance between energy produced by the photosynthetic process and the energetic requirements for cellular maintenance and growth is essential for macroalgae to survive in variable underwater light conditions (Raven et al. 2000, Hanelt et al. 2003, Falkowski and Raven 2007). To maintain photosynthetic plasticity under variable light conditions, especially in temperate coastal seas where irradiance is highly variable both spatially and temporally (Gattuso et al. 2006, Dickey et al. 2011), macroalgae possess a range of adaptations at cellular and morphological levels. For example, the main accessory photosynthetic pigments of the light-harvesting complex (LHC) varies based on macroalga groups. Carotenoids such as fucoxanthin, phycobiliproteins and chlorophylls are typical accessory photosynthetic pigment for Phaeophyceae, Rhodophyceae and Chlorophyceae, respectively (Hanelt et al. 2003). Red macroalgae are particularly adapted to live at a range of light levels (Kain and Norton 1990), as can be deduced from their wide distribution from intertidal to deep sea (Littler et al. 1985, Cole and Sheath 1990). Talarico and Maranzana (2000) suggested that red algae are both light-intensity and light-quality adapters. According to Hanelt et al. (2003) red algae showed the highest photosynthetic rates with green light via absorption by phycobiliproteins within the antenna complex of Photosystem II and efficient electron transfers to both photosynthetic reaction centres. Meanwhile, high electron transport is not induced by blue and red/far red light in red algae because the main antenna pigment in Photosystem I (PSI) is chlorophyll so that the reaction centre PS I is principally activated (Butler 1978, Gantt 1990, Talarico and Maranzana 2000, Hanelt et al. 2003). Apart from differences in pigment components, many marine algae are able to photosynthetically acclimate to variability in the environment by altering photosynthetic pigment concentration of LHC (Ramus et al. 1976) or decreasing the efficiency of photosynthesis (dynamic photoinhibition) to protect photosynthetic apparatus when macroalgae exposed to temporary light stress (Hanelt et al. 2003). However, exposure to high irradiance levels (e.g. full sun light) or the interaction of environmental stresses including light, desiccation and temperature change causes macroalgal bleaching
which results in a degradation and loss of photosynthetic pigments (Irving et al. 2004, Martone et al. 2010).

Photosynthetic activity of macroalgae is associated with morphology, especially with thallus structure (Gacia et al. 1996, Johansson and Snoeijs 2002, Stewart and Carpenter 2003). Species consisting of non-photosynthetic tissues in the medulla have lower light absorption compared to species consisting only of photosynthetically active cells (see Hanelt et al. 2003). Terete (e.g. Desmarestia menziesii, Ballia callitricha) and leathery (e.g. Ascoseira mirabilis, Gigartina skottsbergii) species exhibit lower photosynthetic capacities (P_{max}) than filamentous (e.g. Urospora penicilliformis, Geminocarpus geminatus) or foliose thalli (e.g. Monostroma hariotii, Pyropia endiviifolia, Enteromorpha bulbosa) (Weykam et al. 1996). The differences in photosynthetic activity is not only found between different macroalgae species but also within the same species at different developmental stages. For example, the net photosynthesis rate P_{max} of three-year old Ascoseira mirabilis was lower than that of two-year old A. mirabilis because the young one had a lower percentage of non-photosynthetic tissue, smaller size and faster growth rate (Gómez et al. 1996).

In relation to the effects of depth distribution on photosynthesis of macroalgae, the acclimation of the shallow-water ecotype of Solieria pacifica to the high PAR environment of the upper sublittoral zone exhibited via higher values of P_{max}, E_c, and E_k from shallow (5 m) compared to individuals from deep (35 m) (Borlongan et al. 2017 b). Desmarestia anceps, Kallymenia antarctica, Palmaria decipiens, Gigartina skottsbergii also showed higher P_{max} with algae collected at 10 m and 20 m than at 30 m depth, but the inter-specific variations in photosynthesis characteristics with depth was not clearly detected for this species (Gómez et al. 1997).

To evaluate the photosynthesis ability of different macroalgae under light variation, Photosynthesis versus irradiance (PE) curves are a useful model to reflect photosynthetic responses to the light (Coutinho and Zingmark 1987, Peña et al. 1999, Hurd et al. 2014, Terada et al. 2016). The PE curve can include three definite regions including a light-limited region (α), a light-saturated region (P_{max}) and a photo-inhibited region (β) (Henley 1993, Falkowski and Raven 2007). However, not all macroalgae species show the photoinhibition in the PE curves, especially at experimental low irradiance levels (Figure 5.1) (Coutinho and
Zingmark 1987, Henley 1993, Weykam et al. 1996, Pritchard et al. 2013, Watanabe et al. 2014 a, b, Borlongan et al. 2017 a, b). The irradiance at which photosynthesis balances respiration is called compensation irradiance $E_c$, and the irradiance at which there is intercept between initial slope $\alpha$ and maximum $O_2$ evolution rate at saturating irradiance $P_{\text{max}}$ is called saturation irradiance (Lobban and Harrison, 1997).

The photosynthetic ability of non-calcareous macroalgae is well documented in the previous studies (Peña et al. 1999, Pritchard et al. 2013, Watanabe et al. 2014a, b, Terada et al. 2016, Borlongan et al. 2017 a, b). For calcareous macroalgae, the PE curve has been applied to evaluate the photosynthetic response of coralline algae species. Guenther and Martone (2014) used the PE curves to identify saturating and sub-saturating irradiances of two intertidal articulated coralline algae including $Calliarthron$ $tuberculatum$ and $Corallina$ $vancouveriensis$ distributed among tidepool and non-tidepool habitats in submerged conditions in the North Pacific Ocean. Photosynthetic characteristics of the articulated coralline alga $Ellisolandia$ $elongata$, distributed in intertidal rock pools on the coast of Brittany (France) exhibits three-fold higher values for $P_{\text{max}}$ in summer compared with winter (Egilsdottir et al. 2016). The Mediterranean crustose coralline alga, $Lithophyllum$ $cabiochae$ distributed at 25 m depth also indicated maximal photosynthesis rate $P_{\text{max}}$, saturation $E_k$ and compensation $E_c$ irradiances in summer were higher than that in winter. Photo-inhibition of $L. cabiochae$ was observed in winter (Martin et al. 2013). In the southwestern Pacific Ocean, photosynthesis of coralline algae is poorly understood despite their broad distribution, key ecological role and ability to grow in different light regimes (Harvey et al. 2005, Farr et al. 2009, Nelson 2009, Hepburn et al. 2011, Nelson 2013).

The main aim of this study was to determine photosynthetic activities over irradiance variation of coralline algae which dominate on rocky reefs in a kelp forest in southern New Zealand. This was achieved by measuring oxygen evolution from photosynthesis of coralline algae at different irradiances. The effect of species/group on photosynthesis was investigated by comparing photosynthetic parameters delivered from PE curves of $Arthrocardia$ sp. and crustose coralline algae at the same 2 m depth stratum. The effect of depth (2 m vs. 10 m) on photosynthesis was also incorporated by comparison of photosynthetic parameters delivered from the PE curves of crustose coralline algae. It was hypothesized that $Arthrocardia$ sp. would have higher photosynthetic capacity than crustose coralline algae at
the same stratum based on higher surface area to volume ratio, pigment concentration (chlorophyll $a$ and phycobiliproteins) and three dimensional structure of *Arthrocardia* sp. (see detail in Chapter 3). Moreover, it is expected to find a greater photosynthetic efficiency at low light for deeper coralline algae.
Figure 5.1. Photosynthetic versus irradiance curves. a) Classic PE curve response with and without a downturn at saturating PFD (photoinhibition (1) and no photoinhibition (2)). b) The PE curves without photoinhibition of two individuals having identical $\alpha$ but different $P_{\text{max}}$ and the resultant difference in $E_k$. Different $P_{\text{max}}$ with no change $\alpha$ may result from temperature change or endogenous rhythms (after Henley 1993).
5. 2. Method

5.2.1. In situ irradiance and light treatment levels

The photon flux density (PFD) of photosynthetically active radiation (PAR) was recorded at the surface, 2 m and 10 m below mean low water using Odyssey® (Odyssey, Dataflow Systems Limited, Christchurch, New Zealand) and HOBO® (HOBO Pendant Temperature/Light Data Logger 64k, Onset) Loggers during 616 days between 5th April 2016 to 11th December 2017 (details described in section 2.2.2. In situ irradiance - photosynthetically active radiation (PAR), Chapter 2). After reviewing the in situ PFD record, 25 irradiance levels ranging from 0 to 1800 µmol photon m\(^{-2}\)s\(^{-1}\) (0, 1.3, 1.6, 2.0, 4.3, 7.9, 11.3, 20.9, 26.4, 29.4, 38.4, 46.3, 55.9, 98.9, 114.2, 118.2, 161.0, 312.7, 342.5, 653.8, 694.6, 1133.4, 1295.5, 1575.3, 1794.7 µmol photon m\(^{-2}\)s\(^{-1}\)) were selected to evaluate the photosynthetic performance of coralline algae at 2 m and 10 m strata. The selected irradiances were divided two groups: group 1 ranged from 0, 1.3, 2.03, 11.3, 20.9, 26.4, 46.3, 98.9, 114.2, 312.7, 653.8, 1133.4 to 1295.5 µmol photon m\(^{-2}\)s\(^{-1}\) and group 2 ranged from 0, 1.6, 4.3, 7.9, 29.4, 38.4, 55.9, 118.2, 161.0, 342.5, 694.6, 1575.3 to 1794.7 µmol photon m\(^{-2}\)s\(^{-1}\), to correspond with the light regimes at 2 m and 10 m depths and to avoid long incubation times for these experiments which can result in limitation effects (see detail in Pritchard et al. 2013). All coralline algae were exposed to both irradiance groups above.

5.2.2. Collection and pre-treatment of coralline algae

Entire thalli of Arthrocardia sp. (30-50 cm in length) and sections of crustose coralline algae (4-8 cm diameter per section) at 2 m and 10 m depth strata were gently removed from reefs by SCUBA at Butterfly Bay, Karitāne, South Island, New Zealand (Figure 2.1). Specimens at each stratum were stored in separate plastic bags filled with seawater from the sampling site. They were immediately transported to the Portobello Marine Laboratory in a cool box within one hour.

In the laboratory, coralline algae were stored at a 12:12 light: dark photoperiod in two fibreglass tanks (70 cm x 60 cm x 30 cm) and supplied with natural seawater flow until they were processed. These tanks were set up to mimic the approximate light environment at 2 and 10 m depths by using mesh. The samples were kept in these tanks no more than
seven days until they were used for the photosynthesis *versus* irradiance experiments. Species identification based on DNA analysis was described in the chapter 3 (see detail in 3.2.1. Collection and pre-treatment of coralline algae, Chapter 3). In preparation for photosynthetic incubations, individuals of coralline algae were removed from their light-controlled holding tanks. Coralline algae tissues (1.0 ± 0.5 g) were gently removed of all visible epiphytes by using fine forceps and cleaned by filtered seawater (Whatman GF/C Glass Microfiber Filters, 1.2 µm, hereafter referred to as filtered seawater). The specimens were placed in plastic tank (10 L) of filtered seawater which was supplied with oxygen from an air stone. They were left in the dark in a controlled temperature room at 12°C for at least 16 hrs to acclimate and allow for wound healing (Hepburn et al. 2006).

5.2.3. Photosynthesis *versus* irradiance (PE)

Photosynthesis *versus* irradiance experiments were carried out in September 2017 to determine photosynthetic parameters. All photosynthesis *versus* irradiance experiments on coralline algae were implemented during daytime between 0700 hour to 1800 hour to coincide with natural photosynthetic activity of macroalgae (Coutinho and Zingmark 1987). Incubations were conducted in a controlled temperature room (12°C) using custom made 98 mL acrylic chambers. *Arthrocardia* sp. (1.0 ± 0.5 g) was inserted into holes within a horizontal net platform, while the crustose coralline algae (1.0 ± 0.5 g) were adjusted to stand upright using a vertically support net platform in the middle of each chamber. A magnetic stirring bar was placed underneath the horizontal net platform, which was fixed inside each chamber with a gap of 15mm from the chamber bottom, to prevent the formation of boundary layers around coralline algae. The chambers were then filled with 12°C filtered seawater. Oxygen concentration in these chambers was measured using a NeoFox Phase Fluorometer system (Ocean Optics, Dunedin, USA) connected to a laptop computer installed with NeoFox Viewer Software. Oxygen readings were logged every ten seconds using the Data logging control panel on the main NeoFox Viewer screen. The oxygen sensor probes were calibrated using multipoint calibration purchased from a factory calibration file (Ocean Optics, Dunedin, USA). Calibrated oxygen concentration was selected to display oxygen as DO µM/L. The light system provided for the whole experiment was set up on a horizontal plane with light being produced by two quartz-halogen bulb slide projectors (SAV2000, Kodak, USA). The neutral density filters (Lee Filters, USA) mounted in full-frame 35 mm
glass slides were used to change the levels of irradiance (Figure 5.2). The PFD was measured within each chamber at each light level prior to incubations using a LI - 250A Light meter (LI-COR Inc, Lincoln, Nebraska, USA) connected to LI-190R quantum sensor. Four incubations containing the filtered seawater but without coralline algae were included as controls.

Figure 5.2. Schematic diagram of the photosynthesis *versus* irradiance experiments. a) Oxygen probe, b) Temperature probe connected with NeoFox Phase Fluorometer connected NeoFox Viewer Software installed in a laptop, c) Magnetic stirring bar, d) Raised mesh base, e) *Arthrocardia* sp., f) Crustose coralline algae, and k) Supporting net for crustose coralline algae.

To begin the experiment, the chambers containing the coralline algae were left in absolute darkness for at least an hour or until the respiration of algae was detected in each chamber. Coralline algae were then exposed to each of 12 consecutively higher irradiances for ten minutes according to Pritchard et al. (2013). *Arthrocardia* sp. and crustose coralline algae were removed from the chambers after completion of the light curve, and then photographed (Nikon 1J5, Japan) to measure surface area by Image J (Image J 1.51n Java 1.8.0_66) before blotting dry and weighing (wet weight). The algal tissue (0.03 ± 0.01 g) was then taken for pigment extraction and the rest of tissue was dried to a constant weight.
at 60°C and reweighed (dry weight). Photosynthetic rates were calculated from a linear regression of oxygen concentration *versus* time during the last 360 seconds at each light level as µmol O₂ h⁻¹ and standardised to g⁻¹ wet weight, g⁻¹ dry-weight and mg⁻¹ chlorophyll a as well as cm⁻² surface area.

### 5.2.4. Measurement of photosynthetic pigment concentration

Pigment extraction was conducted as in Chapter 3 (see detail in section 3.2.4. Photosynthetic pigment concentration).

### 5.2.5. Curve fitting and statistical analyses

A nonlinear least squares regression was used to determine the photosynthesis *versus* irradiance model following the equations of Watanabe et al. (2014)

\[
P_{\text{net}} = P_{\text{max}} [1 - \exp(-\alpha E/P_{\text{max}})] - R_d
\]  

(5.01)

\[E_k = P_{\text{max}}/\alpha \text{ and } E_c = P_{\text{max}} \ln \left[P_{\text{max}}/(R_d - P_{\text{max}})\right]/\alpha\]

Where: \(P_{\text{net}}\) is the net O₂ production rate; \(P_{\text{max}}\) is the maximum O₂ production rate; \(\alpha\) is the initial slope of the photosynthesis *versus* irradiance curve; \(E\) is the incident irradiance; \(R_d\) is the dark respiration rate; \(E_k\) is saturation irradiance and \(E_c\) is compensation irradiance.

The PE curves including an inhibition term (\(\beta\)) were also fitted using the following equation of Platt et al. (1980) (see in Coutinho and Zingmark 1987)

\[
P_{\text{net}} = P_s [1 - \exp(-\alpha E/P_s)\exp(-\beta E/P_s)] + R_d
\]  

(5.02)

\[E_b = P_s/\beta\]

Where: \(P_s\) is similar to \(P_{\text{max}}\) which is the maximum O₂ production rate and numerically equal when \(\beta\) is zero and \(\alpha\), \(E\) and \(R_d\) are as described above.

No photoinhibition was found in PE experiments of *Arthrocardia* sp. and crustose coralline algae at 2 m depth (\(\beta < 0\)), but positive \(\beta\) values were determined in PE experiment of crustose coralline algae at 10 m depth (\(\beta = 0.000085\)) and estimated photoinhibition
irradiance $E_b \sim 31058 \, \mu\text{mol photons m}^{-2}\text{s}^{-1}$. This value is 10 times higher than the brightest sunlight on the planet. Therefore, $E_b$ is not defined in this study, so the simpler model without photoinhibition (5.01) was applied. After fitting the PE curves on data, a bootstrapped prediction interval was applied to calculate the confident interval of the PE curves. This was achieved by bootstrapping the underlying data, fitting a curve, generating predictions, and then taking the 2.5% and 97.5% limits of these predictions.

The differences in photosynthetic parameters delivered from the photosynthesis versus irradiance data were analysed using a Student’s t-Test at $P < 0.05$. The Student’s t-Test was used to compare single photosynthetic parameters including $P_{\text{max}}$, $\alpha$, $R_d$, $E_k$ and $E_c$ standardised to g$^{-1}$ dry-weight, g$^{-1}$ wet weight and mg$^{-1}$ chlorophyll $a$ as well as cm$^{-2}$ surface area between Arthrocardia sp. and crustose coralline algae at the same 2 m depth stratum and between crustose coralline algae at 2 m depth and at 10 m depth.

All statistical analyses were conducted by using the R statistical software package (version 1.0.143).
5. 3. Results

5.3.1. Photosynthetic response to irradiance

Photosynthetic responses of all investigated coralline algae at 12°C showed the same pattern (Figure 5.3, 5.4 and 5.5). The net photosynthesis rates initially increased, approached an asymptote ($P_{\text{max}}$) and then saturated to a constant rate at high irradiance. All investigated coralline algae species did not indicate photoinhibition up to the maximum PAR of 1800 µmol photons m$^{-2}$ s$^{-1}$.

The maximum photosynthetic rate of *Arthrocardia* sp. and crustose coralline algae at 2 m depth were 20.38 ± 2.38 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (95% confidence interval (CI) of 16.51 to 23.82 µmol O$_2$. gDW$^{-1}$. h$^{-1}$) and 3.72 ± 0.74 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (CI of 2.27 to 5.13 µmol O$_2$. gDW$^{-1}$. h$^{-1}$), respectively. The difference in the maximum photosynthetic rate between these two coralline algae was significant (Student’s t-Test, df = 5.95, $P < 0.001$, Figure 5.6). Unlike $P_{\text{max}}$, the respiration rates between the two coralline algae at the same depth (2m) were not significantly different ($P > 0.05$). The respiration rate of *Arthrocardia* sp. was 1.60 ± 1.16 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (CI of -1.43 to 3.81 µmol O$_2$. gDW$^{-1}$. h$^{-1}$) and 0.33 ± 0.42 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (CI of -0.96 to 1.72 µmol O$_2$. gDW$^{-1}$. h$^{-1}$) for crustose coralline algae at 2 m depth.

The initial slope $\alpha$ of the PE curves of *Arthrocardia* sp. was 0.46 ± 0.15 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$ (CI of 0.22 to 0.68 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$), whereas crustose coralline algae at 2 m depth was 0.17 ± 0.035 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$ (CI of 0.021 to 0.77 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$). However, there was no significant difference detected in this parameter between these groups (Figure 5.7). The saturation irradiance and the compensation point for photosynthesis, between *Arthrocardia* sp. and crustose coralline algae at the same 2 m depth were also not significantly different (Figure 5.8 and Figure 5.9). *Arthrocardia* sp. reached compensation point and saturation irradiance at 21.24 ± 4.69 µmol photons m$^{-2}$ s$^{-1}$ and 60.01 ± 11.92 µmol photons m$^{-2}$ s$^{-1}$, respectively. These parameters for crustose coralline algae at 2 m depth were 36.43 ± 19.24 µmol photons m$^{-2}$ s$^{-1}$ and 13.43 ± 8.06 µmol photons m$^{-2}$ s$^{-1}$, respectively.
There were no significant differences in all investigated parameters between crustose coralline algae distributed at 2 and 10 m depth (Figure 5.6, 5.7, 5.8, 5.9). Crustose coralline algae at 10 m depth had a maximum net photosynthetic rate was 2.79 ± 0.74 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (CI of 1.78 to 3.38 µmol O$_2$. gDW$^{-1}$. h$^{-1}$) which was 0.92 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ lower than crustose coralline algae at 2 m depth. The other photosynthetic parameters (R$_d$, α, E$_k$, E$_c$) of crustose coralline algae were 0.43 ± 0.52 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (CI of 0.29 to 1.04 µmol O$_2$. gDW$^{-1}$. h$^{-1}$), 0.10 ± 0.04 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$ (CI of 0.03 to 0.22 µmol O$_2$. gDW$^{-1}$. h$^{-1}$ (µmol photons m$^{-2}$ s$^{-1}$)),$86.00 ±33.78 µmol photons m$^{-2}$ s$^{-1}$ and 32.20 ±12.94 µmol photons m$^{-2}$ s$^{-1}$, respectively.

5.3.2. Pigment concentration of coralline algae

Simple main effect analysis showed that the differences between Arthrocardia sp. and crustose coralline algae at 2 m depth had significant effect on the concentration of pigments concentration (F$_{1,30}$ = 84.5, $P < 0.001$), while concentration of pigments of crustose coralline algae at 2 m and 10 m depths were not significantly affected by depth (F$_{1,30}$ = 0.022, $P > 0.05$). Chlorophyll a concentration (0.238 ± 0.044 mg. gWW$^{-1}$) of Arthrocardia sp. were significantly higher than that of crustose coralline algae (0.085± 0.016 mg. gWW$^{-1}$) at the same stratum (Tukey HSD, $P < 0.001$). Chlorophyll a concentration of crustose coralline algae was not significantly affected by depth (Figure 5. 10 a). No considerable difference was observed in phycoerythrin and phycocyanin concentration between two different growth forms of coralline algae at 2 m depth and between two depths (2 m vs. 10 m) of crustose coralline algae (Tukey HSD, $P > 0.05$) (Figure 5. 10b and Figure 5. 10c).

5.3.3. Fitted parameters P$_{max}$, α, E$_k$, E$_c$, R$_d$ for photosynthesis versus irradiance of coralline algae standardised to wet weight, chlorophyll a and surface area

Fitted parameters including P$_{max}$, α, E$_k$, E$_c$, R$_d$ for photosynthesis versus irradiance curves standardised to wet-weight, dry-weight and surface area of coralline algae are presented in Table 5.1. The maximum photosynthetic rate of Arthrocardia sp. standardised to wet-weight or chlorophyll a content was significantly higher than that of crustose coralline algae at 2 m depth (Student’s t-Test, df=5.99, $P<0.001$ and df=8.99, $P=0.024$, respectively). The significant differences in P$_{max}$ between these two coralline algae were not detected to be
statistically significant when this parameter was standardised to surface area (Table 5.1). Of all the remaining PE parameters, there were no significant effects of either growth form or depth stratum on these parameters between the investigated algae regardless of normalising methods utilized (wet weight, chlorophyll a or surface area). The saturation $E_s$ points and compensation $E_c$ points ($\mu$mol photons m$^{-2}$ s$^{-1}$) of all investigated coralline algae were not affected by the units used to standardise the photosynthetic rate.
Figure 5.3. Photosynthesis versus irradiance curves for *Arthrocardia* sp. at 2 m depth at 12°C. The dots indicate the pooled data and 95 % confidence interval of the data (n=6) and the model lines indicate the expected value. The shaded region indicates the 95 % confidence interval of the model.

Figure 5.4. Photosynthesis versus irradiance curves for crustose coralline algae in the order Hapalidiales at 2 m depth at 12°C. The dots indicate the pooled data and 95 % confidence interval of the data (n=6) and the model lines indicate the expected value. The shaded region indicates the 95 % confidence interval of the model.
Figure 5.5. Photosynthesis *versus* irradiance curve for crustose coralline algae in the order Hapalidiales at 10 m depth at 12°C. The dots indicate the pooled data and 95% confidence interval of the data (n=6) and the model lines indicate the expected value. The shaded region indicates the 95% confidence interval of the model.

Figure 5.6. Maximal oxygen production rate of coralline algae derived from the PE curves (mean ± SE) (n =6). Letters denote significant differences between bars (Student’s t-Test, $P < 0.05$, lowercase letters for different species/group, uppercase letters for different depth).
Figure 5.7. Initial slope $\alpha$ derived from the PE curves of coralline algae (mean ± SE) ($n =$6). Letters denote significant differences between bars (Student’s t-Test, $P < 0.05$, lowercase letters for different species/group, uppercase letters for different depth).

Figure 5.8. Saturation irradiance of coralline algae derived from the PE curves (mean ± SE) ($n =$6). Letters denote significant differences between bars (Student’s t-Test, $P < 0.05$, lowercase letters for different species/group, uppercase letters for different depth).
Figure 5.9. Compensation irradiance of coralline algae derived from the PE curves (mean ± SE) (n =6). Letters denote significant differences between bars (Student’s t-Test, $P < 0.05$, lowercase letters for different species/group, uppercase letters for different depth).
Figure 5.10. Pigment concentration (mg. gWW\(^{-1}\)) of coralline algae in photosynthesis *versus* irradiance experiments (mean ± SE) \((n = 6)\). (a) Chlorophyll \(a\), (b) Phycoerythrin, and (c) Phycocyanin. Letters denote significant differences between bars (Tukey HSD, \(P < 0.05\), lowercase letters for different species/group, uppercase letters for different depth).
Table 5.1. Fitted parameters including $P_{\text{max}}$, $\alpha$, $E_k$, $E_c$, $R_d$ for photosynthesis versus irradiance curves (n=6) standardized to wet-weight, chlorophyll $a$ and surface area of coralline algae and the result of a Student’s t-Test between *Arthrocardia* sp. and crustose coralline algae at the same 2 m and between depths (2 m vs. 10 m).

<table>
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<tr>
<th>Parameters</th>
<th>Units</th>
<th>Arthrocardia sp.</th>
<th>Hapalidiales – 2 m</th>
<th>Hapalidiales – 10 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{\text{max}}$</td>
<td>$\mu$mol O$_2$ gWW$^{-1}$ h$^{-1}$</td>
<td>11.80 ± 1.33*</td>
<td>2.24 ± 0.42</td>
<td>1.70 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>$\mu$mol O$_2$ mgChla$^{-1}$ h$^{-1}$</td>
<td>49.93 ± 6.16*</td>
<td>27.82 ± 5.30</td>
<td>20.25 ± 2.85</td>
</tr>
<tr>
<td></td>
<td>$\mu$mol O$_2$ cm$^{-2}$ h$^{-1}$</td>
<td>0.88 ± 0.11</td>
<td>0.66 ± 0.12</td>
<td>0.48 ± 0.13</td>
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<tr>
<td>$R_d$</td>
<td>$\mu$mol O$_2$ gWW$^{-1}$ h$^{-1}$</td>
<td>0.91 ± 0.66</td>
<td>0.18 ± 0.24</td>
<td>0.27 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>$\mu$mol O$_2$ mgChla$^{-1}$ h$^{-1}$</td>
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<td>2.83 ± 3.23</td>
<td>2.13 ± 2.49</td>
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<tr>
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<td>$\mu$mol O$_2$ cm$^{-2}$ h$^{-1}$</td>
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<td>0.07 ± 0.09</td>
</tr>
<tr>
<td>$A$</td>
<td>($\mu$mol photons m$^{-2}$ s$^{-1}$)$^{-1}$</td>
<td>0.26 ± 0.08</td>
<td>0.10 ± 0.02</td>
<td>0.06 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>$\mu$mol O$_2$ mgChla$^{-1}$ h$^{-1}$ ($\mu$mol photons m$^{-2}$ s$^{-1}$)$^{-1}$</td>
<td>1.12 ± 0.37</td>
<td>1.64 ± 0.48</td>
<td>0.77 ± 0.31</td>
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<td></td>
<td>$\mu$mol O$_2$ cm$^{-2}$ h$^{-1}$ ($\mu$mol photons m$^{-2}$ s$^{-1}$)$^{-1}$</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.02 ± 0.01</td>
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<tr>
<td>$E_k$</td>
<td>$\mu$mol photons m$^{-2}$ s$^{-1}$</td>
<td>60.01 ± 11.92</td>
<td>36.43 ± 19.24</td>
<td>86.00 ± 33.78</td>
</tr>
<tr>
<td>$E_c$</td>
<td>$\mu$mol photons m$^{-2}$ s$^{-1}$</td>
<td>21.24 ± 4.69</td>
<td>13.43 ± 8.06</td>
<td>32.20 ± 12.94</td>
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</table>
5.4. Discussion

Macroalgae can acclimate to changes in the environment of their habitat (Hanelt et al. 2003). The acclimation of macroalgae to variation in PAR environment during the course of the day tends to be illustrated by changes in their photosynthetic activity which is essential for the survival and growth of macroalgae (Borlongan et al. 2017 a, b). The capability of several macroalgal species to protect photosynthesis against high irradiation in relation with depth distribution was recorded (Hanelt et al. 1997). According to Hanelt et al. (1997), the reactions of algae to high photon fluence rate varied depending on different algal groups. In particular, algae distributing close to the water surface or in the intertidal were generally not severely stressed. Meanwhile, a decrease in photosynthetic activity during high light stress was observed on algae growing subtidal zone. By measuring fluorescence measurements, the reaction kinetics of photoinhibition and recovery of these algae were slower in compared with algae growing close to the water surface or in the intertidal. Photodamage rather than by dynamic photoinhibition was considered as a main factor causing the decrease in the photosynthetic activity and algae was suggested to possess a certain genetic adaptation to the natural light environment.

This study examined the photosynthetic response to different levels of irradiance of coralline algae that dominate in subtidal rocky reef ecosystems. The comparison of photosynthetic parameters delivered from PE curves of Arthrocardia sp. and crustose coralline algae at the same 2 m depth stratum illustrated the effect of growth form on photosynthesis of coralline algae. The data of this study indicated that the maximum photosynthetic rates for Arthrocardia sp. and crustose coralline algae at 2 m depth were significantly different. The net photosynthesis rate of Arthrocardia sp. normalized to dry mass was considerably higher than that of crustose coralline algae. Given identical experimental conditions, it suggests that the differences in $P_{\text{max}}$ between these coralline algae may be caused by differences in growth form and the structural make-up of the photosynthetic apparatus. Stewart and Carpenter (2003) demonstrated that photosynthetic rates of Dictyopteris undulata and Zonaria farlowii are independently influenced by algal morphology. These authors suggest that morphological plasticity of these species is probably a balance between maximizing photosynthetic ability and limiting susceptibility of their thallus to mechanical damage originating from variation in wave exposure. In addition, the highest photosynthetic rates were normally obtained by algal forms with high surface to
volume ratios, i.e., thin, sheet-like or finely branched species, while lower productivities were generally achieved for thicker, more structurally complex macroalgae and lowest rates were determined for the largely two-dimensional encrusting algae (see in detail Arnold and Murray 1980). The morphology of Arthrocardia sp. has more advanced characteristics in comparison with crustose coralline algae to reach a higher $P_{\text{max}}$ such as stiff, erect fronds and three-dimensional structure of Arthrocardia sp. compared to lies prostrate to the substrate of crustose coralline algae and two-dimensional structure. With three-dimensional structure, Arthrocardia sp. seems to receive more light compared to two-dimensional structure of crustose coralline algae.

The $P_{\text{max}}$ standardised to chlorophyll $a$ and wet weight of Arthrocardia sp. was also significantly higher than that of crustose coralline algae. Chlorophyll $a$ concentration of Arthrocardia sp. $(0.238 \pm 0.044 \text{ mg. gWW}^{-1})$ in this experiment were significantly higher than that of crustose CA $(0.085 \pm 0.016 \text{ mg. gWW}^{-1})$ at the same stratum. In the previous chapter (Chapter 3), the concentration of chlorophyll $a$ of Arthrocardia sp. $(0.252 \pm 0.018 \text{ mg. gWW}^{-1})$ was also significantly higher than crustose coralline algae $(0.099 \pm 0.022 \text{ mg. gWW}^{-1})$ at 2 m depth over the 18 months investigated. Other main pigment concentrations (phycoerythrin and phycocyanin) of Arthrocardia sp. from the field were significantly higher than that of crustose coralline algae at the same stratum. Therefore, the concentration of pigment contents probably explains the higher maximal net photosynthetic rate of Arthrocardia sp. compared to that of crustose coralline algae. Lüder et al. (2001) revealed that increasing pigment concentration increased the number and/or size of light-harvesting antennae and reaction centres to trap more light energy to enhance photosynthesis capacity and advance growth. A positive correlation between pigments and maximal electron transport rate ($ETR_{\text{max}}$) was found in red macroalgae Palmaria decipiens which showed seasonal maximum of photosynthetic capacity followed by maximal concentration of all pigments (Lüder et al. 2001). It should be noted that concentration of chlorophyll $a$ and phycobiliproteins of crustose coralline algae at 2 and 10 m depths were not significantly different in the present experiment and this result is consistent with finding from chapter 3. This is probably one of reasons why there was not significantly different between the net photosynthesis rate of crustose coralline algae at 2 and 10 m depth in this study, even though daily dose at 2 m was significant higher than that at 10 m (see detail in chapter 2).
In terms of photosynthetic efficiency $\alpha$, steeper slopes indicate relatively higher photosynthetic performance, while more gradual slopes indicate lower activity (Arnold and Murray 1980). The $\alpha$ of *Arthrocardia* sp. was not significantly higher than crustose coralline algae. Similarly, there were no significant differences in the photosynthetic efficiency $\alpha$ between these coralline algae when this parameter was normalized to wet-weight or chlorophyll $a$ concentration. Henley (1993) suggested that different $P_{\text{max}}$ with unchanged $\alpha$ may result from temperature change or the alteration of biological processes (endogenous rhythms). The temperature in this study was controlled at 12°C for all the PE experiments of coralline algae. Therefore, the biological alteration of these coralline algae probably led to the difference in photosynthetic response to irradiance. In macroalgae, circadian rhythms have been observed in several green, brown and red algae species (Mishkind et al. 1979, Schmid and Dring 1992, Schmid et al. 1992, Granbom et al. 2001). These rhythms are related to vegetative growth and/or cell division (Lüning 2001) or of photosynthesis (Waaland and Cleland 1972, Lüning 2001). Recently, Jacobsen et al. (2003) reported circadian control of steady-state transcript abundance occurs in *Kappaphycus alvareii* (Rhodophyta). In particular, transcript levels of two plastid-encoded operons, one for phycoerythrin $\alpha$ and $\beta$ subunits and another for ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco) large and small subunits exhibited diurnal regulation under light: dark cycles with maximum transcript abundance during early daytime to midday. Under constant illumination condition, up to three cycles was detected for circadian oscillations of steady-state mRNA abundance and this showing evidence of diurnal and circadian regulation (Jacobsen et al. 2003). Similarly, this physiological rhythm was found to correlate with changes at the level of gene transcription (phycoerythrin $\alpha$ and $\beta$ and Rubisco subunits) in *Grateloupia turuturu* (Goulard et al. 2004). Therefore, in order to clarify my assumption endogenous rhythms can affect relationship between photosynthetic parameters in coralline algae, a study focusing on the expression of gene encoding for photosynthetic parameters should be done in future.

The saturation $E_k$ points and compensation $E_c$ ($\mu$mol photons m$^{-2}$ s$^{-1}$) for photosynthesis are not affected by the units used to standardise the photosynthetic rate and these parameters are useful for comparing minimum light requirement and photoacclimation status both within and between species/group (Henley 1993, Pritchard 2011). $E_k$ indicates the minimum light intensity necessary to produce saturated photosynthesis (Talling 1957, Coutinho and Zingmark 1987). The minimum light intensity required to saturate
photosynthesis was less than 100 µmol photons m\(^{-2}\) s\(^{-1}\). These \(E_k\) values are comparable with those (ranging from 11 to 100 µmol photons m\(^{-2}\) s\(^{-1}\)) of macroalgae in polar regions (Gómez et al. 2009). The \(E_k\) of intertidal and deep-sublittoral algal species were 400-600 µmol photons m\(^{-2}\) s\(^{-1}\) (ca. 20% of full sun) and less than 100 µmol photons m\(^{-2}\) s\(^{-1}\), respectively, while the value of the upper and mid-sublittoral species were 150-250 µmol photons m\(^{-2}\) s\(^{-1}\) (see Hurd et al. 2014). Using these criteria, these coralline algae could be characterized as being between deep sublittoral group and shade adapted.

There are many studies showing effects of vertical distribution on the \(E_k\) of macroalgae. For example, Gómez et al. (2004) indicated that saturation irradiance of 18 macroalgae species from southern Chile varied from 50 to 431 µmol photons m\(^{-2}\) s\(^{-1}\) depending on vertical distribution of the algae. Particularly, the macroalgae growing in the upper parts of the shore tended to have a higher \(E_k\) value than algae located in mid- and infralittoral zones. Similarly, Borlongan et al. (2017 b) found that the red alga *Solieria pacifica* in the upper sublittoral zone (5 m depth) had a higher \(E_k\) (131 µmol photons m\(^{-2}\) s\(^{-1}\)) than those of the deeper sublittoral zone (35 m depth) (15 µmol photons m\(^{-2}\) s\(^{-1}\)) in Kagoshima, Japan. These \(E_k\) values were not statically significant different between these coralline algae and between depths. All \(E_k\) values showed a shade adapted organism with low saturation irradiance (\(E_k\) values are less than 100 µmol photons m\(^{-2}\) s\(^{-1}\)). Hurd et al. (2014) indicated that the photosynthetic performance for example saturating irradiance \(E_k\) show correlation with habitat. However, it is strict to predict the depth distribution of these crustose coralline algae in this study. The significant difference of \(E_k\) parameter of crustose coralline algae at 2 m and 10 m were not observed in the present study although a significant lower light dose was observed at 10 m depth compared to 2 m depth (Chapter 2). Desmond et al. (2017) revealed that greater efficiency (\(\alpha\)) occurred at lower photo flux density in many macroalgae species as a compensatory effect. It is possible that crustose coralline algae at 10 m depth may have a strategy of waiting for clear water and high light at 10 m depth rather than acclimating photosynthesis to low light. On the other hand, there could be enough periods of high light making increased efficiency less important. In addition, it is not possible to rule out that energetic constraints at depth may affect the ability of deeper crustose coralline algae to optimize harvesting structures (see Raven and Hurd 2012). Taken together, the lack of difference in the \(E_k\) of crustose coralline algae between 2 and 10 m depth in the present study is reasonable. It is interesting that basing on higher \(E_k\) value of the Hapalidiales
at 10 m depth compared to the Hapalidiales at 2 m depth, that indicated that the Hapalidiales at 10 m depth can distribute shallower than the Hapalidiales at 2 m depth. It is recommended that additional studies should be conducted on crustose coralline algae distributed over a deeper vertical distribution to evaluate the effects of depth on $E_c$ of crustose coralline algae.

In relation to the levels of compensation irradiance, there were no significant differences in $E_c$ between *Arthrocardia* sp. and crustose coralline algae and between crustose coralline algae at different depths. The compensation point $E_c$ of coralline algae ranged from 13.43 to 32.20 µmol photons m$^{-2}$ s$^{-1}$ which was comparable with other rhodophyte macroalgae from King George Island (Antarctica) that ranged from 4.8 to 21.2 µmol photons m$^{-2}$ s$^{-1}$ (Weykam et al. 1996). At the same 10 m depth stratum, $E_c$ of crustose coralline algae at 10 m depth was higher than that of *Anotrichium crinitum* (1.49-2.25 µmol photons m$^{-2}$ s$^{-1}$) (Pritchard et al. 2013). However, comparisons of $E_c$ values between studies have to be made with caution due to the variation of methodologies used to calculate these parameters (Arnold and Murray 1980, Coutinho and Zingmark 1987).

Based on the results of the oxygenic photosynthesis experiments, no evidence of photo-inhibition was detected in all investigated coralline algae after two hours of exposure to a wide variation of light intensities ranging from 0 to 1800 µmol photons m$^{-2}$ s$^{-1}$. Photoinhibition has been reported for many species of macroalgae in the laboratory and in the field where they were exposed at high PAR with over 1400 µmol photons m$^{-2}$ s$^{-1}$ (King and Schramm 1976, Arnold and Murray 1980, Hanelt et al. 1993, Figueroa et al. 1997, Häder et al. 1998). For example, the photosynthesis of benthic algal species including *Chaetomorpha linum* (saxicolous filaments), *Ulva intestinalis* (thin, tubular parenchymatous), *U. lobata* and *U. rigida* (thin, sheet-like thalli) collected from a rocky littoral shoreline were inhibited as they were exposed to full sunlight (1405 to 1956 µmol photons m$^{-2}$ s$^{-1}$) in the laboratory (Arnold and Murray 1980). According to Hanelt et al. (1993), macroalgae growing in the sublittoral are more sensitive with strong light than upper eulittoral species. These authors demonstrated that *Delesseria sanguinea* (sublittoral species) was totally photoinhibited (100%) when it was exposed to 2500 µmol photons m$^{-2}$ s$^{-1}$ of white light for one hour. Meanwhile, at the same light intensity and period of exposure, the percentage of photoinhibition observed in *Pyropia* sp. (upper eulittoral species) was 75%. In contrast, many Antarctic macroalgae species including red (e.g. *Gigartina*
skottsbergii, Pyropia endiviifolia), brown (e.g. Desmarestia anceps, Halopteris obovata),
green (e.g. Enteromorpha bulbosa, Monostroma hariotii) algae did not show evidence of
photoinhibition in experiments related to irradiance effect on oxygenic photosynthesis with
high PAR (1000 µmol photons m\(^{-2}\) s\(^{-1}\)) (Weykam et al. 1996). Similarly, evidence of
photoinhibition was not found in Eucheuma denticulatum, Kappaphycus alvarezii and red
alga Solieria pacifica collected from two different depths (5 m and 35 m) after one hour of
exposure to 1000 µmol photons m\(^{-2}\) s\(^{-1}\) (Borlongan et al. 2017 a, b). Therefore, based on the
diversity of photosynthetic responses to the high PAR of macroalgae, is not surprising that
there were no evidences of photo-inhibition found in Arthrocardia sp. and crustose coralline
algae at high PAR in the present study. The lack of inhibition could also be something that
suggests the importance of periods of high light for these species: they wait for high light
periods and are able to utilize it without being inhibited. It is interesting that Arthrocardia
sp. was found in tidepools of Fiordland – New Zealand (personal observation, Nguyen, H.
T. T) which suggests this species has probably a high level of tolerance to periods of high
light. Bleaching of coralline algae was also observed in intertidal zone where they were
exposed to full sunlight for a long time in summer. It is recommended that additional long-
term studies should be conducted to examine photosynthetic responses of coralline algae,
especially under a wider range of light regimes and higher irradiance levels to determine the
importance of utilisation of short periods of high light in low light habitats.

In conclusion, the photosynthetic characteristics of coralline algae showed a shade-
adapted organism with low saturation irradiance. There was a difference in the maximal
photosynthesis rate normalized to dry mass, wet weight and chlorophyll \(a\) between coralline
algae at the same depth, which is likely caused by their different growth form. Meanwhile,
the differences in depth distribution (2 m and 10 m) may not have significant effects on the
photosynthetic parameters of crustose coralline algae. The present study also indicated that
the photosynthesis of shallow subtidal coralline algae is relatively tolerant of short periods
of high irradiance. Linking photosynthesis to growth of coralline algae under variable light
especially short periods of higher light intensities (e.g. Desmond et al. 2017) are
recommended for further studies.
Chapter 6

General discussion
6.1. Study rationale

Coralline algae are ecosystem engineers with a broad distribution, found in many different environmental conditions (Nelson 2009, Hepburn et al. 2011, McCoy and Kamenos 2015). The impacts of environmental changes on the physiology of coralline algae are considered to be related to the diversity and structure of marine ecological system (Nelson 2009, McCoy and Kamenos 2015). Articulated (geniculate form) and crustose (non-geniculate form) coralline algae are important components of intertidal and subtidal habitats (Harvey et al. 2005, Shears and Babcock 2007, Farr et al. 2009, Hepburn et al. 2011, Noisette et al. 2013, Guenther and Martone 2014, McCoy and Kamenos 2015), but data on the functional physiology of these two groups is scarce.

In order to contribute a global understanding about nutrient uptake and photosynthetic responses of coralline algae in the context of marine environmental changes, this research investigated the annual variation of environmental conditions including light, temperature and nutrients in the shallow subtidal habitat (2 m and 10 m strata) in southern New Zealand, where coralline algae are a dominant component of rock substrata (Chapter 2). This study investigated the seasonality of nutrient and pigment concentrations of Arthrocardia sp. at 2 m depth and a group of morphologically indistinguishable crustose coralline algae in the order Hapalidiales at 2 and 10 m depths (Chapter 3). The nutrient (ammonium, nitrate, phosphate) uptake kinetics of Arthrocardia sp. and crustose coralline algae distributed at 2 m depth stratum were determined in Chapter 4. Photosynthesis versus irradiance relationships of coralline algae were studied by comparing photosynthetic parameters delivered from PE curves between Arthrocardia sp. and crustose coralline algae at the same 2 m depth stratum, and between crustose coralline algae at 2 m and 10 m depths (Chapter 5). Although the present study was conducted in southern New Zealand, findings generated from this study are relevant to other temperate rocky reef system and coralline algae throughout the world. The main aim of this chapter is to integrate main findings from previous chapters and to provide the implications and strategies for coralline algae of coastal ecosystem today and in context of ocean warming.
6.2. Summary of findings

Light, temperature and ambient nutrient concentration in the shallow subtidal zone dominated by coralline algae under kelp forest significantly varied depending on depth and season. This recording is consistent with previous studies in the same area (Hepburn et al. 2011, Pritchard et al. 2013, 2015, Desmond et al. 2015). Underwater light and temperature showed seasonal patterns with maximal values in summer and minimal values in winter. The highest temperatures over 21-month studies were recorded in December 2017 when a marine heatwave occurred in New Zealand (Sailinger et al. 2019). In the present study, there are no data collected to evaluate the potential negative effects on heatwave on the ecophysiology of the investigated coralline algae due to beyond the scope of this study. However, an increase in seawater temperature caused by heatwave had an unexpected impact on community of crustose coralline algae with high mortality in Western Australia in 2010 – 2011 (Short et al. 2015). It is noticed that the heatwave was predicted to occur more frequently in the future (Law et al. 2017, Shears and Bowen 2017, Alexander et al. 2018, Oliver et al. 2018). Therefore, it is necessary to conduct research on the risk which coralline algae community in the Karitane have to face when the heatwave happen in future due to the important roles of coralline algae in ecosystem. As previous studies in the temperate regions, the ambient nitrate concentration seasonally varied with higher concentration in winter than in summer (Chopin et al. 1989, Fujita et al. 1989, Harrison and Hurd 2001, Phillips and Hurd 2003, Hepburn et al. 2007, Kregting et al. 2008, Pritchard et al. 2015). The well mixing between water layers in winter probably gave favourable conditions for nitrate delivery from deep water where nitrate is very high and is produced by bacteria (Hepburn, 2003). This led to high concentration of ambient nitrate in the winter compared to the summer. In relation to variation of the measured ambient environmental parameters across the depth strata, the light, temperature and nutrient concentration at 2 m depth stratum varied strongly while those at 10 m depth were more stable. These results are consistent with those of the previous studies (Chopin et al. 1989, Fujita et al. 1989, Brown et al. 1997, Harrison and Hurd 2001, Phillips and Hurd 2003, Hepburn et al. 2007, Kregting et al. 2008, Hepburn et al. 2011, Pritchard et al. 2013, Desmond et al. 2015, Pritchard et al. 2015, Rodgers and Shears 2016). The enormous variability in light measured over 10 m depth gradient, within and between days and seasons are characteristics that suggest that Arthrocardia sp. and crustose coralline
algae at 2 m depth may have to face with more challenging environment for photosynthetic organisms to inhabit.

Species/group coralline algae and season influenced nutrient and photosynthetic pigment concentration of coralline algae, whereas depth distribution (2 m vs. 10 m) seemed to be unaffected excepting soluble tissue ammonium, C%, chlorophyll d and chlorophyll c concentration. Data from chapter 3 showed that C% of coralline algae at 2 m depth was significantly higher than that of 10 m depth. Coralline algae can storage carbohydrates produced in photosynthesis process via production of starch grains in perithallial cell compartments (Freiwald and Henrich 1994). A higher light intensity at 2 m depth compared to 10 m depth can lead to more efficient photosynthesis in algae at 2 m depth. This, in turn, probably lead to more carbohydrate stored in tissue of *Arthrocardia* sp. and crustose coralline algae at 2 m depth in comparison with 10 m depth. Similarly, soluble tissue ammonium of coralline algae at 2 m depth were higher than 10 m depth. Ammonium is often available for uptake by macroalgae at constant but relatively low concentrations via local regeneration by fish and invertebrates (Taylor and Rees 1998, Davy et al. 2002, Hepburn and Hurd 2005). It is noticed that the sampling area are modified by anthropogenic activities (Desmond et al. 2015) which can cause a high ammonium in the water column. In addition, according to Pritchard et al. (2015), coralline algae may increase the reliance on ammonium as a nitrogen source as light availability. Taken together, the difference in light intensity between 2 and 10 m depth could be a factor causing the differences in the soluble tissue ammonium concentration between two depths investigated. *Arthrocardia* sp (upright branch thallus) had higher accessory pigment concentration including phycobiliproteins, chlorophyll *a* compared to crustose coralline algae (flattened thallus) at the same stratum. A similar result was found in previous study. In particular, *Corallina elongata*, an articulated coralline alga has compared to *Lithophyllum incrustans*, a crustose coralline alga (Noisette *et al.* 2013). It is noteworthy that this is the first report on the quantity of chlorophyll *c* in pigment extraction of coralline algae (Chapter 3). The existence of small quantity of chlorophyll *c* had been reported in red algae (Sorby 1873). Recently, applying molecular techniques to analyze the light harvesting complex gene sequence of red alga indicated that chlorophyll *c* in existence (Green and Durnford 1996, Tan *et al.* 1997, Bachvaroff *et al.* 2005). Thus, the findings related to chlorophyll *c* in these coralline algae are reasonable.
Coralline algae are able to take up nitrogen and phosphorus in seawater. Ammonium was a preferred nitrogen source for both coralline alga groups compared to nitrate in both summer and winter, whereas phosphate can be taken up by these coralline algae regardless of season. The preferred ammonium uptake of coralline algae in the present study is similar to *Anotrichium crinitum*, which is a light-limited red alga slowly growing without high nitrogen demands (Pritchard et al. 2015). The differences in nitrogen and phosphorus uptake rates between these coralline algae are speculated to be caused by the differences in their growth form. The advantages in morphological structure of *Arthrocardia* sp. with stiff and erect fronds and three-dimensional form possibly exhibited a higher nutrient uptake ability in comparison with the crustose coralline algae which lies prostrate to the substrate and two-dimensional form (Chapter 4). However, this assumption needs to be confirmed by measuring the surface area to volume ratio of these species which have not been done in the present study. A further study should be conducted to clarify this issue.

The photosynthetic characteristics of coralline algae showed a shade-adapted organism with low saturation irradiance (all *E*<sub>k</sub> values are less than 100 µmol photons m<sup>-2</sup> s<sup>-1</sup>). The net photosynthesis rate of *Arthrocardia* sp. was considerably higher than that of crustose coralline algae. Given identical experimental conditions, the differences in *P*<sub>max</sub> between these coralline algae may be caused by differences in growth form and the structural make-up of the photosynthetic apparatus. Many previous studies indicated that photosynthesis activity of macroalgae was shown to be associated with morphology (Gacia et al. 1996, Johansson and Snoeijs 2002, Stewart and Carpenter 2003). The depth (2 m and 10 m) did not seem to have significant effects on the photosynthetic parameters of crustose coralline algae (Chapter 5). It should be noted that concentration of chlorophyll *a* and phycobiliproteins of crustose coralline algae at 2 m depth in the present experiment were not considerably different compared to that of crustose coralline algae at 10 m depth and this result is consistent with finding from Chapter 3. This is probably one of reasons why the net photosynthesis rate of crustose coralline algae at 2 and 10 m depth was not significantly different in the present study. The photosynthesis of these shallow subtidal coralline algae in this present study is relatively tolerant of short periods of high irradiance. Linking photosynthesis to growth of coralline algae under variable light especially short periods of higher light intensities are recommended for further studies.
6.3. Implications and strategies for coastal ecosystem

Coralline algae have received attention as they are considered sensitive to changing ocean pH and useful tools for evaluating the impacts of ocean acidification on the structure and function of ecosystems (Noisette et al. 2013, Comeau et al. 2014, Cornwall et al. 2014, James et al. 2014, Roleda et al. 2015). Much research focuses on elevated CO₂ concentrations in seawater which results in the reduction of net calcification rate, bleaching of coralline algae (Noisette et al. 2013) and potentially negative competition between coralline algae and noncalcified or fleshy algae (Hepburn et al. 2011). Additionally, the increase of energetic costs of calcification likely inhibit biomineralization and new recruitment of coralline algae in the context of ocean acidification (Kuffner et al. 2008). However, Cornwall et al. (2014) suggested that water motion plays an important role in the formation of thick diffusion boundary layers to reduce the negative impacts of ocean acidification. Recently, Johnson and Carpenter (2018) demonstrated the growth of crustose coralline algae in culture conditions combined between high pCO₂ (ocean acidification) and nitrogen enrichment (eutrophication) compensated direct negative effects of near-future ocean acidification on the calcification and photo-physiology of coralline algae.

Eutrophication is considered one of the critical factors causing habitat change and the geographical and temporal expansion of some harmful algal blooms (Smayda, 1990, Anderson et al. 2002) which decrease the cover of coralline algae (Björk et al. 1995, Fabricius 2005). Nitrogen and phosphorus are the main concerns in eutrophication (Glibert et al. 2005, Conley et al. 2009). According to Glibert et al. (2005), nitrogen has received far more attention in comparison with phosphorus due to the global application of nitrogen in synthetic fertilizers and greater impacts of nitrogen in coastal waters. These authors indicated that the rate of nutrients sourcing from chemical fertilizers, atmosphere inputs and anthropogenic activity exported to coastal waters has increased dramatically in recent years. Nutrient enrichment (particularly phosphate) may not favor crustose coralline algae growth and limit their cover (Belliveau and Paul, 2002). The data from Chapter 4 in this study indicated that among nutrient forms and sources investigated, only the uptake of ammonium exhibited saturable kinetics in both Arthrocardia sp. and crustose coralline algae, whereas nitrate and phosphate uptake displayed increased linear uptake. These coralline algae have high affinity for ammonium as they likely use ammonium as a primary source of nitrogen to
sustain growth. Moreover, the low maximal nutrient uptake rates of coralline algae compared to fleshy macroalgae (Rees 2003) suggests that coralline algae are well adapted to low nutrient oligotrophic conditions.

The available information regarding the response of coralline algae to light and temperature in the context of climate change is currently limited. Climate change has led to rising temperatures, increasing lighting intensity and combinations of stress (Harley et al. 2012, Jueterbock et al. 2013) which threatens foundational species (Jueterbock et al. 2013), can relocate macroalgae habitats (Wernberg et al. 2011), and cause the tropicalization of temperate marine ecosystems (Wernberg et al. 2012, Vergés et al. 2014). The physiological responses of coralline algae are more negatively affected by elevated temperature compared to low pH (Vásquez-Elizondo and Enríquez, 2016). During approximate 1.5 years of study, the temperature at the studied area was recorded to increase seasonally, and in December 2017 there were a marine heatwave which was the warmest temperature (in summer 2017/2018) in New Zealand since 1953 (Salinger et al. 2019). These authors also observed an unusual change of marine ecology, with the absence of extensive canopies of the habitat-forming kelp *Macrocystis pyrifera* in southern New Zealand and the appearance of tropical and warm temperate fish species during this time period. The effect of the New Zealand marine heatwave in 2017/2018 on coralline algae beds has not been reported. Hydrolithoideae crustose coralline algae distributed in Western Australia experienced thermal stress following the marine heatwave in the austral summer of 2010–2011. This phenomenon caused the absence of normal seasonal patterns in growth rates and high rates of mortality of coralline algae during summer (Short et al. 2015). The European heatwave in summer 2003 recorded mass mortality in Northwestern Mediterranean rocky benthic communities and the serious bleaching of coralline algae (Schiaparelli et al. 2007, Garrabou et al. 2009). Taken together, it is possible that the physiological responses of these coralline algae could be changed by impacts from environment conditions, and a warming ocean may have a range of direct and indirect effects on coralline algae. Hence, research on physiological responses of coralline algae to variations of marine environment is necessary.
6.4. Future research

6.4.1. Quantifying the sources of environmental condition

The future surface ocean around New Zealand has been predicted to increase 2.5°C, whereas, surface pH and macronutrient may decrease by 0.335 (≈7.77) and 7.5–20% in 2100, respectively (Law et al. 2017). The duration and frequency of global average marine heatwave increased by 17% and 34% from 1925 to 2016 (Oliver et al. 2018). Shears and Bowen (2017) demonstrated the spatial and temporal complexity of warming trends in western boundary currents and widespread expectations of tropicalization in temperature regions under climate changes in future. A marine heatwave has just occurred in New Zealand region in summer 2017/2018 and it is predicted to have widespread effects on marine ecosystems (Salinger et al. 2019). Therefore, variations in environmental parameters need to be regularly investigated with a long-term strategy by setting up environmental monitoring systems at coastal regions. These data will be useful for analysing the correlation between environmental changes and its impacts on coastal ecosystems such as diversity of coralline algae community.

Although data on sea surface temperature has been collected and analysed for a long-term period in New Zealand (Law et al. 2017, Shears and Bowen 2017, Salinger et al. 2019), not much information about the temperature at deeper levels has been recorded. Future research on temperature should focus on the temperature measurement at different depths, temperature stratification with a long-term strategy.

The present study recorded light variation from April 2016 to December 2017 at surface, 2 m and 10 m depths. Seasonal variability of light at this research location were reported in previous years (i.e. in winter (April to May) and summer (October to December) in 2006 at 2 m, 5m and 10 m depths (Hepburn et al. 2011); in December 2009 to July 2010 at 10 m depths (Pritchard et at. 2013); in December 2012 to October 2013 at surface, 2 m and 10 m depths (Desmond et al. 2015)). Future research on light variability should be conducted to understand interactive effects of light, nutrients with the increasing sea surface temperature caused by ocean warming. The potential effects of interaction between temperature and light on macroalgae community should be investigated in future studies as a positive correlation between temperature and light was detected in Chapter 2. Factors
driving light attenuation in the water column, the changes in total photosynthetically active radiation and spectral composition of light also need to be studied further.

There are many factors driving concentrations of nutrients in coastal seawater such as vertical mixing and upwelling from the deep ocean (Dugdale and Goering 1967, Jackson and Williams 1985, Boyd and Hurd 2009), anthropogenic inputs (Herbert 1999, Glibert et al. 2005, Seitzinger et al. 2005, Den Haan et al. 2016), atmospheric dust deposition (see more detail in Boyd and Hurd, 2009) and excretion from aquatic animals and heterotrophic bacteria (Banse 1974, Herbert 1999, Hepburn and Hurd 2005). Among these factors, nutrients sourced from anthropogenic activities has increased dramatically (Seitzinger et al. 2005, Glibert et al. 2005, Conley et al. 2009). Future investigations on ambient nutrients should focus on the source of nutrients exported to coastal regions and correlation between these nutrient sources and ambient nutrient concentration in seawater. This work will be crucial important for planning to reduce the increase of anthropogenic sourced nutrients in shallow subtidal zone.

6.4.2. Investigating the ecology of coralline algae under changing ocean environment

A wide diversity in species composition of coralline algae has been documented (Harvey 2005, Farr et al. 2009, Nelson et al. 2015, Gabrielson et al. 2018) and their ecological services are well reviewed (Nelson 2009, McCoy and Kamenos 2015). At present, much attention has been paid to evaluate the impacts of ocean acidification on coralline algae in the structure and function of ecosystems (Noisette et al. 2013, Comeau et al. 2014, Cornwall et al. 2014, James et al. 2014, Roleda et al. 2015). The present study contributed a fundamental understanding about nutrient and photosynthesis physiology of an articulated coralline alga *Arthrocardia* sp., and a group of crustose coralline algae. Therefore, research on the ecophysiology of coralline algae is still needed in the future in the context of changes of oceanic environment. Especially, more species of coralline algae, belong to articulated coralline algae group in comparison with crustose coralline algae group to examine how morphology between two groups affect to nutrient and photosynthesis characteristics of coralline algae. The impact of summer 2017/2018 New Zealand heatwave to marine ecology was documented (Salinger et al. 2019). However, its impacts on coralline algae has not been reported yet, especially the percentage of the covering of coralline algae in the shallow
subtidal after summer New Zealand heatwave. Future investigation on diversity of growth forms of coralline algae at different depths and the proportion between the covering of coralline algae and other species macroalgae and invertebrates should be conducted to evaluate the impacts of summer New Zealand marine heatwave to coralline algae communities.

6.4.3. Ecophysiology of coralline algae in functioning of coastal ecosystem

6.4.3.1. Nutrient ecophysiology of coralline algae

The present study evaluated the uptake ability of coralline algae to single nutrient. The results from chapter 4 showed that ammonium is the preferred nitrogen source for coralline algae to uptake. The uptake ability of coralline algae to the combination of different nutrient types should be investigated. Understanding the preferred form of inorganic nitrogen and phosphorus and the optimal nutrient concentration for growth of coralline algae is still limited and it needs further research. Additionally, future work on potential nutrient sources for growth of coralline algae and the potential uptake of coralline algae of variable and multiple nutrient types should be conducted for longer time period instead of short time as in the present study. The main factor driving differences in nitrogen and phosphorus uptake rates and uptake kinetics between Arthrocardia sp. and a group of crustose coralline algae was predicted causing by the difference in morphology between these species/group. However, this study only observed the external morphology of these coralline algae. Therefore, the internal tissue structure of these coralline algae should be examined, and effects of functional morphology related to genetic variation on nutrient uptake of these coralline algae should be conducted in future.

6.4.3.2. Photosynthetic physiology of coralline algae

Studies on light-harvesting complex of coralline algae should be performed to explain why coralline algae can survive at very low light environment. The complexity of pigment contents of coralline algae was found in present study with the presence of chlorophyll c (Chapter 3). Therefore, these pigment contents need to be investigated in other coralline algae species. It is recommended that more diversity of growth forms of coralline algae and deeper depth distribution should be conducted to examine the potential
photosynthetic ability of coralline algae under a wider range of light regimes and higher irradiance levels. This information would allow more accurate evaluation the effects of diversity of different morphology and depth distribution to coralline algae community. The effects of morphological characters and depth distribution associated with light intensity on photosynthetic efficiency of coralline algae (the effective quantum yield ($\Phi_{PSII}$) could be explored further by measuring electron transport rate (ETR) with a Pulse Amplitude Modulation-PAM system in the field and in the laboratory (e.g. Wilson et al. 2004, Burdett et al. 2012, Terada et al. 2016).

6.4.3.3. The physiological response of temperate coralline communities in functioning of coastal ecosystem today and in a warmer ocean

Future climate change effects on the surface ocean around New Zealand could be considerable (Law et al. 2017). It is important to build up plans for management of important coastal habitat and resources within southern New Zealand addressing the negative influence of climate change. Future investigations on the physiology of coralline algae under extreme climate such as extremely high temperature or light or nutrient concentration and combinations of these multiple stressors should be conducted in order to predict potential effects of climate changes on communities of coralline algae and the structure of coastal ecosystem. Coralline algae are used as a model species to evaluate the impacts of climate change on marine species. This database could be then used to develop specific coralline algae protection plan.

Coralline algae are a foundational component of kelp forests and play an important ecological role in subtidal rocky habitats (Shears and Babcock 2007, Nelson 2009, Hepburn et al. 2011, McCoy and Kamenos 2015). Environmental change is strongly impacting on habitat forming kelp species (e.g. Schiel et al. 2004, Schiel and Foster 2006, Johnson et al. 2011, Wernberg et al. 2012) with a range of flow on effects that will undoubtably influence coralline algae recruitment, growth and competitive interactions. For example, loss of kelp forest canopies and reduced light attenuation and patchiness in the light environment could have strong flow on effects for coralline algal communities (e.g. Reed and Foster 1984). Our understanding of the influence of environmental change and stressors on kelp ecosystem engineers should be linked to studies that explore how loss of
kelp and other associated change impacts the structure and species components of coralline algal communities. Indirect effects could have fundamental impacts on services provided by corallines, such as food and habitat provision, larval settlement surfaces, grazing areas for numerous fish and invertebrates, and in supporting reef frameworks (Bosence 1983, Harrold and Reed 1985, Gherardi and Bosence 1999, Huggett et al. 2006). In kelp forest communities, coralline algae are considered a foundation species and also autogenic ecosystem engineers (Steneck et al. 2002, Nelson 2009). The foundational role of corallines and their apparent sensitivity to environmental stressors highlights the importance of understanding how these poorly understood groups function physiologically and how and if they can acclimate and adapt to a changing ocean.
References


Ecology, 92(2–3), 283–301.


Gao, X., Endo, H., Nagaki, M., and Agatsuma, Y. (2017). Interactive effects of nutrient availability and temperature on growth and survival of different size classes of


Hepburn, C. D., Pritchard, D. W., Cornwall, C. E., Mcleod, R. J., Beardall, J., Raven, J. A.,


mass outdoor cultures. *Botanica Marina*, 22(8), 529–538.


pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*. *Polar Biology*, 24(8), 598–603.


McGlathery, K. J. and Pedersen, M. F (1999). The effect of growth irradiance on the


Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V,


Pritchard, D W. (2011). The ecophysiology of the deep-water macroalga *Anotrichium*


A.1 Appendix 1

Figure A.1.1. Calibration of natural log-transformed data from LiCor and Odyssey data loggers over thirteen individual campaigns at different sites and under different underwater light conditions.
Figure A.1.2. Calibration of natural log-transformed data from LiCor and Odyssey data loggers over seven individual campaigns at different sites and under different light conditions in air.

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\text{PAR} = \exp\left(\frac{\log(ODY/600)+3.216}{0.944}\right)
\]
Figure A.2.1. *Arthrocardia* sp. (a, c, e) and crustose coralline algae in the order Hapalidiales (b, d, f) in the field and in the laboratory.
Table A 2.1. The names of coralline algae in this study in GenBank (see detail in Twist, 2019)

<table>
<thead>
<tr>
<th>Alternate name</th>
<th>Algae Number</th>
<th>Genbank</th>
<th>Name in PhD Thesis (Twist, 2019)</th>
<th>Genus</th>
<th>Order</th>
<th>Species</th>
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<td>20/07/2016 Shallow</td>
<td>NZC5268</td>
<td>MK413473</td>
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<td>MK413470</td>
<td>Arthrocardia_sp3</td>
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<td>#N/A</td>
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Figure A.2.2. Location of Odyssey and HOBO loggers in underwater in the field (a). The divers changed periodically the underwater loggers (b). Location of Odyssey and HOBO loggers at shore (c). Underwater calibration of Odyssey loggers (d).
Figure A.2.3. Extraction of pigments of coralline algae (phycobiliproteins and chlorophylls) (a). The colourless pellets of coralline algae after pigment extraction (b). Extraction of soluble tissue nutrients of articulated coralline algae (c). Preparing ground coralline algae samples for tissue organic carbon and nitrogen analysis.
Figure A.2.4. Nutrient uptake kinetics experiment of coralline algae (a). Seawater samples from time-course nutrient deplete experiments (b) Analysing nutrient seawater samples by a QuickChem 8500 Series 2 Automated Ion Analyser (Lachat Instruments, Hach Company, USA).
Figure A.2.5. Photosynthesis *versus* irradiance experiment (a). *Arthrocardia* sp. (b) and crustose coralline algae in the order Hapalidiales (c) in the P vs. E experiment.