Predicting the impact of future climate on ecologically important macroalgae

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Predicting the impact of future climate change on ecologically important macroalgae

Master of Science (Biological Sciences)
Thesis Prepared by
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Abstract

Macroalgae play an important role in coastal reef systems and are often referred to as ecosystem engineers. They serve as primary producers, supporting a diverse range of organisms, and are a sink for atmospheric CO₂. Water acidification and ocean warming caused by anthropogenic activities are affecting many marine flora and fauna, potentially impacting the physical and chemical performance of macroalgae and the consumption rates of associated herbivores. Many studies have focused on ocean acidification or ocean warming individually but there is an overall lack of research investigating the combined effects and the ensuing repercussions on consumer-prey relationships.

Three species of ecologically important macroalgae (*Ecklonia radiata*, *Sargassum linearifolium* and *Laurencia brongniartii*) were subjected to elevated temperature and increased pCO₂ conditions and observed for alterations in algae physiology and chemical production, in terms of growth, toughness, bleaching, density, blade mass, quantum efficiency yields, carbon: nitrogen (C:N) ratios and phenolic content. A series of feeding assays were conducted with two abundant marine herbivores, an amphipod (*Allorchestes compressa*) and a gastropod (Family Trochidae), to examine the indirect impact of climatic stressors on the palatability of the algae.

The overall impact of climate change on macroalgae was species-specific, with each algal species having distinct physical and chemical responses to the changes in environmental conditions. *S. linearifolium* functioned poorly at high temperatures, exhibiting high levels of bleaching, lower quantum efficiency yields and, when ground, was less palatable to Trochidae. Overall, *E. radiata* was less affected by the projected climate change conditions, with only the C:N ratios being impacted in the combined increased temperature and increased pCO₂ treatment. The palatability of *E. radiata* was also altered with the gastropod consuming a greater amount of the ground algae exposed to the combined temperature and pCO₂ conditions. Finally, *L. brongniartii* was impacted in all the performance tests measured across all treatments, showing increases in levels of bleaching, density, and C:N ratios and decreases in growth, quantum efficiency yields, blade toughness and total phenolics. Uniquely, this study shows the vulnerability of
understory red algal species, such as *L. brongniartii* to changes in climatic conditions. Surprisingly, these alterations in algal performance for *L. brongniartii* did not change the consumption rates of either herbivore.

This study indicates that extreme climatic events have the potential to affect the performance and health of three abundant habitat-forming temperate algal species. The loss in health and performance seen in each species could have key implications for benthic communities in temperate Australian reefs, through processes such as changes in herbivory rates, competition with invasive species or simply through algal death. A possible implication of these stressors is the facilitation of range shifts along the west coast which could lead to the retraction of distribution ranges for many temperate Australian species.
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1. Introduction

1.1 Ecological importance of habitat forming macroalgae

Plants form the foundation of most ecosystems by providing structure and habitat for fauna (Wiens 1973), playing a key role in nutrient cycling (Bernot et al. 2009) and establishing the basis of food webs for both herbivores and omnivores (Coll & Guershon 2002). Examples include grasslands, forests and savannahs, all of which support a vast number of bird (Wiens 1973), mammal (Grant et al. 1982) and insect species (Steffan-Dewenter & Tscharntke 2002). Within these landscapes plants play an important role in the cycling of nutrients. For example, in terrestrial wetland systems plants are often exposed to high nutrient inputs from riverine, terrestrial and groundwater sources (Bernot et al. 2009) where they respond by rapid growth. The increase in plant material leads to an increase in herbivory and in degradable litter, facilitating the nutrient cycling processes (Hobbie 1992). In addition, plants are integrally linked to the carbon cycle through photosynthesis whereby carbon is withdrawn from the atmosphere and fixed to produce carbohydrates (Hopkins & Hüner 2008).

In marine systems macroalgae and seagrasses are the most ecologically important primary producers (Harley et al. 2012; Yamasaki et al. 2014). They are the foundation of the marine food web (Koch et al. 2013), providing a principal food source and a three-dimensional habitat for many marine taxa, and bacteria (Egan et al. 2013). Macroalgae also play an important role in nutrient retention and cycling, and CO2 storage (Koch et al. 2013) and are culturally and economically important through the provision of food and medicines (Harley et al. 2012).

Macroalgae are under constant survival pressure by biotic factors such as grazing by herbivores, viruses, bacteria and other microorganisms as well as abiotic factors such as competition for light availability, substrate accessibility for settlement and pressure from water currents and drag (La Barre et al. 2004). These stressors seldom operate alone and algae could potentially face the cumulative addition of climate change induced stresses such as increases in water temperature and acidification. Understanding the effect that climate change conditions may have, and the response by algae in terms of defences to combat these complex interactions, is vital (Campbell et
This knowledge will underpin implementation of management and conservation practices to assist with the survival of macroalgae themselves as well as the diverse and economically and environmentally important habitats they support (Connell & Russell 2010; Campbell et al. 2011).

### 1.2 The role of herbivores

Herbivores in marine and terrestrial environments can have a substantial impact on the growth, survival and reproductive success of plants (Steinberg & van Altena 1992). Herbivore abundance can affect the biomass of individual plants, the diversity of plant species and the number of individuals in higher trophic levels (Pearse et al. 2013). Levels of herbivory, both terrestrial and aquatic, are often related to the nutritional value of the plant material and the number of herbivores present on the plant (Cebrian et al. 2009). Experiments that have compared the abundance of primary producers with and without herbivores have shown that grazing causes an average of 68% reduction in the biomass of benthic primary producers (Poore et al. 2012). Additionally, in marine systems where herbivores are particularly abundant, overgrazing on kelp sporophytes can cause sterility and the complete collapse in productivity within kelp forests (Graham 2002).

Mesograzers are small, mobile, primary consumers, found in high abundances in marine benthic communities (Duffy & Hay 2000). This diverse group includes animals such as amphipods, isopods, gastropods, polychaetes, small crabs and shrimps (Cruz-Rivera & Hay 2000). Some mesograzers are found within the water column, sand and rocky substrata, but most are found closely associated within the seagrasses and macroalgae (Huang et al. 2006). Mesograzers inhabit, consume and construct nests on macroalgae (Poore et al. 2013) using the macroalgae as a shelter for protection from the physical stresses of the ocean (Gestoso et al. 2011) and as a refuge from predators (Huang et al. 2006). Marine mesograzers can consume between 2-23% of their body mass daily, with the exception of amphipods which have been recorded to ingest >100% of their body weight (Ruesink 2000). Individually, mesograzers have a small impact on their host algae (Ruesink 2000). In high densities, often in the tens of thousands per square meter, their combined consumption can impact algal biomass significantly (Poore et al. 2009).
In both terrestrial and marine environments many plants and macroalgae produce secondary compounds as a chemical defence to suppress herbivory and protect the host (Steinberg 1985). However, in response herbivores can develop a high tolerance for the chemicals produced by their host macroalgae (Steinberg 1995) with tolerance levels often varying greatly between taxa (Van Altena & Steinberg 1992). For example, chemicals produced by the brown alga *Sargassum vestitum* significantly deterred feeding of the echinoid *Tripneustes gratilla* but did not deter the gastropod *Turbo undulat* (Van Altena & Steinberg 1992). The close interaction between macroalgal defences and herbivore consumption has led to a continual arms race: macroalgae develop new chemical defences to guard against predation, which then induces a counter adaptation in herbivores to overcome these deterrents, and so on. These ecological responses demonstrate the strong link between herbivores and algae (Leimu *et al.* 2012).

1.3 Impact of climate change
Climate change can affect all ecosystems globally, including polar, terrestrial and marine environments. It impacts all levels of organisational hierarchies, encompassing individual species of flora and fauna to entire communities and ecosystems (Walther *et al.* 2002). Many of these global ecosystems are an essential part of the carbon cycle, acting as a natural ‘sink’ whereby marine (Connell & Russell 2010) and terrestrial ecosystems absorb approximately 30% of the anthropologically produced CO$_2$ that has been released into the atmosphere (Cramer *et al.* 2001). As greenhouse gases increase, CO$_2$ being the main contributor, there is a concern that this rise will correspond with an acceleration in the rate at which the CO$_2$ is released (Lough & Hobday 2011). Prior to the industrial revolution atmospheric CO$_2$ levels were between 180 and 300 ppm (Fabry *et al.* 2008); they are currently 394 ppm. Under the ‘business as usual’ scenario CO$_2$ concentrations are predicted to reach 1000 ppm by the end of the century (Fabry *et al.* 2008). Similarly, the rising partial pressure of CO$_2$ ($p$CO$_2$) in seawater was 280 µatm during preindustrial times and this has increased to a current level of 380 µatm (Pörtner 2008).

Current evidence indicates that the effect of $p$CO$_2$ in seawater varies, depending on the organism of interest (Ries *et al.* 2009). One known effect is that as pH decreases, $p$CO$_2$
increases, this can reduce the calcification rate in calcifying organisms such as corals and gastropods (Ries et al. 2009). Although, increases in pCO₂ can have a positive effect on organisms, an example of this is where under increased pCO₂ the cephalopod mollusc Sepia officinalis maintains its calcification and experiences increased growth and metabolism (Gutowska et al. 2008). Non-calcifying algae also have a varying reaction to elevated pCO₂. Where many species of fleshy algae have shown a positive response to increased pCO₂ (Porzio et al. 2011), the brown alga Fucus vesiculosus experienced reduced growth and lowered C:N ratio when exposed to high pCO₂ (Gutow et al. 2014). It is estimated that at the end of the century if pCO₂ reaches 1400 µatm and atmospheric CO₂ reaches 1000 ppm the pH in oceanic waters will drop between 0.3-0.4 pH units, having a profound effect on marine ecosystems (Caldeira & Wickett 2003; Fabry et al. 2008; Koch et al. 2013).

In addition to the direct impact of rising levels of particular gases, atmospheric greenhouse gases also confine heat energy that would otherwise re-radiate into space, resulting in increased global air and sea surface temperatures (SST) (Harley et al. 2006). In the previous century, SST rose between 0.4-0.8°C (Harley et al. 2006) and future predictions suggest that long term SST will continue to rise, increasing by 3-4°C by the end of the century (Koch et al. 2013). A rise in temperature of 3-4°C is predicted to have a significant impact on all organisms (Hughes 2003).

SST can be strongly affected by ocean currents; the Leeuwin Current is the most extensive boundary current in the world (Gurgel et al. 2014). This current originates in the Indo-Pacific, flows down the coastline of Western Australia and culminates eastward along the Great Australian Bight (Smale et al. 2011). The Leeuwin current is often stronger during the autumn and winter months when it transports tropical warm, nutrient poor waters polewards down the coast (Pearce & Feng 2007; Smale et al. 2011). The impact that climate change is having on this natural process is relatively speculative. However, it is anticipated that the El Niño-Southern Oscillation cycle, including La Niña, will not only be occurring more frequently, but will increase in severity with heavier rainfall over time (Caputi et al. 2010; Stocker 2015). Furthermore, when La Niña, the Leeuwin Current and long-term warming events coincide with heat waves or major temperature anomalies it can have disastrous effects on the Western Australian
marine life, including macroalgal assemblages (Pearce et al. 2011; Smale et al. 2011). The importance of examining the responses organisms have to unexpected temperature events was highlighted in 2011, when at the peak of La Niña, the west coast of Australia experienced a high magnitude warming event (Feng et al. 2013). The average SST increased by 2-4°C for more than ten weeks (Wernberg et al. 2012b). This anomaly resulted in the loss of many habitat-forming macro algae and a subsequent shift in community structure towards a reduced diversity of species (Wernberg et al. 2012b). Although this was a discrete climatic event, it is expected that there will be more frequent and severe weather events occurring in the future making it valuable to predict how these extreme climatic events will affect natural ecosystems (Swanson & Fox 2007).

Climate change is predicted to have a negative effect on terrestrial environments by increasing the intensity, duration and frequency of abiotic factors such as drought, fire, landslides and insect outbreaks (Dale et al. 2001). Changes in these abiotic factors lead to disturbances in the ecosystem, and the biota are then required to cope with these disturbances that are exacerbated by the increase in temperature and pCO₂ (Dale et al. 2001). In the marine environment climate change is expected to change the major wind currents across the globe, possibly changing the distribution and abundance of many organisms (Hoegh-Guldberg & Bruno 2010). Unless plants are able to rapidly adapt to these changes in climate conditions the consequences are likely to be increased stress (Pearson & Dawson 2003), a reduction in growth (Allen et al. 2010) and reduced survival (Campbell et al. 2011). A primary goal of climate change research is to develop an understanding of how climatic changes can affect ecosystem dynamics and from this, forecast the possible effects climate change may have in the near future (Colvard et al. 2014).

1.4 Western Australian temperate reefs
Australian oceans are biodiversity hotspots, containing between 30-40% of the world’s species of macroalgae and of these, 50% are thought to be endemic to Australia (Wernberg et al. 2011a). Similarly, the temperate reefs of Western Australia have one of the most species-rich assemblages of algae in the world (Kendrick et al. 2004). It is speculated that this high biodiversity in Western Australia is due to the unique climatic association with the Leeuwin current, and the absence of large natural disturbances
such as tectonic plate movement and glaciation (Foster et al. 2014). These reefs are made up of limestone and granite and line more than 1600 kilometres of the coast of Western Australia. The dominate habitat is *E. radiata* kelp forests (Wernberg et al. 2003b; Bennett et al. 2015a). The temperate *E. radiata* forests found around Perth support eighty two species of brown, green and red macroalgae (Kendrick et al. 1999). The main foundation species on these reefs are the canopy-forming kelps (e.g. *E. radiata*) and fucoids (e.g. *Sargassum*), which are often abundant (Wernberg et al. 2011b).

The Great Southern Reef is the name recently given to the southern coastline of Australia spanning from Kalbarri in Western Australia to Brisbane in Queensland, and including Tasmania. This reef is estimated to contribute $40 billion dollars each year to Australia’s economy through tourism and fisheries (Bennett et al. 2015a). Considering The Great Southern Reef comprises a large proportion of Western Australia’s reefs, it suggests that this area of coastline is an extremely valuable commodity. Thus, with many endemic species and a high net worth, the potential loss of biodiversity through climate-mediated changes is very alarming (Wernberg et al. 2011a; Bennett et al. 2015a).

### 1.5 Impact of climate change on macroalgae

Most species’ distribution ranges are highly climate dependent. Therefore, changes in the natural distribution, and possible contraction, of species ranges are a likely consequence of climate change and this phenomenon is expected to be seen across all ecosystems (Pearson & Dawson 2003). In terrestrial ecosystems the predicted consequence of climate change is the shift of plant species in a progressive movement to higher altitudes and latitudes (Jump & Penuelas 2005). In marine environments, temperature determines the boundary and distribution of macroalgal dominated habitats (Staehr & Wernberg 2009). Macroalgae such as *E. radiata* can have varying rates of respiration and photosynthesis depending on the environmental temperature conditions. *E. radiata*’s photosynthetic output decreased by 50% in warmer latitudes and increased its respiration by more than 90% compared to the same algae from cooler latitudes (Staehr & Wernberg 2009). Overall, macroalgae are limited in their ability to acclimatise to increases in temperature and it is suggested that as climate change
conditions intensify the distribution of many macroalgal species will progressively shift poleward, migrating according to the algae’s thermal tolerances (Harley et al. 2006; Bearham et al. 2013). Evidence of this shifting process has been observed in the temperate bay regions of western Japan. The abundance of temperate Sargassum spp., previously dominating the region declined significantly over the past 30 years, with tropical Sargassum spp. now dominating (Yamasaki et al. 2014). A similar shift was also observed during the aforementioned warming anomaly along the west coast of Western Australia in 2011. The dominant kelp E. radiata reduced in cover in the coastal region of Jurien bay (the uppermost limit of E. radiata’s thermal tolerance), allowing algal turf to occupy the region. The mats of algal turf are less structurally diverse than the kelp, representing a shift in the region to a less productive ecological state (Wernberg et al. 2012b). The potential impact of changes in climate on the south Western Australian macroalgal species is a shift from the endemic temperate species E. radiata to the subtropical or tropical species Laurencia. This is likely to have a dramatic effect on the benthic community of the west coast, impacting the associated food web (Wernberg et al. 2012c).

1.6 Macroalgal performance in changing conditions

Macroalgae possess many different mechanisms to defend against herbivory including physical toughness (Verges et al. 2008), low nutrient content (Harley et al. 2012) and secondary metabolites (Stern et al. 1996). These mechanisms and others including photosynthetic rate, growth (Li et al. 2013), blade mass (Niinemets 2001), and bleaching (Irving et al. 2004) can be altered by changes in temperature and \( p\text{CO}_2 \) caused by climate change conditions (Kirschbaum 2004). One possible detrimental impact on plants is that more plant tissue will be consumed by herbivores due to changes in plant features (e.g. chemical defences or physical toughness) (Harley et al. 2012).

The physical toughness of the algae can prevent herbivores from removing and masticating the algae tissue, thereby influencing the herbivore’s food preferences (Duarte et al. 2010). The preference of the herbivore may sway towards the tissue that is easily consumed (Duarte et al. 2010). Interestingly, there is evidence for within-plant variation in plant toughness where sections of the one plant may be tougher and less
palatable than other parts (Pansch et al. 2008). Feeding experiments carried out by Taylor et al. (2002) indicated that chemical defences played a strong role in deterring herbivore consumption in the meristematic tissue of Sargassum filipendula, but tissue toughness in the base stipe played a stronger role in deterring herbivore consumption. However, plant toughness is difficult to manipulate and evaluate as different herbivores feed in different ways (Pennings & Paul 1992). Pennings and Paul (1992) found that tissue toughness strongly correlated with the feeding preferences of the gastropod Dolabella auricularia. These results were contradicted by Steinberg (1985) who suggested that when testing the consumption rates of the gastropod Tegula funebralis consuming Dictyota flabellata, thallus toughness did not contribute to food preference. However, the tissue toughness of D. flabellata was similar to that of the preferred species Nereocystis luetkeana, indicating that tissue toughness should not be used exclusively as a feeding preference indicator (Steinberg 1985).

The carbon and nitrogen balance is essential to optimise plant growth and biomass and to assist in the response to increased atmospheric $p$CO$_2$ (Zheng 2009). As previously stated, macroalgae function as a sink for atmospheric carbon (Chung et al. 2011), and as atmospheric $p$CO$_2$ increases, macroalgae increase their carbon uptake (Hungate et al. 1997). The quantity of CO$_2$ absorbed by the plant can depend on the amount of nitrogen available and in increased CO$_2$ conditions this would be important (Hungate et al. 2003). The increased carbon uptake puts a greater demand on nutrient intake (Schulze et al. 1994) and if this demand is not met it results in lower algal nitrogen levels (Rusterholz & Erhardt 1998) and therefore an increased C:N ratio (Hungate et al. 2003). One possible outcome of an increased C:N ratio would be limitations in growth and leaf surface area, resulting in a reduction in photosynthetic rate and the ability to fix CO$_2$ (Reich et al. 2006), thereby reducing the plant’s sink strength (Schulze et al. 1994). Along with CO$_2$ levels (Gutow et al. 2014) a change in the C:N ratio can result from stresses including UV radiation (Pavia et al. 1997), light availability (Cronin & Lodge 2003) and temperature (Murray et al. 2013). Phenolic concentrations can decrease when growth is limited by alterations in either the nutrient or light availability (Yates & Peckol 1993) as seen in the freshwater macrophyte Potamogeton amplifolis (Cronin & Lodge 2003). Under low light conditions and subsequent low C:N ratios in P. amplifolis the result was lower phenolic
concentrations. However, when low nutrients were the cause of the low C:N ratio this led to high phenolic content in *P. amplifolis* (Cronin & Lodge 2003).

Climate change conditions and photosynthesis are strongly linked, given that plants require CO$_2$ for photosynthesis. Photosynthetic rates can increase with elevated pCO$_2$, however, extremes in temperature can negatively impact the biochemical processes of photosynthesis, and this may reduce enzyme activity (Kirschbaum 2004). Over a long term basis (weeks) the effects of increased CO$_2$ can be variable and may be dependent on nutrient availability (Xu et al. 2010) and in terrestrial environments, water availability (Kirschbaum 2004). Generally, as the plant is supplied with more CO$_2$ the growth rate and blade mass increases, and the requirement for more nutrients is amplified (Xu et al. 2010), and as temperatures increase the demand for water increases (Kirschbaum 2004). In marine ecosystems Johnson et al. (2012) found that when exposing the brown alga *Padina pavonica* to elevated CO$_2$, the Chlorophyll a and Chlorophyll c content increased, indicating a greater photosynthetic capacity.

Bleaching can be a sign of stress or disease (Marzinelli et al. 2015). Stressors include temperature, UV radiation or bacteria and often these factors interact (Martone et al. 2010; Case et al. 2011; Wieters et al. 2013). Bleaching is a result of the loss of algal pigmentation, which is often irreplaceable; this can reduce photosynthetic rates and result in algal death (Martone et al. 2010). An indication of the causative stressor can be made from the site of the bleaching. For example, UV radiation often results in bleaching being uniformly distributed across the entire thallus, whereas bacterial bleaching initiates from the infected site of the bacteria and expands across the algal blades (Case et al. 2011). It is vital to know the causes of physiological stress, as large bleaching events can result in mass disturbances across a reef system and affect the distribution of many macroalgae (Wieters et al. 2013).

### 1.7 Macroalgal chemistry

Many plants, both marine and terrestrial, produce polyphenolic secondary metabolites as a chemical defence to reduce the impacts of herbivory (Stern et al. 1996), to protect against UV radiation (Tierney et al. 2014), to reduce fouling by epiphytes and to enhance adhesion to the substrate (Kamiya et al. 2010; Egan et al. 2013). The production of
defensive chemicals can either be inducible or constitutive, with both having their own advantages. The advantage of induction is that it saves resources that could otherwise be used for growth and reproduction (Rohde & Wahl 2008) and to reduce the potential of the plant tissues to be exposed to self-toxicity (Molis et al. 2008). From the initial grazing period the induction of these defences can occur in 3 - 14 days and continue for 2-7 days after grazing, indicating that these defences can be rapidly initiated and lost (Rohde & Wahl 2008).

Phenolics differ between species, are highly variable and have different effects based on the chemical composition (Bidart-Bouzat & Imeh-Nathaniel 2008). Phlorotannins, polymeric structures of the simple phenolic phloroglucinol (1,3,5-trihydroxybenzene), are abundant in brown algae (Steevensz et al. 2012). These phenolics can comprise of up to 25-30 % of dry weight within the plant (Tierney et al. 2014). In red algae such as Laurencia, secondary metabolic production can be highly diverse, but it’s the halogenated sesquiterpene compound that is believed to protect against herbivory and fouling (Oliveira et al. 2013; de Oliveira et al. 2015). The metabolites in Laurencia are thought to be stored within the algal vesicles. The metabolite production is inducible and the compounds are transported from the vesicles to the surface when stimulated by herbivore consumption, or fouling (Oliveira et al. 2013). The red alga Delisea pulchra generates halogenated furanones as a natural defence mechanism. These furanones prevent the communication-regulated processes between bacterial cells, reducing the formation of large bacterial colonies and biofilm (Harder et al. 2012). When these furanones are reduced, bacterial-induced bleaching disease can occur. This bleaching reduces the algal pigmentation and causes overall algal decay (Fernandes et al. 2012).

Both abiotic and biotic factors can influence a plant’s chemical content. Abiotic factors such as light quality, salinity, nutrient availability and temperature alter phenolic concentrations as well as biotic factors including epiphyte attachment and grazing pressures (Kamiya et al. 2010). The concentration of phenolics can vary between algal species and this variation can differ for species that are located at different latitudes. For example, tropical species have been shown to produce higher levels of phenols than temperate species (Steinberg 1985). Phenolic content can also fluctuate annually;
Kamiya et al. (2010) observed that *Sargassum confusum* produced the highest tannin concentrations during autumn and lower concentrations during winter.

Climate change conditions can have varying effects on the phenolic production in plants. Cronin and Hay (1996b) subjected the brown alga *Dictyota ciliolata* to desiccation through increased temperatures and found that the concentrations of phenolics lowered significantly, allowing for an increased rate of grazing by the sea urchin *Arbacia punctulata*. Elevated CO$_2$ can have a positive effect on phlorotannin production. After exposure to increased CO$_2$ the kelp *Nereocystis leutkeana* experienced enhanced growth, possibly due to an increase in carbohydrates, which lead to a threefold increase in phenolic production (Swanson & Druehl 2002).

### 1.8 Aims

The aims of this research are to:

- Determine how increased temperature and decreased pH affects *Ecklonia radiata*, *Sargassum linearifolium* and *Laurencia brongniartii* performance in terms of growth, bleach cover, quantum efficiency yield, blade toughness, mass per square centimetre, and nutrient content;

- Determine if the feeding rates of *Allorchestes compressa* and Trochidae (Gastropoda) are impacted by *E. radiata*, *S. linearifolium* and *L. brongniartii* that have been subjected to increased temperature and increased pCO$_2$ conditions; and

- Establish the role of plant chemicals and total phenolics in affecting herbivores after *E. radiata*, *S. linearifolium* and *L. brongniartii* have been subjected to increased temperature and increased pCO$_2$ conditions.
2. Methods

2.1 Study site and organisms

*S. linearifolium* (Turner) C. Agardh is a dominant fucoid brown macroalga that occurs in rocky tropical (Schaffelke 1999) and temperate regions worldwide (Stiger *et al.* 2004). *Sargassum* spp. are important primary producers, generally possessing complex foliage structures made up of dense thalli which provide a habitat for small invertebrates and juvenile fish (Phillips & Blackshaw 2011). *Ecklonia radiata* (C. Agardh) J. Agardh is a warm-temperate, canopy-forming, brown kelp found on the rocky reefs of Australia, New Zealand and South Africa (Phillips *et al.* 1997; Wernberg *et al.* 2003*a*), and *Laurencia brongniartii* (J. Agardh) is a subtropical red algal species found in subtidal zones in Australia, Japan and the Caribbean Sea (Nishihara *et al.* 2004). All three species are abundant within their ranges.

*S. linearifolium, E. radiata* and *L. brongniartii* specimens were collected and used in three independent experiments in February (*S. linearifolium*), March (*E. radiata*) and August (*L. brongniartii*) 2015. *S. linearifolium* and *E. radiata* were chosen haphazardly from shallow regions (0.5 - 2 m depth), off the limestone groyne at Hillarys Boat Harbour, Perth, Western Australia (31° 49’ 14” S. 115° 44’ 12” E). *L. brongniartii* were collected similarly from Point Peron, Rockingham, Western Australia (32° 16’ 18” S. 115° 41’ 17” E). Twenty healthy, medium sized (approximately 250 – 530 mm in total length for *E. radiata* and *S. linearifolium* and 125 – 195 mm in total length for *L. brongniartii*) individuals were collected with their holdfasts intact. Algae were dipped in fresh water for one minute, shaken gently to dislodge attached fauna, and placed in an indoor aquaria system at Edith Cowan University (ECU). Each alga was initially measured for quantum efficiency yield (QEY) (See 1.3.3) and immediately placed in the aquaria in conditions representative of the mean summer temperatures in Perth (20.5 °C) (Navy Metoc 2015. [http://www.metoc.gov.au/products/data/ausst.php](http://www.metoc.gov.au/products/data/ausst.php)) for an acclimation period of 48 hours.

*Allorchestes compressa* (Dana) is a semi-aquatic amphipod found abundantly in coastal macroalgal and seagrass habitats (Crawley *et al.* 2007). The amphipods were collected from the surf zone at Quinns Rocks, Western Australia and reared on *E. radiata*
throughout the year to supply new individuals for each experiment. Gastropods from the family Trochidae (Rafinesque, 1815) are abundant and found within reefs worldwide (Hickman & McLean 1990). The gastropods were collected from macroalgal reefs in the subtidal zone in Rockingham, Western Australia, 24 hours prior to feeding experiments.

2.2 Experimental conditions
The aquaria design consisted of four reservoir tanks with a 2 x 2 design. Two environmental variables were manipulated: temperature and ocean acidification, each at two treatment levels: control and elevated. The treatments included (1) the control: 20.5°C, pH 8.1, from here on referred to as PaTA, (2) decreased pH: 20.5°C and pH 7.6, from here on referred to as PeTA, (3) increased temperature: 25.5°C, pH 8.1, from here on referred to as PaTE and (4) combined increased temperature and decreased pH: 25.5°C, pH 7.6, from here on referred to as PeTE (Figure 1). The combination of 25.5°C and pH 7.6 represent the upper threshold for the near future (2,070-2,100) (IPCC 2007; Poore et al. 2013).

The aquaria room was temperature controlled to achieve the ambient temperature (20.5°C). To adjust and regulate the temperature conditions within the experiment, aquarium heaters (200W; Aqua One) were placed within the necessary reservoir tanks to supply heated water to the increased temperature treatment tanks. pH was adjusted with a self-regulating CO₂ controller (Aquatronica). Both pure CO₂ and water from the reservoir tanks flowed through the CO₂ controller where they were mixed to the desired pH and returned to the reservoir tank. After the 24 hour acclimation period, the aquaria heaters and the CO₂ controller were turned on. Warming of the water and the mixing of the CO₂ was a gradual process which took approximately 12 hours to reach the desired
treatment conditions. Salinity levels (35.6 ppt) were maintained daily with the addition of fresh rainwater to reservoir tanks as required. The experiments were controlled in a semi-recirculating seawater system. The treated seawater was pumped (using an AQUAPRO AP1050 water pump) from the reservoir tank into each of the five treatment tanks at ~ 40 L/hr using irrigation dripper valves. This then independently provided each replicate tank with reflected conditions of the reservoir tank. The treatment tanks were designed with an overflow pipe, which passaged the movement of water through aquaria wool (Mipet, filter wool for aquarium filters), for filtration, and then returned the water to the reservoir tank in a re-circulation system (similar to methods in Widdicombe & Needham 2007; Spicer et al. 2011; Tait et al. 2013). This setup is pseudo-replication and not the most desired setup for aquaria experiments due to the lack of independence and potential contamination among tanks, however as previously mentioned the filtration system used in this experiment is designed to reduce any contamination. This design was chosen because of logistic reasons, the Aquatronica is limited to the number of probes (2) available to monitor fluctuations in pH conditions. This suited the two larger reservoir tanks but this would not be feasible to monitor the pH levels in the 10 treatment tanks. The water within the reservoir tanks was continuously circulated using 10.5 W (using an AQUAPRO AP550) pumps to maintain even temperatures and pH levels. To replenish nutrients, maintain consistent carbon chemistry and provide a clean environment, 100 L of water was removed and fresh seawater added to the reservoir tank at a very slow rate every four days. During the water changing process the treatment tanks were monitored to ensure there were no alterations in pH and temperature levels. The tanks walls and irrigation pipes were wiped down with a clean cloth every second day. Air stones connected to the building’s central air compressor were placed in each treatment tank adding a constant flow of fresh air which also enhanced water circulation.

For each experiment, one alga was placed in each of the treatment tanks (n = 5 per treatment tanks, total of 20 algae per experiment). Fluorescent lights (Sylvania 36W) providing ca 40 µmol m⁻² s⁻¹ were placed above the treatment tanks to ensure photosynthesis continued on a 14:10 hr light:dark cycle as per the average Perth sunset/sunrise hours for summer (Perth Observatory 2014). The pH, temperature and
salinity in each tank were measured daily and total µ was measured weekly. Total alkalinity was measured by taking water samples (100.0 mL) from each treatment tank, which were titrated against 0.02 M sulphuric acid using a Metrohm 716 Titrino (method adapted from (Williams et al. 2009)). pCO₂ was calculated with CO2SYS (Pierrot et al. 2006) using measurements of pH, temperature, salinity and total alkalinity. After the 14 day treatment period the algae were removed from the tanks, subjected to the final plant performance measures (see below) and replicate pieces from each treatment were stored at -80°C for plant chemistry analysis, nutrient analysis and for use in the ground feeding experiment. A pilot study was conducted prior to this research. The pilot study indicated a rapid depletion in E. radiata’s health after a period of 14 days, suggesting that 14 days is the limit for the algae to be maintained in the aquaria conditions. No herbivores were subjected to climate change testing conditions.

2.3 Algal performance
To determine the response of each algal species to the treatments, algal performance was measured prior (Day 1) and post (Day 14) exposure to the treatment conditions. In addition, the quantum efficiency yield was tested immediately after collection from the field, and bleaching was recorded daily. Analyses included: measurement of growth rate, bleaching, quantum efficiency yields, blade toughness, density and nutrient analysis. All performance tests were completed using the new growth blades for each species.

2.3.1 Growth rates
Growth occurs in different sections of the algae for each species, requiring two different measurements for growth. New growth occurs in the apex of each frond within S. linearifolium and L. brongniartii, so the length from the holdfast to apical tip along the main frond axis was measured as total length (Baer & Stengel 2010). The total length on Day 14 was subtracted from the total length on Day 1, giving a proxy for growth rate. The growth rate for E. radiata was measured using the hole punch method (adapted from Mann and Kirkman (1981)). This involved piercing the blade with a hole (3 mm diameter) 20 mm from where the blade connects to the stipe (Figure 2). As new growth occurred the hole moved up the blade. The distance between the final destination of the hole and the position 20 mm from the blade joint represents the new growth. The
initial length was measured at day 1, prior to treatment and subtracted from the length on day 14 to obtain a proxy for growth.

2.3.2 Bleaching
Similar to methods in Marzinelli et al. (2015), bleaching was characterised as visible whitening of the blade tissue due to the loss of surface integrity. Bleaching was observed daily and recorded as a percentage of total cover or severity per algae (Rasher & Hay 2010). The bleaching observed in each species was not uniform, so visual observations were conducted by placing the algae against a white background which was used as standard.

2.3.3 Quantum efficiency yields
$F_v/F_m$ is a measurement of the efficiency of photosystem II, this measurement can give an indication of photo-physiological stress when these values drop below the threshold (Genty et al. 1989). A Diving – PAM Fluorometer (Walz, Effeltrich, Germany) was used to measure the fluorescence and obtain quantum efficiency yields. This was given as a ratio of $F_v/F_m$, where $F_v$ is the variable fluorescence and measured as the minimum fluorescence ($F_m$) and divided by the maximum fluorescence ($F_o$) (Necchi Jr 2004). Dark-leaf clips were placed on the new growth blades and prepared in the dark-acclimation setting for 15 minutes prior to taking quantum efficiency readings (Beer et al. 2006). Quantum efficiency yields were taken at three stages: (1) immediately post collection from the field, (2) after acclimation, prior to commencement of treatment conditions (day 1) and (3) after the treatment period (day 14).
2.3.4 Blade toughness
To estimate tissue toughness, a custom made penetrrometer (following method developed by Duffy and Hay (1991)) measured the force needed to penetrate the algal frond using a metal pin (Figure 3). After the treatment period (day 14), five fresh new growth blades from each algae were selected at random and tested for blade toughness. The frond was placed in the centre of the penetrrometer, the pin, topped with a plastic dish, was placed above the frond. Silicone beads were slowly added to the top dish and once the pin pierced the frond entirely the beads were weighed, giving a proxy for tissue toughness.

2.3.5 Blade mass per square centimetre
Blade mass per square centimetre (BM/cm\(^2\)) measurements were taken to determine the thickness of the blades. Fresh new growth blades were randomly chosen, patted dry with paper towel and then cut to fit accurately into a 100 mm\(^2\) area (Figure 4). The blades were then weighed to gain a measurement of g/mm\(^2\) fresh weight, a proxy for BM/cm\(^2\) (methods adapted from McClendon (1962)).

2.3.6 Nutrient analysis
Nutrient content was determined by measuring the total carbon and total nitrogen content by gas chromatography (GC) stable isotope-ratio mass spectrometry (IRMS) (Thermo Delta V IRMS with EA, University of Western Australia) to gain the percentage of total carbon and nitrogen, and expressed as a C:N ratio following the methods in Farmer et al. (2005). Briefly, between 1.1 and 1.2 mg of dried algae (weighed on a Sartorius AG Goettingen (Germany) analytical balance) was transferred to a tin capsule and introduced to the IRMS. The sample was then combusted at high temperature and the N\(_2\) and CO\(_2\) gases evolved were separated and quantified by mass spectrometry.
2.4 Feeding assays

2.4.1 Fresh feeding assay
To determine how the treatments effected herbivore consumption rates, no-choice fresh feeding assays were conducted. Fresh (accurately weighed (± 0.001 g) using A&D Company GR-200 (Japan) analytical balance) pieces of algae weighing approximately 0.1 g (± 0.07 g) from each treatment were presented to the herbivores. Herbivores were provided with a representative mix of fresh algae for 24 hours prior to the feeding essay to prevent impulsive feeding behaviour occurring (Cronin & Hay 1996a). One gastropod was added to each treatment dish (n = 1 gastropod per dish, 5 dishes per treatment tank, 20 treatment tanks, total of 100 dishes) and six amphipods were added to additional treatment dishes (n = 6 amphipods per dish, 5 dishes per treatment tank, 20 treatment tanks, total of 100 dishes) with a pre-weighed section of algae with static seawater at PₐTₐ conditions (20.5°C, pH 8.1). No-herbivore controls were also included. This involved placing fresh algal material in feeding disks (n = 2 disks per treatment, total 40 disks) containing no herbivores, but using the same weighing techniques as the herbivore disks. The feeding assay ran for a 36 hour period to ensure that the algal piece was not entirely consumed but long enough so that an adequate feeding rate could be determined (Poore 1994; Duarte et al. 2011). Each algal piece was reweighed after this period to determine the total mass of algae consumed. The total mass consumed was calculated using the equation \[\frac{H_f - H_0 - (\bar{W}_i/n)}{n}\], where \(H_0\) and \(H_f\) is the initial and final tested algal weights, the \(W_i\) is the “no-herbivore” control algal weights and n = the number of live herbivores.

2.4.2 Ground feeding assay
The algal blades not used in the fresh feeding assay were placed in the -80°C freezer for 48 hours, then freeze dried (Esco, Sublimate 2) for an additional 48 hours. The algae was then ground to a fine powder with a mortar and pestle, then a portion of ground algae was removed to be used in a ground feeding assay. The ground algae (approximately 0.1 g) was accurately weighed and placed evenly into a 5 mL petri dish that was lined with filter paper (for support). Agar was prepared by adding agar powder (plant TC, micropropagation grade (Phyto Technology Laboratories, US)), 3.2 g (4%) to 80.0 mL water and heated in a microwave until boiling (method adapted from (Pennings et al. 1998)). This agar mix (3.0 mL) was pipetted into the petri dish and stirred until an even
consistency of algae was achieved throughout the disk. The agar dishes were cooled to room temperature, the filter paper were removed from the petri dish and then evenly divided into 12 agar pieces (weighing approximately 0.1 g (± 0.07 g) (Figure 5) (Pennings et al. 1998). The agar pieces were then placed in individual static sea water containers overnight. All pieces of agar were then accurately weighed and used in a feeding assay for 36 hours as described above (section 2.4.1), including the no herbivore controls.

Figure 5: Algae/agar piece used in ground feeding assay.

2.5 Determination of total phenolic content

2.5.1 Chemicals
Folin & Ciocalteu’s phenol reagent, phloroglucinol and sodium carbonate (Na$_2$CO$_3$) were obtained from Sigma-Aldrich (Steinheim, Germany). Ethanol (analytical grade), was obtained from Rowe-Scientific (Australia). The H$_2$SO$_4$ used for titrations was analytical reagent grade, and all other solvents were of high performance liquid chromatography grade.

2.5.2 Preparing crude extract
Crude extracts were prepared according to methods in Wang et al. (2012) with some modifications. Freeze-dried, ground algae (0.1 g) was added to a 10.0 mL centrifuge tube containing 3.0 mL of ethanol: water (80:20 v/v). The tube was agitated (using Ratek, Orbital mixer) on Level 4, for 15 minutes and then centrifuged at 1250 rpm (Hettich Universal, Hettich-zentrifugen, D-7200 Tuttlingen, Germany) for 5 minutes. The supernatant was collected and placed into a separate 10 mL centrifuge tube and placed in the dark. A fresh aliquot of solvent (3.0 mL) was added to the plant residue and the process repeated a further two times. This process produced approximately 9 mL of crude extract supernatant for each sample.

2.5.3 Total phenolic content
Total phenolic content (TPC) was determined using the Folin-Ciocalteu method using the protocol adapted from Long and Trussell (2007) with some modifications. To a 10.0 mL centrifuge tube, crude extract (0.5 mL) was added (for S. linearifolium and E. radiata the extract was first diluted 1:10, (80:20 ethanol: water) solution), then Folin-Ciocalteu
phenol reagent (0.5 mL) and Milli Q (7.5 mL) were added to the tube. The mixture was agitated for 30 seconds and left to rest for 10 minutes. Then 0.5 mL of 2 M sodium carbonate was added, mixed well and left to stand for 60 minutes. Using a spectrophotometer (Shimadzu, UV-Vis mini 1240) the absorbance of the samples was recorded at 700 nm. Fresh phloroglucinol standards in the range 0-100 µg/mL were prepared prior to every analysis with ethanol as the diluting solvent. An aliquot of each standard was treated similarly to the samples and used to generate a calibration curve.

2.6 Statistical analysis
The results from the macroalgae and herbivores for all plant performance and feeding assay data were analysed separately. All datasets were pre-treated by completing an overall square root transformation followed by normalising the data. A permutational multivariate analysis of variance (PERMANOVA) was used to compare the results of growth, blade toughness, BM/cm², C:N ratios and total phenolic content, per treatment. The bleaching and quantum efficiency yields were analysed using the data from the post treatment (Day 14), averaged per treatment and compared using PERMANOVA. Feeding rates were calculated by subtracting the final algal weight from the initial algal weight, which was added to the averaged ‘no herbivore’ weight and then averaged per live herbivore, per treatment. The treatments were compared using a PERMANOVA. All PERMANOVA tests were followed by PERMANOVA pairwise comparisons. PERMANOVAs were carried out using PRIMER v.6 and PERMANOVA (PRIMER-E Ltd. 2013). Paired-sample t tests were carried out to compare the QEYs between post collection and day 1. The data was square root transformed and tested for normality prior to the t test. This analysis was completed using SPSS (IBM Corp. 2013). The significance level for all datasets was P < 0.05.

For each species of macroalgae a multi-dimensional scaling (MDS) plot was created based on the Euclidean distance to visualize the patterns using all the data including the performance measurements, ground and fresh feeding assays, and the phenolic content and compared against the four treatments (P_E T_A, P_E T_E, P_A T_E and P_A T_A). These plots were generated using PRIMER.

Datasets were evaluated using the LnRR (logarithmic response ratio) analysis to obtain the individual effects of the algae within treatments. LnRR is the natural log of the mean
treatment response divided by the mean ambient (control) response. The mean LnRRs
and 95% confidence intervals of each dataset were ascertained by bootstrapping (R =
999). A LnRR value of zero indicates no significant response to the tested treatment. A
positive and negative value signifies a positive and negative response to the treatments,
respectively. When the 95% confidence intervals overlap zero, this indicates that the
treatments did not indicate a significant response (Pansch et al. 2008). LnRR analysis was
conducted using R, Version 3.2.1 (R Development Core Team 2013). Datasets with
negative values (e.g. L. brongniartii growth measurements and all the feeding assays)
were unable to be naturally logged and therefore were omitted from LnRR analysis.
3. Results

3.2 Algal performance

3.1.1 Growth rates

The experimental treatments had varying effects on the average growth rate, in the different algal species tested. The average ambient growth rate of *S. linearifolium* was 6 mm/week (1.2 ±SE), *E. radiata*, 10.3 mm/week (0.7 ±SE), and *L. bronniartii* 1.7 mm/week (± 0.6) (Figure 6.).

For *S. linearifolium* there were no statistically significant differences in growth rate for algae treated with increased $p$CO$_2$, increased temperature, or the combination of treatment conditions (PERMANOVA, Table 1, $p >0.05$). However, there were significant responses between individual algae in the P$_{ET}$A and P$_{TE}$ treatments which indicated an increase in growth compared to individuals in the control treatment. The upper and lower bound 95% confidence intervals displayed increases in growth rates between individuals for *S. linearifolium*, ranging from 0.03 % to 11.9 % (P$_{ET}$A) and 5.6 % to 11.1 % (P$_{TE}$) (LnRR analysis, Figure 7).

Similarly, there were no significant differences in growth rate between treatments for *E. radiata* (PERMANOVA, Table 1, $p >0.05$). Bootstrapping revealed significant differences between individuals within the treatment. The 95% confidence intervals indicated that the P$_{ATE}$ treatment had a significantly decreased growth rate between individuals within the treatment, from -2.3 % to -8.2 % (LnRR analysis, Figure 7).

A significant difference in the growth rate of *L. bronniartii* between the treatments was observed (PERMANOVA, $p < 0.05$). *L. bronniartii* in the P$_{ET}$ treatment experienced a high growth rate, increasing by 5 mm/week (1.3 ±SE) (pairwise comparison, $p < 0.05$) (Figure 6c). When *L. bronniartii* was grown in the P$_{ATE}$ treatment, the algae experienced a decrease in total length by 3.4 mm/week (0.9 ±SE) compared to 1.7 mm/week (0.6 ±SE) in the control treatment (pairwise comparison, $p >0.05$). The average amount of *L. bronniartii* was similar in both temperature treatments, where total length decreased by 3.2 mm/week (3.5 ±SE) (P$_{TE}$) and 3.4 mm/week (0.9 ±SE) (P$_{ATE}$) through
deterioration. The P<sub>E</sub>T<sub>E</sub> treatment also had a high variability within the treatment compared to the control treatment (P<sub>E</sub>T<sub>E</sub> = 3.5 ±SE, P<sub>A</sub>T<sub>A</sub> = 0.6 ±SE). This dataset contained negative growth values which were unable to be naturally logged and therefore were omitted from LnRR analysis.
Figure 6: Growth rate of: A. S. linearifolium, B. E. radiata and C. L. brongniartii in the four treatments (P_E_T_A, P_E_T_E, P_A_T_E, and P_A_T_A). Data are mean ± SE of the total growth, calculated by taking measurements after the 14 day experiment period and subtracting it from algal length at day 1 of experimental period. Growth rates of S. linearifolium and L. brongniartii were measured as total length measurement of the algae averaged over seven days. E. radiata growth was measured using a hole punch method as described in section 2.3.1.

Table 1: PERMANOVA contrasting growth rate with different treatment conditions (P_E_T_A, P_E_T_E, P_A_T_E, and P_A_T_A) for each algal species. Significant values (p < 0.05) shown in bold.

<table>
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<th>Growth</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
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<tr>
<td>S. linearifolium</td>
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<td>1.6758</td>
<td>1.919</td>
<td>0.135</td>
</tr>
<tr>
<td>E. radiata</td>
<td>3</td>
<td>2.044</td>
<td>2.5415</td>
<td>0.097</td>
</tr>
<tr>
<td>L. brongniartii</td>
<td>3</td>
<td>3.1644</td>
<td>5.3256</td>
<td>0.007</td>
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</table>

Figure 7: Average log response ratio (LnRR) (± 95% CI) of growth rates for S. linearifolium and E. radiata, by calculating the natural log of treatment (P_E_T_A, P_E_T_E, P_A_T_E) response divided by the control (P_A_T_A) response.
3.1.2 Bleaching
The percentage of bleaching observed was not uniform between species, with *S. linearifolium* and *L. brongniartii* displaying high levels of bleaching (up to 95%) and *E. radiata* more resistant to bleaching (between 10% and 16% for all treatments). However, all three species of algae exhibited the greatest percentage of bleach cover in the elevated temperature treatments (P\textsubscript{ETE}, P\textsubscript{ATE}) at day 14 (Figure 8). The bleach cover observed in *S. linearifolium* was significantly different between treatments (PERMANOVA p < 0.05) (Table 2). *S. linearifolium* was strongly affected by bleaching in the P\textsubscript{ATE} treatment, with a mean increase in the percentage of bleaching of 94.4% when compared to the control treatment (pairwise comparison, p >0.05). Additionally, the upper and lower bound 95% confidence intervals showed increased bleaching between individuals within the treatments (0.9% to 10.2% P\textsubscript{ETE}, 2.4% to 9.4% P\textsubscript{ETE} and 3.4% to 12.3% P\textsubscript{ATE}) (LnRR analysis, Figure 9). *S. linearifolium* also bleached early in the experiment, with the first observation of bleaching at day 3 in the P\textsubscript{ATE} treatment (Figure 8a).

There were no differences in the amount of bleaching among *E. radiata* treatments (P\textsubscript{ETE}, P\textsubscript{ETE}, P\textsubscript{ATE} and P\textsubscript{ATE}) (Table 2). However, bootstrapping showed an increase in bleaching between individuals in the P\textsubscript{ETE} treatment (0.6% to 7.9%) (LnRR, Figure 9). In contrast to the bleaching observed in *S. linearifolium* and *L. brongniartii*, which showed the lowest bleach cover in the control treatment, the lowest percentage of bleach cover at day 14 was observed in *E. radiata* in the P\textsubscript{ATA} treatment (Figure 8b). For *E. radiata*, the first sign of bleaching occurred at Day 6 in the P\textsubscript{ATA} treatment (Figure 8b).

There were significant differences between the amount of bleaching and the treatments for the alga *L. brongniartii* (PERMANOVA, p < 0.05, Table 2). Bleaching was highest in the P\textsubscript{ETE} and P\textsubscript{ATE} treatments, with an approximate 200% increase in bleaching observed in both treatments, compared to the control treatment (pairwise comparison, p <0.05, Figure 8c). The P\textsubscript{ATA} treatment also showed a statistically significant difference, with an approximate 120% increase in bleaching compared to the control (pairwise comparison, p <0.05). The individuals in the P\textsubscript{ETE} and P\textsubscript{ATE} showed similar, increased bleaching results between individuals, using the LnRR analysis (5.9 % to 20.8 % P\textsubscript{ETE}, 5.8 % to 20.8 % P\textsubscript{ATE}). High levels of variability were observed in the upper and lower bound 95% confidence
intervals in the \( P_{E A} \) treatment which resulted in no significant differences between individuals within this treatment (-2.2 % to 16.7 %) (LnRR analysis, Figure 9). The first observation of bleaching was early in the experiment, at day 3 in the \( P_{E E} \) treatment (Figure 8c).
Figure 8: Bleaching level of A. *S. linearifolium*, B. *E. radiata* and C. *L. brongniartii* in the four treatments (P$_{ET}$A, P$_{ETE}$, P$_{ATE}$, and P$_{ATA}$). Data is recorded as the mean bleaching level for each treatment, calculated by taking daily observations throughout the 14 day experiment period.

Table 2: PERMANOVA contrasting bleaching level with different treatment conditions (P$_{ET}$A, P$_{ETE}$, P$_{ATE}$, and P$_{ATA}$) for each algal species. Significant values (p < 0.05) shown in bold.

<table>
<thead>
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<th>F</th>
<th>P</th>
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<td>3.6454</td>
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<td>3</td>
<td>5.4283</td>
<td>31.989</td>
<td>0.001</td>
</tr>
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</table>

Figure 9: Average log response ratio (LnRR) (± 95% CI) of percentage of bleaching on day 14 for *S. linearifolium*, *E. radiata* and *L. brongniartii*, by calculating the natural log of treatment (P$_{ET}$A, P$_{ETE}$, P$_{ATA}$) response divided by the control (P$_{ATA}$) response.
3.1.3 Quantum efficiency yields (QEY)

The three species of algae responded differently to the treatments in terms of QEY. *S. linearifolium* had a loss of QEY with increased temperature, *E. radiata* experienced a significant loss in QEY across treatments and *L. brongniartii* experienced a loss in QEY in the *P*<sub>E</sub>*T*<sub>E</sub> treatment.

Significant differences were observed between the QEY among treatments in *S. linearifolium*, measured at day 14 (PERMANOVA, *p* < 0.05) (Table 3). The *P*<sub>A</sub>*T*<sub>E</sub> treatment had a significant reduction of 0.5 (0.1 ±SE) Fv/Fm value compared to the control treatment (pairwise comparison, *p* <0.05). For *S. linearifolium* and using the data from day 14, the upper and lower bound 95% confidence intervals showed significantly decreased QEYS between individuals in the *P*<sub>E</sub>*T*<sub>A</sub> (-0.2% to -3.2%), *P*<sub>E</sub>*T*<sub>E</sub> (-0.2% to -1.0%) and *P*<sub>A</sub>*T*<sub>E</sub> (-20.5% to -91.1%) treatments and the control *P*<sub>A</sub>*T*<sub>A</sub>, with the *P*<sub>A</sub>*T*<sub>E</sub> treatment showing the highest level of variability (LnRR analysis, Figure 11). The differences in Fv/Fm values observed between post collection and day 1 for *S. linearifolium* were slight, the differences were 0.07 (<0.1 ±SE) (*P*<sub>E</sub>*T*<sub>A</sub>), 0.009 (<0.1 ±SE) (*P*<sub>E</sub>*T*<sub>E</sub>), 0.001 (<0.1 ±SE) (*P*<sub>A</sub>*T*<sub>E</sub>) and 0.01 (<0.1 ±SE) (*P*<sub>A</sub>*T*<sub>A</sub>) Fv/Fm values (Figure 10a).

No significant differences were measured in QEY in *E. radiata* between treatments (*P*<sub>E</sub>*T*<sub>A</sub>, *P*<sub>E</sub>*T*<sub>E</sub>, *P*<sub>A</sub>*T*<sub>E</sub> and *P*<sub>A</sub>*T*<sub>A</sub>) on day 14 (Table 3). However, the upper and lower bound 95% confidence intervals showed a significantly decreased QEY between the individuals in the *P*<sub>A</sub>*T*<sub>E</sub> treatment, with a reduction of 0.04 Fv/Fm value compared to the control treatment (LnRR analysis, Figure 11). When comparing the post-collection readings to the day 1 readings, *E. radiata* showed only minor variations with differences of 0.01 - 0.04 (<0.1 ±SE) Fv/Fm for all treatments (Figure 10b).

For *L. brongniartii*, using the data from day 14; a statistically significant difference was observed between the QEY and the treatments *P*<sub>E</sub>*T*<sub>A</sub>, *P*<sub>E</sub>*T*<sub>E</sub>, *P*<sub>A</sub>*T*<sub>E</sub> and *P*<sub>A</sub>*T*<sub>A</sub> on day 14 (PERMANOVA, *p* < 0.05, Table 3). The *P*<sub>E</sub>*T*<sub>E</sub> treatment had a significantly lower QEY value which reduced by 0.46 (<0.1 ±SE) Fv/Fm compared to the control treatment (pairwise comparison, *p* <0.05, Figure 10c). Surprisingly, it was only the individuals in the *P*<sub>A</sub>*T*<sub>E</sub> which were significantly reduced in the upper and lower bound 95% confidence intervals, varying from 13.7% to 73.3% (Figure 11). Considerable differences were observed between the Fv/Fm readings at post collection compared to Day 1 for *L.
brongniartii. These differences included 0.06 (<0.1 ±SE) (P_{ET}A), 0.15 (<0.1 ±SE) (P_{ET}E), 0.22 (<0.1 ±SE) (P_{ATE}) and 0.15 (<0.1 ±SE) (P_{ATA}) Fv/Fm values (Figure 10c).

For S. linearifolium there was a statistically significant difference, indicating an increase in Fv/Fm value between the post collection, and the day 1 data in the P_{ET}A treatment \( p < 0.05 \) (P_{ET}A). All other treatments showed no significant difference between the two data sets (post collection and day 1), \( p > 0.05 \) (P_{ET}E, P_{ATE} and P_{ATA}) (paired-sample t test). There were no significant differences between the post collection and day 1 data for E. radiata in all treatments (P_{ET}A, P_{ET}E, P_{ATE} and P_{ATA}) (paired-sample t test). Although, for L. brongniartii, both the P_{ET}E and P_{ATE} treatments showed a significant difference between post collection and day 1 (\( p < 0.05 \)) denoting a decrease in Fv/Fm value in both instances. The P_{ATE} and P_{ATA} treatments indicated no significant differences between datasets (paired-sample t test).
Figure 10: Quantum efficiency yields of **A** *S. linearifolium*, **B** *E. radiata* and **C** *L. brongniartii* in the four treatments (PETA, PETE, PATE, and PATA). Data are mean ± SE of the quantum efficiency yields, calculated by taking Fv/Fm readings post collection, on day 1 and day 14.

Table 3: PERMANOVA contrasting quantum efficiency yields measured on Day 14, with different treatment conditions (PETA, PETE, PATE, PATA) for each algal species. Significant values (p < 0.05) shown in bold.

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<td>2.891</td>
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3.1.4 Blade toughness

The blade toughness results were not uniform between species; *S. linearfolium* had the toughest blades, which required 97.3 g (6.7 ±SE) of weight to penetrate the blades. *E. radiata* was the second toughest of the three species, and it needed 67.8 g (3.5 ±SE) to pierce the blades, with *L. brongniartii* being the softest, only needing 32.6 g (3.0 ±SE) to penetrate the blades (Figure 12).

None of the treatments affected the toughness of *S. linearfolium* or *E. radiata* (Table 4). However, for *E. radiata* the upper and lower bound 95% confidence intervals indicated a significant increase in toughness (0.4% to 3.4%) within the individuals in the *P*₆*T₆* treatment (LnRR analysis, Figure 13).

The blade toughness in *L. brongniartii* was significantly different between treatments (PERMANOVA, *p* < 0.05) (Table 4). *L. brongniartii* in the *P*₆*T₆* treatment had the least tough blades, 45.7% less than the control treatment (pairwise comparison, *p* <0.05 (Figure 12c). For the *P*₆*T₆* and *P*₆*T₅* treatments, the upper and lower bound 95% confidence intervals indicate a decrease in toughness between individuals (-1.1% to -3.8% and -0.03% to -5.8%, respectively) (LnRR analysis, Figure 13).
Figure 12: Weight needed to penetrate blades of **A. S. linearifolium**, **B. E. radiata** and **C. L. brongniartii** in the four treatments (PETA, PETE, PATE, and PATA). Data are mean ± SE of the weight required to penetrate blades, measured using a penetrometer, after the 14 day experiment period.

Table 4: PERMANOVA contrasting blade toughness with different treatment conditions (PETA, PETE, PATE, and PATA) for each algal species. Significant values (p < 0.05) shown in bold.

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3.1.5 Blade mass per square centimetre
Density results were not uniform between species. The average blade mass per square centimetre for untreated algae was highest in *E. radiata* (19.3 mg/cm²) compared to *S. linearifolium* (8.7 mg/cm²) and *L. brongniartii* (7.8 mg/cm²) (Figure 14). In *S. linearifolium* blade density varied between treatments (PERMANOVA, *p* < 0.05, Table 5). The average blade mass per square centimetre for algae in the PₐTₑ treatment significantly decreased by 12.1% compared to the control treatment (pairwise comparison, *p* < 0.05, Figure 14). For *S. linearifolium*, individuals in the PₑTₐ treatment reduced in density, observed in the upper and lower bound 95% confidence intervals (-0.4% to -3.2%) (LnRR analysis, Figure 15).

No significant differences between blade densities among treatments were observed for *E. radiata* (Table 5). Despite this, the upper and lower bound 95% confidence intervals showed that some individuals in the PₑTₑ treatment had higher blade mass, ranging from 0.3% to 3.0% (LnRR analysis, Table 5). *L. brongniartii* blade density varied between treatments (PERMANOVA, *p* < 0.05) (Table 5). *L. brongniartii* in the PₑTₐ and PₐTₑ treatments increased in average blade density by approximately 15.6% and 9.1% more than controls, respectively, (pairwise comparison, *p* < 0.05) (Figure 14c). Similarly, the upper and lower bound 95% confidence intervals indicated that there were
increases in blade density between individuals in the PETA (0.2% to 1.6%) and PATE (0.4% to 2.3%) treatments for L. brongniartii (LnRR analysis, Figure 15).
Figure 14: Blade mass per cm$^2$ of A S. linearifolium, B E. radiata and C L. brongniartii in the four treatments (P$_a$T$_A$, P$_a$T$_E$, P$_a$T$_E$, and P$_a$T$_A$). Data are mean ± SE of the blade mass per cm$^2$ conducted after the 14 day experiment period.

Table 5: PERMANOVA contrasting blade mass per cm$^2$ with different treatment conditions (P$_a$T$_A$, P$_a$T$_E$, P$_a$T$_E$, and P$_a$T$_A$) for each algal species. Significant values (p < 0.05) shown in bold.

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<td>L. brongniartii</td>
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Figure 15: Average log response ratio (LnRR) (± 95% CI) of percentage of blade mass per cm² after 14 day experimental period for *S. linearifolium*, *E. radiata* and *L. brongniartii*, by calculating the natural log of treatment (P<sub>E</sub>T<sub>A</sub>, P<sub>E</sub>T<sub>E</sub>, P<sub>A</sub>T<sub>E</sub>) response divided by the control (P<sub>A</sub>T<sub>A</sub>) response.

### 3.1.6 Nutrient analysis

The treatments produced different results for *E. radiata* and *L. brongniartii*. *E. radiata* showed differences in the P<sub>E</sub>T<sub>A</sub> and P<sub>E</sub>T<sub>E</sub> treatments, whereas *L. brongniartii* produced higher C:N ratios in the treatments P<sub>E</sub>T<sub>A</sub>, P<sub>E</sub>T<sub>E</sub> and P<sub>A</sub>T<sub>E</sub> compared to the control treatment. Overall, *L. brongniartii* showed a considerably lower C:N ratio 12.54:1 (0.6 ±SE) compared to *S. linearifolium* 51.45:1 (3.9 ±SE) and *E. radiata* 56.2:1 (4.4 ±SE) observed in the control treatments (Figure 16).

There were no significant differences between C:N ratios and the treatments for *S. linearifolium* (PERMAVOVA, p>0.05, Table 6), the upper and lower bound 95% confidence intervals indicated that the C:N ratio in *S. linearifolium*, for some individuals C:N increased in both the P<sub>E</sub>T<sub>E</sub> (1.9% to 5.5%) and P<sub>A</sub>T<sub>E</sub> (0.1% to 6.4%) treatments relative to controls (LnRR analysis, Figure 17).

Significant differences were observed in the C:N ratio among treatments in *E. radiata* (PERMANOVA, p<0.05, Table 6). The C:N ratio significantly increased (by approximately 40%) in the P<sub>E</sub>T<sub>A</sub> treatment compared to the P<sub>E</sub>T<sub>E</sub> treatment (pairwise comparison, p < 0.05, Figure 16b). *E. radiata* C:N ratios varied among treatments. Similarly, the upper and lower bound 95% confidence intervals showed a significantly increased C:N ratio in the P<sub>E</sub>T<sub>A</sub> treatment (0.7% to 2.8%), and a lower C:N ratio in the P<sub>E</sub>T<sub>E</sub> treatment (-0.2% to -3.4%) (LnRR analysis, Figure 17).

The C:N ratio in *L. brongniartii* was significantly different among treatments (PERMANOVA, p < 0.05, Table 6). The C:N ratio increased by approximately 32% in the P<sub>E</sub>T<sub>A</sub> treatment compared to the control treatment (pairwise comparison, p < 0.05, Figure 16c). The upper and lower bound 95% confidence intervals indicated that the P<sub>E</sub>T<sub>A</sub> (2.2% to 3.5%) and P<sub>A</sub>T<sub>E</sub> (0.4% to 1.4%) treatments, C:N increased between individuals within treatments (p <0.001, LnRR analysis) (Figure 17). *L. brongniartii* showed high variability in the P<sub>E</sub>T<sub>E</sub> treatment between individuals as seen in the standard error (4.8 ±SE) (Figure 16c).
Figure 16: Carbon/ nitrogen ratios of A. S. linearifolium, B. E. radiata and C. L. brongniartii in the four treatments (P_{E\,T_A}, P_{E\,T_E}, P_{A\,T_E}, and P_{A\,T_A}). Data are mean ± SE of the total carbon and total nitrogen as a percentage ratio, conducted after the 14 day experiment period.

Table 6: PERMANOVA contrasting carbon/ nitrogen ratios with different treatment conditions (P_{E\,T_A}, P_{E\,T_E}, P_{A\,T_E}, and P_{A\,T_A}) for each algal species. Significant values (p < 0.05) shown in bold.

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Figure 17: Average log response ratio (LnRR) (± 95% CI) of percentage of carbon / nitrogen ratio after 14 day experimental period for S. linearifolium, E. radiata and L. brongniartii, by calculating the natural log of treatment (P_{E\,T_A}, P_{E\,T_E}, P_{A\,T_E}) response divided by the control (P_{A\,T_A}) response.
3.3 Feeding assays

3.2.1 Fresh feeding assay

The average amount of fresh material consumed by the herbivores varied with each species, with *A. compressa* consuming 5.9 mg (0.8 ±SE) *S. linearifolium*, 0.7 mg (0.1 ±SE) *E. radiata* and 1.9 mg (0.9 ±SE) *L. brongniartii* in the control treatment. Trochidae consumed on average 0.01 mg (1.1 ±SE) *S. linearifolium*, -0.8 mg (0.5 ±SE) *E. radiata* and 0.5 mg (2.2 ±SE) *L. brongniartii* in the control treatment. When presented with fresh algal material, Trochidae consumed more algae in the P<sub>E</sub>T<sub>A</sub> and P<sub>E</sub>T<sub>E</sub> treatments compared to the P<sub>A</sub>T<sub>E</sub>, and the control in all species of algae. However, no comparable results were observed between species of algae consumed by *A. compressa* and the treatments (P<sub>E</sub>T<sub>A</sub>, P<sub>E</sub>T<sub>E</sub>, P<sub>A</sub>T<sub>E</sub>, and P<sub>A</sub>T<sub>A</sub>) (Figure 18). Additionally, no significant results were found for *S. linearifolium* or *L. brongniartii* between herbivore consumption (*A. compressa* and Trochidae) and treatments (Table 7).

When fresh *E. radiata* material was presented to Trochidae, significant differences in the consumption rates of the algae between treatments were observed (PERMANOVA, \( p < 0.05 \)) (Table 7). Trochidae consumed approximately 1.88 mg (P<sub>E</sub>T<sub>A</sub>) and 1.85 mg (P<sub>E</sub>T<sub>E</sub>) more mass of *E. radiata* in the 36 hour assay, compared with the control treatment (pairwise comparison, \( p < 0.05 \)) (Figure 18b). Whereas, there were no significant differences in *E. radiata* consumed by *A. compressa* between treatments (Figure 18b).

![Graph showing feeding assays results](image-url)
Figure 18: Fresh feeding assay, performed with *A. compressa* and Trochidae using *S. linearifolium*, *E. radiata* and *L. bronniartii* in the four treatments (PETA, PETE, PATE, and PATA). Data are mean ± SE of the mass loss per herbivore conducted over a 36 hour period, conducted after the 14 day experiment period.

Table 7: PERMANOVA contrasting fresh feeding assay and herbivore with different treatment conditions (PETA, PETE, PATE, and PATA) for each algal species. Significant values (p < 0.05) shown in bold.

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3.2.2 Ground feeding assay

Similar to the fresh feeding assay, the average amount of ground material consumed by the herbivores varied between species, with *A. compressa* consuming 13.2 mg (2.2 ±SE) *S. linearifolium*, 13.4 mg (5.0 ±SE) *E. radiata* and 3.0 mg (2.0 ±SE) *L. brongniartii* in the control treatment. Trochidae consumed on average 4.4 mg (3.0 ±SE) *S. linearifolium*, 2.8 mg (1.1 ±SE) *E. radiata* and 12.8 mg (7.4 ±SE) *L. brongniartii* in the control treatment.

*S. linearifolium* produced statistically significant results between the amount of ground algae consumed by Trochidae and the treatments (PERMANOVA, *p* < 0.05, Table 8). Trochidae consumed significantly different amounts of ground alga between treatments, with the gastropods consuming approximately 10.0 mg less within the 36 hour feeding assay than the control (pairwise comparison, *p* < 0.05) (Figure 19a).

Significant differences were also identified between the amount consumed by Trochidae and the treatments for the ground *E. radiata* material (PERMANOVA, *p* < 0.05) (Table 8). Trochidae consumed 5.7 mg (0.1 ±SE) less of the *E. radiata* in the *P*₆*Tₐ* treatment and 3.7 mg (0.4 ±SE) less in the *P*₆*Tₚ* treatment across the 36 hours of the feeding assay (Figure 19b).

No significant results were found between the mass consumed by Trochidae and the treatments for ground *L. brongniartii* (*P*₆*Tₐ*, *P*₆*Tₚ*, *P*₆*Tₚ*, and *P*₆*Tₐ*). Similarly, no significant results were found for the amount of ground algae (*S. linearifolium, E. radiata* of *L. brongniartii*) consumed by *A. compressa*, between the treatments (Figure 14, Table 8).
Figure 19: Ground feeding assay, performed with *A. compressa* and Trochidae using *A. compressa*, *S. linearifolium*, *E. radiata* and *C. brongniartii* in the four treatments (PETE, PETA, PATE, and PATA). Data are mean ± SE of the mass loss per herbivore conducted over a 36 hour period, conducted after the 14 day experiment period.

Table 8: PERMANOVA contrasting ground feeding assay and herbivore with different treatment conditions (PETE, PETA, PATE, and PATA) for each algal species. Significant values (p < 0.05) shown in bold.

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3.4 Total phenolic content

The concentration of phenolics varied between species; *E. radiata*, irrespective of the treatment, had the highest mean concentration, ranging from 5.7–6.5% dry mass (0.4 - 0.9 ±SE). The phenolic mean concentration in *S. linearifolium* was almost half of that of *E. radiata* and ranged from 32.76 to 27.55 mg/g dry wt (1.5 - 4.8 ±SE). *L. brongniartii* had much lower mean phenolic concentration which ranged from 1.51 - 5.07 mg/g dry wt (0.1 - 0.6 ±SE). No significant results were found between the total phenolic content and the treatments in *S. linearifolium or E. radiata* (Table 9).
Significant differences were found between the total phenolic content and the treatments observed in *L. brongniartii* (PERMANOVA, *p* < 0.05) (Table 9). Algae in the temperature treatments (PETE and PATE) significantly reduced phenolic production by approximately 70.2% (PETE) and 68.7% (PATE) compared to the control (pairwise comparison, *p* < 0.05) (Figure 20c). Significantly reduced phenolic content was observed in the upper and lower bound 95% confidence intervals between individuals in the PETE (-8.4% to -15.4%) and PATE (-9.7% to -13.2%) treatments (LnRR analysis, Figure 21).
Figure 20: Total phenolic content of **A** *S. linearifolium*, **B** *E. radiata* and **C** *L. brongniartii* in the four treatments (PETA, PETE, PATE, and PATA). Data are mean ± SE of the total phenolic content as milligrams per gram of dry weight, conducted after the 14 day experiment period.

Table 9: PERMANOVA contrasting total phenolic content with different treatment conditions (PETA, PETE, PATE, and PATA) for each algal species. Significant values (p < 0.05) shown in bold.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total phenolic content</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. linearifolium</em></td>
<td>3</td>
<td>0.428</td>
<td>0.369</td>
<td>0.816</td>
</tr>
<tr>
<td><em>E. radiata</em></td>
<td>3</td>
<td>0.317</td>
<td>0.281</td>
<td>0.840</td>
</tr>
<tr>
<td><em>L. brongniartii</em></td>
<td>3</td>
<td>5.359</td>
<td>29.319</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Figure 21: Average log response ratio (LnRR) (± 95% CI) of percentage of total phenolic content after 14 day experimental period for *S. linearifolium*, *E. radiata* and *L. brongniartii*, by calculating the natural log of treatment (PETA, PETE, PATE) response divided by the control (PATA) response.
3.5 Pattern of results
The MDS plots for *S. linearifolium* show a distinct pattern of similarity amongst the datasets in the P<sub>E</sub>T<sub>E</sub> treatment, however a high amount of variability between the data for all other treatments (P<sub>E</sub>T<sub>A</sub>, P<sub>A</sub>T<sub>E</sub> and P<sub>A</sub>T<sub>A</sub>) (Figure 22a). The MDS plots for *E. radiata* indicate that the treatments are highly dissimilar, indicating large differences between the data within the performance/feeding/phenolic measurements (Figure 22b). The MDS analysis performed on *L. bronniartii* show a clearer pattern of similarity than the other two algal species. The P<sub>E</sub>T<sub>A</sub> and P<sub>A</sub>T<sub>A</sub> treatments displayed a very high level of similarity between the datasets. Although not as similar, the P<sub>E</sub>T<sub>E</sub> and P<sub>A</sub>T<sub>E</sub> treatments still show a high level of likeness between datasets for *L. bronniartii* (Figure 22c).
Figure 22: MDS-plot of the pattern of similarity between all datasets, including performance measurements, feeding experiments and phenolic content for A S. linearifolium, B E. radiata and C L. brongniartii.

3.6 Summary of results

To summarise the previous results showing the statistically significant differences in algal performance, chemical analysis and feeding results and comparing the three species of algae examined a table (Table 10).
Table 10: Summary of the results, combined conditions, ocean acidification, ocean warming. ↑ statistically significant increase compared to another treatment, ↓ statistically significant decrease compared to another treatment.

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth rates</th>
<th>Density</th>
<th>Blade toughness</th>
<th>Bleaching</th>
<th>QEY’s</th>
<th>Total phenolics</th>
<th>C:N ratios</th>
<th>Ground FA</th>
<th>Fresh FA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. linearifolium</em></td>
<td>↓</td>
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<tr>
<td><em>E. radiata</em></td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td><em>L. brongniartii</em></td>
<td>↓</td>
<td>↑</td>
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<td>↑</td>
<td>↓</td>
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</tr>
</tbody>
</table>

3.7 Water Chemistry

The total alkalinity was measured weekly for each species in each treatment. The $pCO_2$ in the increased pH treatments fluctuated at 1080 -1380 µatms depending on the water conditions including, temperature, salinity and pH. The $pCO_2$ levels in the treatments without altered pH conditions fluctuated at 112 – 410 µatms (Figure 23). The temperature was fairly stable throughout the experiment (Figure 24). The salinity wavered daily (at 35.7 -36.3 ppt) depending on the water temperature, the filtration and the amount of fresh rainwater added the previous day (Figure 25).

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Figure 23: Total alkalinity of *S. linearifolium*, *E. radiata* and *L. brongniartii* in the four treatments (PETA, PETE, PATE, and PATA). Data are mean ± SE of the total alkalinity measured as micro-atmospheres. The water sample was taken in the second week of the experiment period for each species.
Figure 24: Water temperature in the four treatments (P\text{ET}A, P\text{ET}E, P\text{AT}E, and P\text{AT}A). Data are mean ± SE of the water temperature as Degrees Celsius, measured daily throughout the 14 day experiment period.

Figure 25: Water salinity in the four treatments (P\text{ET}A, P\text{ET}E, P\text{AT}E, and P\text{AT}A). Data are mean ± SE of the water salinity as parts per thousand (ppt), measured daily throughout the 14 day experiment period.
4. Discussion

There are varying opinions on how fleshy macroalgae will respond to climatic changes: some studies comparing terrestrial studies to macroalgal studies suggest that elevated \( p\text{CO}_2 \) may be uniformly beneficial (Ainsworth & Long 2005), and that elevated temperatures will have a negative effect (Kirschbaum 2004), however, the combined effect is relatively unknown (Harley et al. 2012). Having an understanding of the combined effect of increased temperature and ocean acidification is vital, as together they are likely to have a dramatic effect on the dynamics and distribution of many organisms in all marine ecosystems (Hale et al. 2011). It is also essential when conducting projected climate change research to not only consider how the stressors temperature and \( p\text{CO}_2 \) interact but how they could potentially impact multiple species (Wernberg et al. 2012a; Poore et al. 2013). Also, there are few studies that show how multiple species of algae are affected by these climate change stressors using multiple health and performance indicators.

This study examined how predicted changes in temperature and ocean acidification may affect three species of ecologically important macroalgae in terms of algal performance and health, nutrient and phenolic content, and potential changes in biomass through shifts in consumption. This is the first study that has (1) evaluated the impact of both increased temperature and increased \( p\text{CO}_2 \) on multiple macroalgal species and (2) considered multiple performance indicators for each alga. This study will increase current knowledge in this field by providing a comprehensive insight into how both canopy forming and understory macroalgae could respond, physically and chemically, to projected climate change conditions. Although the data from each species cannot be statistically compared, the results demonstrate that macroalgal responses to potential climate change are likely to be diverse, and that comprehensive predictions regarding macroalgal responses to environmental changes will not be possible.

4.1 Treatment effects on \( S. \text{linearifolium} \)

Temperatures above the optimum functioning level can affect the physiological processes of all living organisms (Poore et al. 2013). When exposed to temperature
levels 5°C above ambient, *S. linearifolium* was substantially bleached, produced low quantum efficiency yields and had a higher density in high temperature conditions. This response to high temperatures is unexpected as the species has a temperate to subtropical distribution range. Campbell *et al.* (2011) suggest that increases in temperature generally result in increases in the amount of bleaching in macroalgae, as found here for *S. linearifolium*. Surprisingly, bleaching in the control treatment was also relatively high, suggesting high amounts of stress in the algae within all treatments, possibly due to the removal of the algae from the reef and placement in tanks. Despite the background levels of bleaching in the control, the treatments simulating climate change conditions resulted in a significantly higher amount of bleaching, above that of the controls, suggesting that bleaching is likely to occur in response to warming seawater. Interestingly, bleaching of this type may not be a direct response to temperature, as indicated in a study by Campbell *et al.* (2011) who examined bleaching in the red alga *Delisea pulchra*. They found that at high temperatures, the chemical defences in *D. pulchra* were reduced, allowing for increased colonisation by bacterial pathogens, which then resulted in bleaching of the algae. This demonstrates the complexity of the interactions that occur in algal functioning and how changes in temperature can facilitate other mechanisms that may also detrimentally impact macroalgal health. Similar studies are exploring whether bleaching in brown algae is caused from a shift in associated microbial communities similar to that observed in *D. pulchra* (BOM 2014; Marzinelli *et al.* 2015). Given that macroalgae are the ecosystem engineers of temperate rocky shores, the loss of species through disease induced bleaching would be parallel in effect to the masses of bleached coral observed in the tropics (Marzinelli *et al.* 2015).

The effect temperature has on bleaching and photosynthetic rates synergistically and adversely affected the health of *S. linearifolium*. Similar to the results found in Marzinelli *et al.* (2015), which saw a reduction in photosynthetic rates with bleaching in *E. radiata*, bleaching in *S. linearifolium* resulted in significantly lower quantum efficiency yields (QEYs) indicating increased stress in algae under high temperature treatments. Photosynthesis functioning is dependent on the temperature being within an optimal range (Berry & Bjorkman 1980) and it has been well established that with slight increases
in temperature, photosynthetic rates increase (Eggert 2012). However, at the algae’s optimum temperature the photosynthetic rate plateaus. Beyond this the photosynthetic rate rapidly decreases and when macroalgae are exposed to extreme temperatures the integrity of the photosynthetic system is ultimately damaged (Eggert 2012). The consequence is a reduction in the functioning of the photosynthetic system, inhibiting the release of energy from the fluorescent pathway which results in a diminished QEY output. My study shows that at 25.5°C the QEY decreased to 0.169 Fv/Fm, indicating that this temperature is beyond the optimum temperature for *S. linearifolium*. This information is especially important for individuals surviving in regions at the edge of the specie’s thermal limits. By having an understanding of the tolerances and adaptive ability of the algae this will assist in predicting the full impacts of climate change (Koch *et al.* 2013), in this case in relation to projected range shifts from areas near thermal limits.

The impact that elevated $p$CO$_2$ conditions will have on macroalgae is still relatively unknown. Where many species of algae have shown a positive response to increased $p$CO$_2$ (Porzio *et al.* 2011), others have shown either an unfavourable reaction or no measurable response (Israel & Hophy 2002; Gutow *et al.* 2014). Although there were no significant differences between the mean response in the increased $p$CO$_2$ treatments and the other treatments for *S. linearifolium*, individuals within the elevated $p$CO$_2$ treatments ($P_{ET_A}$ and $P_{ET_E}$) were significantly bleached (as indicated by the LnRR analysis). Bleaching was so high for these individuals in the increased $p$CO$_2$ treatments that they were unable to produce a QEY reading. This reduction in QEY may be influenced by the mechanism used by the algae to absorb CO$_2$. Studies are showing that different species of macroalgae are absorbing CO$_2$ by one of two mechanisms: (1) actively, through CO$_2$ concentration mechanisms (CCM) or (2) through simple diffusion (Case *et al.* 2011; Cornwall *et al.* 2012). All three species examined in this study possess the first mechanism described and actively absorb CO$_2$ through the CCMs. It would be expected that with increases in CO$_2$ concentrations and constant light availability there would be higher photosynthetic rates and therefore a higher QEYs in the treatments with increased $p$CO$_2$ ($P_{ET_A}$ and $P_{ET_E}$). Surprisingly, this was not the case for *S. linearifolium*. Similar to the results in my study, the red alga *Palmaria palmata* has been
shown to have a decreased photosynthetic rate under increased $pCO_2$ (Nunes et al. 2015).

Maintaining optimal leaf density is important for many reasons. Increased density is thought to increase the photosynthetic capabilities of the leaves (Niinemets 2001) and provide a longer leaf lifespan due to the extra structural strength (Westoby et al. 2002). If density is reduced the leaves are more susceptible to tearing and puncturing (Wright et al. 1989) and less dense blades also make the leaves easier to be consumed by herbivores (Westoby et al. 2002). Although common in many terrestrial studies, very few marine studies have examined the link between increased temperature and increased $pCO_2$ on leaf density. In terrestrial plants, leaf densities can be altered due to temperature, water availability, nutrients and sulphate availability, light intensity, herbivory, altitude, atmospheric concentrations of CO$_2$, season and leaf age (Witkowski & Lamont 1991). The results of my study indicate that increased temperature reduces the blade density of $S$. linearifolium. Similarly, Ku and Hunt (1973) observed a reduction in leaf density when growing alfalfa ($Medicago saliva$ L.) in increased temperatures. A reduction in leaf density would not be expected as higher temperatures usually accompany higher metabolic rates (Bearham et al. 2013) and therefore higher densities. However, similar to the mechanisms affecting temperature and QYE, the reduction in blade density may have been caused by the temperature exceeding the optimum threshold. Once the temperature has exceeded this threshold the metabolic rate is lowered and therefore the blade density reduced. Producing denser blades provides the algae with a physical deterrent, therefore protecting against herbivory, as the blades become less dense this protection is reduced and the likelihood of blade breakage and deterioration increases (Westoby et al. 2002). A loss in blade density could therefore facilitate biomass loss in macroalgae species through herbivory and breakage. It is plausible that this decreased blade density may have been a contributing factor to the reduction of algal diversity such as was observed off the Western Australian coast during the summer of 2011 (Wernberg et al. 2012b).

Despite the high levels of bleaching and decreased density observed in this experiment, the treatment conditions had no significant effect on the consumption rates of fresh $S$. linearifolium by either herbivore ($A$. compressa or Trochidae). This is in contrast to
results found in a similar experiment by Poore et al. (2013). They found that herbivores showed a lower preference for \textit{S. linearifolium} treated with increased temperature and decreased pH. However, my experiments did show a significant difference in consumption rates when Trochidae were fed on \textit{S. linearifolium} in the ground feeding assay. Trochidae consumed less of the ground algae treated with the combination of increased pCO$_2$ and increased temperature treatment compared to the control treatment. Changes in consumption rates have been observed in the gastropod, \textit{Norrisia norrisi} (Trochidae) which consumed more algae based on its morphology and toughness (Wakefield & Murray 1998). The purpose of producing the ground material was to remove the physical attributes of the algal blades, such as mass per square centimetre and toughness, from affecting the consumption rates of the herbivores, while maintaining the nutritional and phenolic content. Unlike many herbivores, gastropods have limited mobility and it is understood that they will consume the food that is reliably available rather than selecting food based on its nutritional quality (Wakefield & Murray 1998). In contrast to the consumption rates of \textit{N. norrisi} (Wakefield & Murray 1998), the algae in the present study had comparable nutritional quality to controls. Furthermore, all algae were readily available to the gastropods, so it is not surprising that these results do not correspond with previous studies. Similarly, the reduction in the consumption in the ground feeding assay does not align with the results of severe bleaching or the shifts in QEYs, suggesting that these are also not influencing consumption rates. Instead, other defensive or nutritional mechanisms such as fatty acids or proteins may be affecting the consumption rates of Trochidae (Poore \textit{et al.} 2013).

There were no significant differences in growth rates in \textit{S. linearifolium} among treatments. This was unexpected considering the increased temperature treatment experienced up to 70\% bleaching and a correspondingly low QEY. It is predicted that in increased CO$_2$ conditions the algal metabolic rate and growth rate would increase (Bearham \textit{et al.} 2013). Xu \textit{et al.} (2010) placed the red alga \textit{Gracilaria lemaneiformis} in increased CO$_2$ conditions and found that the growth rate increased during the 16 day treatment period. As observed in the LnRR analysis, an increase in growth in high CO$_2$ conditions was seen in some individual algae within the increased pCO$_2$ treatment and the combined increased pCO$_2$ and increased temperature treatment. This suggests that,
as expected, *S. linearifolium* were growing faster in the elevated *pCO₂* conditions but only for some individuals, and that this increase in growth varied among the algae in the treatments. This increase in growth rate may be beneficial for *S. linearifolium* inhabiting reefs in southern Australia where ocean temperature are predicted to remain within the *S. linearifolium* optimum growth conditions, but *pCO₂* levels are still expected to increase. However, for algae at the edge of the distribution range where both temperatures and *pCO₂* levels are likely to be high, *S. linearifolium* is expected to be affected by a reduction in growth rates.

Overall, the C:N results found in this study for *S. linearifolium* (51.46:1) were higher than the results found for the related species *Sargassum flavicans* (30.36:1) (Angell *et al.* 2012). However, the variation between species may be due to simple differences between algal species or, as many studies suggest, due to seasonal variations (Villares *et al.* 2013; Nunes *et al.* 2015). There were no significant differences in the mean C:N ratios in *S. linearifolium* among the treatments. Yvon-Durocher *et al.* (2015) compiled a meta-analysis of approximately 700 global datasets of macroalgal nutrient information, which included 17 studies of varied temperature analysis. Similarly, they found no difference in the C:N ratios of macroalgae at higher temperatures. Interestingly, they did find differences in the C:P and N:P ratios in higher temperatures, indicating that phosphorus may be an important factor in changing climatic conditions (Yvon-Durocher *et al.* 2015). Nunes *et al.* (2015) also found phosphorous and nitrate to be significantly variable among algal species in high *CO₂* conditions, indicating that these nutrients would be worthwhile analysing in future studies on the impact of climate change.

Although there were no overall significant differences between the C:N ratios among treatments, there were observed differences between individuals in the both of the increased temperature (P<sub>ET</sub> and P<sub>AT</sub>) treatments (LnRR analysis). Brown *et al.* (2014) did a similar study, examining the possible effects of increased temperature and elevated *pCO₂* on the giant kelp *Macrocystis pyrifera*. They found that in increased temperatures the C:N ratio of *M. pyrifera* increased by approximately 50%. A higher C:N ratio, as observed by individuals in the increased temperature treatments, suggests that they experienced a depletion of nitrogen content (Schulze *et al.* 1994). This reduced nitrogen content is likely to be a contributing factor to the decrease in blade density
observed in *S. linearifolium*. A comparable study on the species *S. linearifolium* tested under increased temperature and decreased pH conditions showed a similar pattern of no change in C:N ratios among treatments (Poore *et al.* 2013). Stable nutrient content may explain why increases in water temperature and increases in ocean acidification had no effect on consumption rates of *A. compressa* in both feeding assays.

This study has shown that a persisting warming anomaly of +5°C, as was experienced in Western Australia for several weeks during the summer of 2011 (Wernberg *et al.* 2012b), could have damaging effects on *S. linearifolium*. In terms of bleaching, the physiologically damaging effects on the algal pigmentation, the high amount of bleach cover and early onset of bleaching in high temperatures indicate that individuals persisting near the edge of their distribution range may be at risk from bleaching disease similar to that observed in *D. pulchra* (*Campbell et al.* 2011). Furthermore, this study indicates that 25.5°C is beyond the optimum temperature for *S. linearifolium* to photosynthesise. At the edge of the distribution range, this increase in temperature could possibly result in the mass removal of this canopy forming algae and facilitate the growth of other more resilient species such as algal turf. There is also a concern for individuals with higher C:N ratios. An increase in C:N ratio could indirectly increase herbivory rates by generating nutrient deficient algae and reducing the blade density. Encouragingly, *S. linearifolium* under future climate change scenarios may not be at risk from increased herbivory by mesograzers such as *A. compressa* or Trochidae.

**4.2 Treatment effects on *E. radiata***

Temperate reefs in Western Australia have historically been dominated by the canopy-forming kelp *E. radiata*. However, in 2011 a summer heatwave caused a shift in the benthic macroalgal community whereby kelp cover at sites in the northern temperate regions of the coast reduced significantly, allowing for the proliferation of turf-forming algae (Wernberg *et al.* 2012b). Surprisingly, my study indicates that physiological responses by the alga may not have been the determining factor in this loss of kelp cover. Increases in temperature did not have the severe bleaching effect on *E. radiata* that it did on *S. linearifolium*. This was unexpected as *E. radiata* is a truly temperate species and it would be anticipated that this species would be more susceptible to stress under increased temperature than the tropical-to-temperate *S. linearifolium*. For *E.*
*E. radiata*, the treatment with elevated pCO₂ had significantly higher C:N ratios compared to the increased pCO₂ and elevated temperature treatment. As previously observed for gastropods feeding on *S. linearifolium*, changes in nutrient levels can have a direct effect on herbivory rates and this was also observed in the fresh feeding assay for *E. radiata*. Remarkably, this is the first study to show that under projected climate change conditions changes in C:N influences consumption rates in gastropods resulting in greater amount of the key canopy forming species *E. radiata* being consumed. In contrast, when the algae material was ground, less *E. radiata* was consumed by Trochidae in the elevated pCO₂ and the combined elevated pCO₂ and increased temperature treatments, indicating that the physical features of *E. radiata* also play a role in the consumption rates of algae treated with future climate change conditions. These results suggest that under increased ocean acidification conditions, *E. radiata* will experience changes in nutrient levels and increased rates of herbivory by gastropods. *E. radiata* is the main canopy forming species in temperate ecosystems, and a loss in canopy through over grazing will cause a retraction in species range, allow for the establishment of invasive species and have a flow on effect to the commercial fishing industry (Wernberg et al. 2012c).

*E. radiata* had a higher C:N ratio in the elevated pCO₂ treatment. This is not surprising as increases in CO₂ can result in higher carbon allocation and therefore higher C:N ratios (Harley et al. 2012). High C:N ratios result in the algae placing a higher demand on nitrogen uptake and when this demand is not met it generates an overall deficiency in algal health (Rusterholz & Erhardt 1998). Similarly, temperature had a reducing effect on nutrient levels in *E. radiata* in natural conditions. It has been observed that as the kelps’ distribution range reduces in latitude the C:N ratio also reduces (Staehr & Wernberg 2009). My study indicates that the combination of increased temperature and increased pCO₂ reduced the C:N ratio in *E. radiata*, indicating that temperature influenced a significant reduction in the carbon allocation. These results agree with the observation in Poore et al. (2013) which suggests that temperature has a negative effect on the C:N ratios in macroalgae. Changes in the nutritional content of primary producers can also alter herbivory rates and affect the biomass of herbivores. Herbivores consuming primary producers with high nutritional quality (low C:N ratios), result in the
associated herbivores having accelerated metabolic and growth rates through the consumption of the additional nutrients (Cebrian et al. 2009). However, this increase in herbivore abundance and increased herbivory rates can result in lower producer biomass as the primary produces are over grazed. Although this trend is greater in terrestrial environments it is still detected in aquatic ecosystems (Cebrian et al. 2009). This reduction in C:N ratio caused by the addition of temperature in the combined elevated pCO2 and increased temperature treatment is unusual as the exclusively temperature treatment in this study did not show the same effect. This result is important as it indicates that the combination of the two stressors is having a unique effect on the C:N ratio in E. radiata.

Trochidae consumed greater amounts of fresh E. radiata material that were subjected to increased pCO2 in comparison to controls. When comparing the two treatments with increased pCO2 (PET and PET2), Trochidae consumed similar amounts, however, the C:N ratios of the pCO2 treatments differed, with the elevated pCO2 treatment increasing in C:N ratio and the combined increased pCO2 and temperature treatment decreasing in C:N ratio. This suggests that for E. radiata C:N ratios were not the contributing factor in the consumption rates on Trochidae on fresh E. radiata. Similarly, Molis et al. (2015) concluded that C:N levels had no effect on the feeding rates of gastropods when consuming the algae Fucus vesiculosus. As for S. linearifolium, the ground feeding results in E. radiata showed conflicting results to the fresh feeding assay. The treatments with increased pCO2 in the ground feeding assay were significantly less consumed than controls, suggesting again that there may be other influencing factors, such as proteins or fatty acids, contributing to the changes in observed feeding rates (Poore et al. 2013). The LnRR analysis indicated an unexpected increase in toughness for some individuals in the elevated pCO2 and increased temperature treatment for E. radiata; this was unusual considering that the Trochidae consumed more of this material. It was expected that if the algal blades became tough the consumption rates by herbivores would be reduced, (Wakefield & Murray 1998). This may suggest, similar to S. linearifolium that increases in temperature may be having an impact on other nutritional mechanisms such as fatty acids and proteins and these nutritional changes are affecting consumption rates in Trochidae. This is the first study to demonstrate that mesograzers will consume
more *E. radiata* in projected climate change conditions. After climate mediated disturbance, the gastropod *Turbo undulates* can increase its feeding rates on invasive turfing algae. This high level of grazing can reduce turf growth and promote new algal recruits (Ghedini *et al.* 2015). Although, when herbivory rates are too high they can be detrimental, recent evidence suggests that one strategy for coping with high levels of grazing is to ‘hide’ new macroalgal recruits. In areas in Portugal, juvenile *E. radiata* are able to persist only in reef crevices where light conditions are less optimal and growth rates are reduced, but the crevice protects algae from herbivory (Franco *et al.* 2015). Tropical fish consumption on new algal recruits is one of the driving factors of range shifts in *E. radiata* (Bennett *et al.* 2015b). In projected climate change conditions, tropical fish grazing combined with high rates of mesograzer consumption would accelerate the *E. radiata* range decline in Western Australian temperate reefs. It would be beneficial for future studies to examine the role of crevices in hiding *E. radiata* recruits from both fish and mesograzer herbivores along the coast of Western Australia and determine potential recruit survivorship.

*A. compressa* displayed no variations in their feeding preferences among treatments for both the fresh and ground *E. radiata*. Similar results were found by Gutow *et al.* (2014) when feeding isopods with fresh samples of the brown alga, *Fucus vesiculosus*, which was exposed to higher pCO$_2$ and temperatures. Comparable to *S. linearifolium*, there were no differences in phenolic content in *E. radiata* when exposed to the varying treatments. The consistency in the phenolic content, toughness and density across treatments may explain the lack of variation in feeding rates observed for *A. compressa* across the treatments. *E. radiata* also had lower percentages of bleaching compared to the other species of macroalgae and very little variation in the QEY. This supports the idea that, similar to what was observed for *S. linearifolium*, bleaching and QEY are both factors which impact macroalgae. The later onset of bleaching at day 6 in *E. radiata*, rather than day 3 as observed in *S. linearifolium* (and *L. brongniartii*, see below), was also an indication of the severity of bleaching. These results suggest a greater physiological resilience to projected climate change conditions in *E. radiata* compared to *S. linearifolium* and *L. brongniartii*. 
My results indicate that *E. radiata* was more resilient to physical changes in projected climate change conditions than may have been predicted due to the current shifts in the range of this species down the coast of both East and Western Australia (Verges *et al.* 2014). For the first time, I have demonstrated that environmental changes may result in increased herbivory from mesograzers, in this case gastropods from the family Trochidae. Similar to Jurien Bay, prior to 2011, *E. radiata* was the dominant algal species in Port Gregory, Western Australia (Bennett *et al.* 2015b). However, due to the increased water temperatures, as a result of the heatwave which removed the *E. radiata* from the area, invasive tropical fish are now preventing the recruitment and establishment of new kelps (Bennett *et al.* 2015b). The reduction of this distribution range is concerning as canopy forming kelps such as *E. radiata* are extremely valuable to the benthic community. They influence the temperate reef community structure and if lost, the kelp forest dynamics would be substantially altered.

### 4.3 Treatment effects on *L. brongniartii*

This study is one of a few studies to investigate the impacts of elevated pCO$_2$ and increased temperature on a fleshy, red understory algae. I found that *L. brongniartii* was physically and chemically altered by changes in ocean acidification and increased temperature, displaying significant alterations in plant health for all the performance parameters measured. Growth rates and phenolic content decreased, and bleaching increased in both treatments with increased temperature (P$_E$T$_E$, and P$_A$T$_E$). QEYs and toughness both decreased in the combined increased temperature and elevated pCO$_2$ treatment (P$_E$T$_E$). Nutrient content and growth increased in the elevated pCO$_2$ treatment (P$_E$T$_A$). Density gave unexpected results and increased in the elevated pCO$_2$ treatment (P$_E$T$_A$) and in the increased temperature treatment (P$_A$T$_E$) but not the combined treatment (P$_E$T$_E$). There was a high similarity between the datasets (as observed in the MDS plot), this indicates that each performance measurement was showing a recognisable response to the treatment conditions. Considering the numerous physical and chemical alterations observed in *L. brongniartii*, it was unexpected to find no changes in the feeding behaviour of the herbivores in either of the feeding assays.

The percentage of bleached tissue in *L. brongniartii* was significantly higher in all the treatments (P$_E$T$_A$, P$_E$T$_E$, and P$_A$T$_E$) compared to controls. Similar to *S. linearifolium*, there
was an overall higher percentage of bleaching in the increased temperature treatments ($PE_{TE}$ and $PA_{TE}$). As previously mentioned, a link has been made between the bleaching prevalence in the red alga $D. pulchra$ and increases in temperature. This increase in temperature reduces the halogenated furanone production by the alga and allows for an increase in colonisation of bacterial pathogens which in turn cause algal bleaching (Case et al. 2011). For future studies it would be valuable to determine if temperature will result in a reduction of chemical defences leaving the algae open to infections of bacterial causing bleaching disease in brown algae such as $S. linearifolium$ and $E. radiata$. Bleaching was not as intense in the elevated $pCO_2$ treatment when compared to the increased temperature treatments ($PE_{TE}$ and $PA_{TE}$). Therefore, in high latitudes, or at higher depths, where prevailing temperatures are lower, understory red algal species may be less impacted by bleaching even under future projected climate change conditions. Bleaching was observed early on in the experiment in $L. brongniartii$, at day 3, similar to $Sargassum sp.$ and this species was highly effected by bleaching at day 14. This suggests that $Sargassum sp.$ and $L. brongniartii$ are both susceptible to the onset of bleaching disease caused by increased temperature and that it could only take a few days of intensive weather conditions to result in the potential loss of individuals. Furthermore, the LnRR analysis indicated that individuals in the increased temperature and elevated $pCO_2$ treatment ($PE_{TE}$) were significantly bleached in all three species tested, and therefore it is expected that under projected climate change conditions bleaching will be a significant issue effecting all of these key temperate species.

Bleaching was most severe in the combined increased temperature and elevated $pCO_2$ treatment and this treatment also produced the lowest QEY. In a similar study, Connell and Russell (2010) found that projected climate change conditions allowed turving algae to expand, grow and occupy up to five times more available substratum area than in control treatments. My results indicate that under projected climate change conditions $L. brongniartii$, which also occupies the understory, will instead experience reduced QEYs, be susceptible to bleaching disease, have reduced toughness and reduced phenolic content. Reduced toughness could lead to overall deterioration in algal tissue as was observed in $L. brongniartii$. Similarly, reduced blade toughness and reduced phenolic content the algae could be more prone to herbivore consumption and with
lower QEYs, a lower metabolic rate would occur, which may impact reproductive output (O’Connor 2009). Therefore, we can predict that under future climate change scenarios *L. brongniartii* are likely to be out competed by faster growing and invasive species of turfing algae (Connell & Russell 2010).

The large variations in the QEY between post collection and day 1 in the P<sub>e</sub>T<sub>e</sub> and P<sub>e</sub>T<sub>a</sub> treatments suggest that *L. brongniartii* was stressed in the aquaria system prior to the initial treatment period. However, algae in the controls regained their yield readings to a state which were not significantly different to the post collection QEY readings. These results suggest that *L. brongniartii* requires longer than a 48 hour period to acclimatise to tank conditions, but that after this time the algae returns to a state of good health. It was surprising that *E. radiata* did not show a similar response and a decline in QEY as a response to the tank conditions, considering it is the largest of the three species. This again supports the idea of high resilience in *E. radiata* and high vulnerability in *L. brongniartii*.

*L. brongniartii* was the only species studied that showed variation in phenolic content among treatments. When examining the effect of phenolic content and temperature on macroalgae, Figueroa et al. (2014) suggested that shifts in phenolic content were variable with reduced levels occurring under increased temperature for some species of algae and increased concentrations for others. Furthermore, some studies have shown an inverse relationship between the increase in levels of bleaching and the reduction of phenolic content in red algae (Campbell et al. 2011). This was consistent with the bleaching and phenolic content observed in this study with both treatments with increased temperature showing large reductions in phenolic content. This reduction in phenolic content could be contributing to the high amounts of bleaching recorded in the increased temperature treatment. The reduction of these secondary metabolites may increase the colonisation of bacteria on the algae, stimulating thallus bleaching (Case et al. 2011). Red algal metabolites also have a number of other ecologically important roles. For example, the red alga *D. pulchra* produces the metabolite, histamine, which is a settlement cue to the sea urchin *Holopneustes purpurascens*. The urchin larvae settle on *D. pulchra*, which then provides shelter and food to the vulnerable urchin larvae (Swanson et al. 2004). In addition, the gastropod *Aplysia parvula* sequesters metabolites
from *Laurencia obtusa* that aid it in avoiding predation (Rogers *et al.* 2002). These studies demonstrate the importance of understory algae and their associated chemical compounds, in marine ecosystems. A reduction in metabolites such as histamine or terpenes (metabolites common in *L. obtusa* (Rogers *et al.* 2000)) could interfere with these key ecological roles. Therefore, a loss of chemical compounds associated with red algal species in the understory, caused by increasing climate change conditions, would be likely to have ecosystem wide impacts.

Overall, *L. brongniartii* had a lower C:N ratio (12.54:1) when compared to both brown algal species. This is not unusual for red algal species, with similar C:N ratio results observed in *Laurencia majuscula* (16.15:1) (Angell *et al.* 2012). In the elevated pCO₂ treatment, *L. brongniartii* experienced a high QEY and, similar to *E. radiata*, the increased pCO₂ treatment increased the C:N ratio in *L. brongniartii*. This again could be due to the high CO₂ allocation associated with increases in photosynthetic processes (Harley *et al.* 2012). This high C:N ratio may contribute to the blade density observed in the pCO₂ treatment, with higher carbon contributing to structural growth. In terrestrial sugar maple, *Acer saccharum*, Ellsworth and Reich (1992) recorded photosynthetic rates, carbon increase and leaf mass. They found that as the photosynthetic rates of *A. saccharum* increased there was a positive relationship between carbon accumulation and leaf mass. Although there was no statistically significant increases in QEY observed in *L. brongniartii* suggesting that it may be carbon saturated under current pCO₂ conditions, there was an increase in C:N and leaf density relationship. To assist in attaining a high photosynthetic rate, in increased pCO₂ conditions, *L. brongniartii* allocated the available carbon (high C:N ratio) to structural leaf density and growth. Algal physiological processes can be complex, while high QEYs can be advantageous for algal growth and increased density (Witkowski & Lamont 1991; Wu *et al.* 2008), alterations in the C:N ratio can be detrimental. Over time, if the demand for nitrogen is not met, the growth rates and blade density could eventually decrease, making the algae more susceptible to herbivory (Schulze *et al.* 1994).

Similar to *S. linearifolium*, *L. brongniartii* was greatly impacted by increased temperature. *L. brongniartii* deteriorated and blade material was lost from the apical tips in both treatments where temperature was increased (PₖTₑ and PₐTₑ), reducing the
measurable total growth. These results complement the findings of Flukes et al. (2015) who also noted a reduction in growth rates in the brown algae *Phyllospora comosa* when exposed to high temperature conditions. However, in my study, growth rates increased in the elevated pCO$_2$ treatment, again, this may be due to higher uptake of CO$_2$ observed in this treatment which provided the algae with more structural carbon to be utilised for growth (Harley et al. 2012). Algal blades were significantly softer in the combination of increased pCO$_2$ and increased temperature. When viewing all *L. brongniartii* treatments, an inverse relationship between the increasing percentage of bleaching and the blade toughness is observed. The reduction in the physical attributes of the algae including the softer and deteriorated blade could make the algae vulnerable to herbivory by tropical species and competition with other algal species (Schiel et al. 2004). If projected climate change conditions reduced the biomass of canopy forming species such as *E. radiata* and *S. linearifolium*, this will directly impact the understory species including *L. brongniartii*. When the dominate canopy forming kelp species are removed, it often facilitates the increase in abundance of understory species through the increase of light and available substrate (Clark et al. 2004; Flukes et al. 2014). As the performance of *L. brongniartii* was intensively impacted by increases in temperature and elevated pCO$_2$ it is expected that a decrease in *L. brongniartii* health and abundance would be observed. Therefore, if the canopy species was removed and a shift in understory species occurred, it would be expected that the increased light and substrate availability would allow for the prevalence of turfing algae, rather than fleshy red algae such as *L. brongniartii*.

No significant differences were observed in the consumption of *L. brongniartii* in either the fresh or the ground feeding assay for either herbivore examined. This result was unexpected considering the many differences observed in the various performance parameters measured. Duarte et al. (2016) observed an increase in herbivory when they exposed the brown alga, *Durvillaea antarctica* to increased ocean acidification conditions for 10 days. Using the treated algae they competed a series of no choice feeding assays using the amphipod *Orchestoidea tuberculata* and found an increase in consumption rate of approximately 30% in the elevated pCO$_2$ conditions. When observing consumption rates among species, the mean amount of *L. brongniartii* material consumed was similar to the amounts consumed in *S. linearifolium* and *E.*
radiata, although the phenolic content in L. brongniartii is sizably less. This suggests that phenolics are not responsible for deterring herbivory in L. brongniartii, and that other secondary metabolites such as halogenated monoterpenes might be playing this role (Raven 2015). For future studies, conducting a high-performance liquid chromatography analysis on L. brongniartii may identify the compounds present and responsible for generally deterring herbivory (Swanson et al. 2004). Within the fresh feeding assay, both A. compressa and Trochidae showed high levels of variability in all of the treatments measured, indicating that these herbivores are responding differently to the individual material presented to them, rather than the treatment as a whole. Unlike the results found for S. linearifolium and E. radiata, the ground feeding assay did not show opposing results to the fresh feeding assay, in the increased pCO₂ treatments (P̄₇TA and P₇TE). Although my results indicate that L. brongniartii may not be impacted by herbivory by small mesograzers such as amphipods and gastropods, larger herbivores including fish may exploit the reduction of L. brongniartii physical health and reduce the algal biomass.

L. brongniartii was greatly impacted both physically and chemically by changes in projected climatic conditions. As indicated by the fresh and ground feeding assays, in projected climate change conditions, herbivory by mesograzers may not cause the removal of this species. However, it is expected that L. brongniartii densities would still be in decline due to the reduction in the algal physical health. Understory red algal species play an important role in benthic reef communities. Red algae, as a chemically rich species, can provide associated herbivores with a chemical defence with herbivores that consume the algae able to sequester the secondary metabolites which in turn provides protection from mesograzer predators (Rogers et al. 2002). Sea urchin larvae can be induced to settle on red algae by the compound histamine and without this settlement cue recruitment of certain sea urchins is reduced (Swanson et al. 2004). As a chemically rich species, L. brongniartii may have a relationship with an associated herbivore which could be impacted if the species is retracted in distribution. A shift in L. brongniartii’s distribution range could also provide a substrate for invasive species such as turfing algae (Connell & Russell 2010), which could potentially affect any associated mesograzers and impact fauna in higher trophic levels.
5. Conclusion

The first aim of this study was to determine how the three species of macroalgae respond to increases in temperature and $p$CO$_2$ conditions. Overall, there is no one definitive response to the effects of climate change on the three species of macroalgae examined here. Each species displayed distinct responses to the treatment conditions in terms of plant performance, phenolic content and nutrient analysis. *S. linearifolium* had a strong response in the temperature treatments including significant increases in the percentage of bleaching, reduction in QEYs and increase in consumption rates by gastropods in the ground feeding assays. *E. radiata* was impacted by increases in $p$CO$_2$ and showed a significant increase in C:N ratios in the elevated $p$CO$_2$ treatment and a decrease in the combined treatment, and the consumption rates of the gastropods in both the fresh and the ground feeding assays. Despite these results, this study has shown that *E. radiata* may be more resilient to projected climate change conditions than was previously anticipated. *L. brongniartii* was significantly influenced by all three of the treatment conditions and displayed increased bleaching, decreased QEYs, increased C:N levels, decreased total phenolic content, decreased algal growth, decreased blade toughness and increased density in the projected climate change conditions. Within the increased temperature conditions, all three species of algae showed a similar pattern of bleaching throughout the experiment. These consistent results indicate that these findings are a genuine response to the treatment conditions rather than a response to the tank effects of tank design.

The second aim was to determine if the feeding rates of two species of mesograzers altered on macroalgae that had been subjected to projected climate change conditions. In the ground feeding assay for both *S. linearifolium* and *E. radiata*, feeding rates decreased under the various treatment conditions. For *E. radiata* the decline in consumption rates was observed in both elevated $p$CO$_2$ treatments, and for *S. linearifolium* it was observed only in the combined elevated $p$CO$_2$ and increased temperature treatment. *L. brongniartii* displayed no differences in feeding rates for either herbivore among the treatments, showing again the species specific nature of the impact of environmental change to macroalgae. For the first time, I observed an increase
in feeding rates by a mesograzer on *E. radiata* subjected to projected climate change conditions. Therefore, it may be expected that in future climate change conditions, overgrazing by gastropods or other marine herbivores may occur and reduce the biomass of this species. This overgrazing by herbivores in temperate reefs has already been observed in Port Gregory in Western Australia (Bennett *et al.* 2015b). Tropical fish are shifting down the coast to temperature water and consuming *E. radiata* at high rates. These rates of consumption are often so high that the fish are maintaining algal turf dominance and preventing the reestablishment of the kelp species (Bennett *et al.* 2015b).

The final aim of this study was to determine the role of plant chemicals in altering the consumption rates of herbivores in algae that has been exposed to projected climate change conditions. *L. brongniartii* was the only species to have variations in phenolic content and also the only species to show no variations in consumption rates. These results indicate that there may not be a strong link between shifts in algal chemistry and herbivory by mesograzers under environmental change. However, red algae are known to be chemically rich (Swanson *et al.* 2012), and this study only examined the presence of phenolics. Through further analysis, using methods such as high-performance liquid chromatography, many other compounds may be detected. Overall, this study indicates that phenolic content had no effect on the feeding rates of the two herbivores tested.

One suggestion for future research is to examine the dispersal patterns of macroalgae under climate change conditions. Macroalgae reproduce and disperse in many different ways and in changing climatic conditions it would be expected that these responses would vary among species. The green alga *Ulva intestinalis* is very seasonal in growth and when weather conditions are less favourable the algae will physically respond to maintain survival. If the abundance of adult *U. intestinalis* is reduced due to extremes in winter weather conditions, these algae will utilise all their resources for growth rather than for reproduction (Martins *et al.* 2008). However, under situations of high irradiance and prolonged light, the spores of giant kelp *M. pyriforma* were able to swim for longer periods of time and therefore dispersed over a greater distance than when irradiance was reduced (Reed *et al.* 1992), suggesting that this alga might increase in abundance and possibly become invasive. To date, there have been no studies that have examined
the impact of environmental change on the reproductive success or dispersal abilities of any of the species examined in this thesis, indicating a knowledge gap that is important to address in future studies.

Warming anomalies, such as the one that occurred in Western Australia in 2011, are expected to re-occur and the frequency at which they will arise is also predicted to increase (Smale & Wernberg 2013). The potential for macroalgal species to survive in future climate change conditions depends on their plasticity and ability to adapt physiologically to environmental changes. Many species of algae adapt every year to large changes in seasonal weather conditions. Therefore, slow and constant increases in temperature and ocean acidification may not have a large impact on the algae (Wernberg et al. 2010). However, if algae are unable to acclimate to the intense, prolonged warming anomalies, in conjunction with the slow increase in projected climate change conditions, they will most likely perish. This will most likely facilitate a range shift in many species, promote the establishment of turfing algae (Connell & Russell 2010) and prevent recruitment of new individuals from overgrazing by invasive herbivores (Bennett et al. 2015b). Therefore, having an understanding of how warming anomalies and projected climate change conditions will impact marine organisms is important. The results of this study allow for a clearer understanding of how these three ecologically important species may respond to altered conditions, assisting with more accurate predictions of how temperate marine ecosystems as a whole may respond to predicted changes in environmental conditions into the future.

This study agrees with many others, which suggest that projected climate change will have species specific impacts (Bidart-Bouzat & Imeh-Nathaniel 2008). Although, for the first time, this study has shown how the red algal species, L. brongniartii will be greatly affected physically and chemically by projected climate change. This alga may be vulnerable to a reduction in health due to increased temperature and elevated pCO₂, which may influence a shift in distribution range. However, the loss of biomass or abundance of the L. brongniartii would not be due to herbivory by amphipod and gastropod mesograzers. E. radiata proved to be more resilient to projected climate change conditions than initially predicted. Interestingly, this study is fundamental in showing that E. radiata would be vulnerable to increased consumption by gastropods in
projected climate change conditions. This could impact this canopy forming species by reducing the abundance of this kelp and facilitate competition for habitat with invasive tropical species. Similar to L. brongniartii, herbivory would not influence a reduction in abundance and biomass of S. linearfolium in projected climate change conditions. Although increases in temperature and $pCO_2$ will greatly impact the physical performance of S. linearfolium, this study is unique in suggesting that due to a reduction in physical health, this semi-tropical to tropical species would not be a dominant invasive species in future climatic scenarios.
6. References


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